Vol. 129: 165–174, 2018
https://doi.org/10.3354/dao03248

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published August 14

Morbillivirus infection in Risso's dolphin *Grampus griseus*: a phylogenetic and pathological study of cases from the Canary Islands

Eva Sierra[1,2], Antonio Fernández[1,*], Daniele Zucca[1], Nakita Câmara[1], Idaira Felipe-Jiménez[1], Cristian Suárez-Santana[1], Yara Bernaldo de Quirós[1], Josué Díaz-Delgado[2], Manuel Arbelo[1]

[1]Department of Veterinary Pathology, Institute of Animal Health, Veterinary School, University of Las Palmas de Gran Canaria, 35413 Las Palmas, Spain

[2]Department of Anatomy and Comparative Pathology, Veterinary Faculty, University of Córdoba, Animal Health Building, Campus de Rabanales, Ctra. Madrid-Cádiz km. 396, 14014 Córdoba, Spain

ABSTRACT: The earliest evidence of cetacean morbillivirus (CeMV) infection dates from 1982, when the dolphin morbillivirus strain (DMV) was identified in bottlenose dolphins *Tursiops truncatus* stranded in the mid-Atlantic region. Since then, CeMV has been detected globally in at least 26 species of mysticetes and odontocetes, causing widespread mortality and a wide range of pathological effects. In the Canary Islands, DMV and pilot whale morbillivirus have been detected in cetacean species, including short-finned pilot whales *Globicephala macrorhynchus* and bottlenose dolphins. Risso's dolphins *Grampus griseus* have been reported year-round in waters of the Canary Islands and are considered a resident species. No information is currently available on CeMV prevalence in this species in this ocean region. We searched for evidence of CeMV infection in 12 Risso's dolphins stranded in the Canary Islands from 2003 to 2015 by means of histopathology, PCR and immunohistochemistry. PCR revealed 2 CeMV-positive animals (16.6%). Phylogenetic analysis showed that the strains from the 2 positive specimens were phylogenetically quite distant, proving that more than 1 strain infects the Risso's dolphin population in this region. We also determined that the strain detected in one of the specimens mainly circulated in the northeastern Atlantic Ocean from 2007 to 2013.

KEY WORDS: Central eastern Atlantic Ocean · Cetaceans · Morbillivirus · Non-suppurative meningoencephalitis · Risso's dolphin

INTRODUCTION

Since the late 1980s, cetacean morbilliviruses (CeMVs) have caused lethal disease outbreaks in odontocete and mysticete populations worldwide (Van Bressem et al. 2014, Morris et al. 2015, Kemper et al. 2016). CeMVs, as well as other viruses of the genus *Morbillivirus* (canine and phocine distemper viruses in carnivores, rinderpest and peste des petits ruminant viruses in artiodactyls and measles viruses in humans and other primates), are very contagious and cause severe infections of the respiratory, nervous and immune systems. Lesions attributed to CeMV include broncho-interstitial pneumonia, non-suppurative meningoencephalitis (N-S ME) and lymphoid depletion, which can lead to fatal secondary infections in susceptible hosts (Domingo et al. 1992, Duignan et al. 1992, Kennedy 1998).

CeMVs have been detected by serology, immunohistochemistry and/or molecular methods in at least

*Corresponding author: antonio.fernandez@ulpgc.es

26 different cetacean species, including both mysticetes and odontocetes (Van Bressem et al. 2001, 2014, Bento et al. 2016, Centelleghe et al. 2017). Susceptibility to disease caused by CeMV varies among cetaceans, with striped dolphins *Stenella coeruleoalba* and bottlenose dolphins *Tursiops truncatus* dolphins being the most common species involved in outbreaks (Van Bressem et al. 2014). Four different strains of CeMV (Bolt et al. 1994) (family *Paramyxoviridae*) have been well characterized: porpoise morbillivirus (PMV), isolated from harbour porpoises *Phocoena phocoena* that died along the coast of Ireland and the Netherlands (McCullough et al. 1991, Barrett et al. 1993, Visser et al. 1993); dolphin morbillivirus (DMV), first identified in striped dolphins from the Mediterranean Sea (Van Bressem et al. 1991, Barrett et al. 1993, Visser et al. 1993); pilot whale morbillivirus (PWMV), isolated from a long-finned pilot whale *Globicephala melas* stranded in New Jersey, USA (Taubenberger et al. 2000); and Longman's beaked whale morbillivirus (LBWMV), isolated from a Longman's beaked whale *Indopacetus pacificus* stranded in Hawaii, USA (West et al. 2013). In addition, 2 novel sequences or strains infecting 2 different species, the Indo-Pacific bottlenose dolphin *T. aduncus* (Western Australia) and the Guiana dolphin *Sotalia guianensis* (Brazil), have recently been reported in the southwestern Atlantic of the Southern Hemisphere (Groch et al. 2014, Stephens et al. 2014).

Although the volume of available information has increased, obtained through the combination of traditional (microscopy, serology and culture) and molecular techniques (Groch et al. 2014, Stephens et al. 2014, Van Bressem et al. 2014, van Elk et al. 2014, Mazzariol et al. 2016, 2017, Centelleghe et al. 2017), the virulence of CeMV in some species remains unclear.

The Canary Islands are located in the central eastern (CE) Atlantic Ocean, where CeMV has been detected in 12 stranded cetaceans from 4 different species: bottlenose dolphin (n = 1), common dolphin *Delphinus delphis* (n = 1), short-finned pilot whale *G. macrorhynchus* (n = 4) and striped dolphin (n = 5) (Bellière et al. 2011a, Sierra et al. 2014a,b, 2016). Three species within the family Delphinidae are considered resident in the Canary Islands: bottlenose dolphin, short-finned pilot whale and Risso's dolphin *Grampus griseus* (Vonk & Martel 1988, Carwardine 1995, García et al. 2002, Tobeña et al. 2011). We investigated the occurrence and prevalence of CeMV in Risso's dolphins from the Canarian archipelago by means of histopathology supplemented with immunohistochemistry and molecular tools. For this purpose, we tested for evidence of CeMV and explored the relationship of partial sequences of viral strains from Risso's dolphins with those detected in other species within the archipelago and other geographical regions.

MATERIALS AND METHODS

Risso's dolphins were found either dead (n = 17) or stranded alive (n = 4) along the coasts of the Canarian archipelago from 1994 to 2015. Live-stranded animals died shortly after stranding (n = 2), after several attempts of refloating (n = 1) or after/during transportation to a rehabilitation centre (n = 1). A complete standardized necropsy (Kuiken & García Hartmann 1993) was performed on 14 of the 21 (66.6%) dolphins. The required permission for the management of stranded cetaceans in the Canarian archipelago was issued by the environmental department of the Canary Islands' government. During the necropsy, representative tissue samples were collected and stored in a 10% neutral buffered formalin fixative solution for histological and immunohistochemical (IHC) analysis. Fixed tissue samples were trimmed, routinely processed, embedded in paraffin, sectioned at 5 µm thickness and stained with haematoxylin and eosin for examination by light microscopy. The decay stage of the carcasses (Geraci & Lounsbury 1993) allowed for the sample collection of skin, skeletal muscle, lung, liver, kidney, spleen, mesenteric lymph nodes and brain in 12 of the 14 specimens (Table 1). Samples were stored at −80°C until processing for molecular virology. DNA/RNA was extracted from a 300 µl macerated sample using a DNA Tissue Kit S (QuickGene) according to the manufacturer's instructions. Molecular detection of CeMV was performed by 3 different PCRs: a 1-step real-time RT-PCR method to detect a conserved region (192 bp) of the fusion protein (F) gene (Sacristan et al. 2015) and 2 conventional 1-step RT-PCRs amplifying a 426 bp conserved region of the phosphoprotein (P) gene (Reidarson et al. 1998) and a 230 bp region of the nucleoprotein (N) gene (Lipscomb et al. 1996). The presence of other aetiological agents responsible for central nervous system inflammation in cetaceans, such as α herpesviruses and *Brucella*, was examined by conventional nested PCR using degenerate primers designed to amplify a region of the DNA polymerase gene that contains highly conserved amino acid motifs within the family *Herpesviridae* (VanDevanter et al. 1996) and by real-time PCR to detect *Brucella* spp. (Wu et al. 2014). Additionally, 1-step real-time RT-PCR for the housekeeper genes glyceraldehyde 3-phosphate dehydrogenase (G3PDH) (Pe-

Table 1. *Grampus griseus* specimens included in the present study. SD: stranding date; ST: stranding state; DS: decay stage (Geraci & Lounsbury 1993) ranging from 1 (alive) to 5 (bare bones or mummified). Lesions classically attributed to cetacean morbillivirus (CeMV) range from bronchointerstitial pneumonia (BIP) to non-suppurative meningoencephalitis (N-S ME), and meningitis (N-S M), and lymphoid depletion (LD). NE: non-evaluable; NA: not applicable; –: not present; LN: lymph nodes; HV: herpesvirus. Tissues that tested positive for CeMV are indicated in **bold**

ID	Sex	Age class	SD	ST	DS	Lung lesions	Lymphoid lesions	Brain lesions	Samples tested for PCR CeMV	PCR HV and *Brucella* in brain
CET 199	Male	Adult	19/01/2003	Dead	3	BIP	–	–	Lung, kidney, brain, spleen	NA
CET 431 (Specimen 1)	Male	Juvenile	20/04/2008	Alive	2	BIP	LD	N-S ME	Skin, skeletal muscle, lung, liver, mesenteric LN, kidney, **brain**, spleen	Negative
CET 456	Female	Adult	17/06/2008	Alive	2	BIP	LD	N-S ME	Skin, skeletal muscle, lung, liver, intestine, mesenteric LN, kidney, brain, spleen	Negative
CET 472	Female	Calf	07/11/2008	Dead	2	BIP	LD	–	Skin, skeletal muscle, lung, liver, mesenteric LN, kidney, brain, spleen	NA
CET 483	Male	Adult	06/03/2009	Dead	2	BIP	–	–	Skin, skeletal muscle, lung, liver, kidney, brain, spleen	NA
CET 533	Male	Adult	20/04/2010	Dead	3	–	–	–	Skin, skeletal muscle, lung, liver, mesenteric LN, kidney, brain, spleen	NA
CET 534	Male	Subadult	22/04/2010	Alive	2	BIP	LD	N-S M	Skin, skeletal muscle, lung, liver, mesenteric LN, kidney, brain, spleen	Negative
CET 549	Female	Adult	17/09/2010	Dead	2	BIP	–	–	Skin, skeletal muscle, lung, liver, mesenteric LN, kidney, brain, spleen	NA
CET 565	Female	Adult	22/03/2011	Dead	3	BIP	–	–	Lung, mesenteric LN, kidney, brain, spleen	NA
CET 578	Female	Adult	29/05/2011	Alive	2	BIP	LD	N-S ME	Lung, mesenteric LN, kidney, brain, spleen	Negative
CET 634	Male	Subadult	03/11/2012	Dead	3	–	–	–	Lung, mesenteric LN, kidney, brain, spleen	NA
CET 751 (Specimen 2)	Female	Adult	16/03/2015	Dead	4	NE	NE	NE	Lung, **mesenteric LN**, kidney, **brain**, spleen	Negative

ters et al. 2003) and β-actin (Toussaint et al. 2007) were used as an internal control in all samples. Two negative controls (non-template) for extraction and amplification were included. A cycle threshold greater than 35 was not considered significant.

CeMV PCR products were purified using a Real Clean spin kit (REAL) and sequenced. A BLAST search (www.ncbi.nlm.nih.gov/blast/Blast.cgi) was conducted to compare sequenced products with morbillivirus sequences described in GenBank. MEGA 6.0 software was used to construct maximum likelihood phylogenetic trees, and bootstrap resampling (1000 replicates) was used to assess their reliability.

IHC was performed on the selected brain, lymph node, lung and kidney samples from PCR-positive specimens, as previously described (Sierra et al. 2014b). A monoclonal antibody, raised against the nucleoprotein of canine distemper virus (MoAb CDV-NP; Veterinary Medical Research and Development) and known to react with DMV and PWMV, was used as the primary antiserum (Rubio-Guerri et al. 2013, Sierra et al. 2014b, 2016). Tissue sections in which the primary antibodies were replaced by phosphate-buffered saline or nonimmune serum served as negative controls (Ramos-Vara et al. 2008).

RESULTS

Nine of 12 specimens showed lesions that could be attributable to CeMV. The lesions included broncho-interstitial pneumonia (9/12), lymphoid depletion (5/12) and N-S ME (3/12) (Table 1). However, PCR only detected CeMV in the brain of 2 of the specimens (Specimens 1 and 2) and in one lymph node (Specimen 2) (Table 1). CNS inflammatory lesions detected in other specimens (for more details, see Table 1) were also negative for herpesviruses and *Brucella*. CeMV cDNA could be amplified from a juvenile male stranded alive in Playa de Bajamar, Tenerife (28° 40′ 00″ N, 17° 46′ 00″ W) on 21 April 2008 (Specimen 1) and an adult female stranded in Abades (Arico), Tenerife (28° 8′ 28″ N, 16° 26′ 23″ W), on 26 March 2015 (Specimen 2). The rest of the samples tested negative for

Fig. 1. Gross findings associated with cetacean morbillivirus (CeMV) in Risso's dolphin, Specimen 1. Left lateral view. Note the post-nuchal depression, indicative of poor body condition. Inset shows the skin, where ventrolateral ulcerative lesions were present in the neck area (arrowhead)

CeMV (Table 1). RNA integrity was confirmed for all samples.

Specimen 1

Grossly, Specimen 1 was in poor body condition (Fig. 1) and presented some ulcerative skin lesions scattered along the neck region (Fig. 1, inset). Diffuse pulmonary atelectasis was observed with some peripheral emphysematous areas. Multifocal ulcerative pharyngitis, oesophagitis and gastritis were also present. Gastric contents included anisakids and foreign bodies such as ropes, wires and plastics. Severe, multifocal areas of

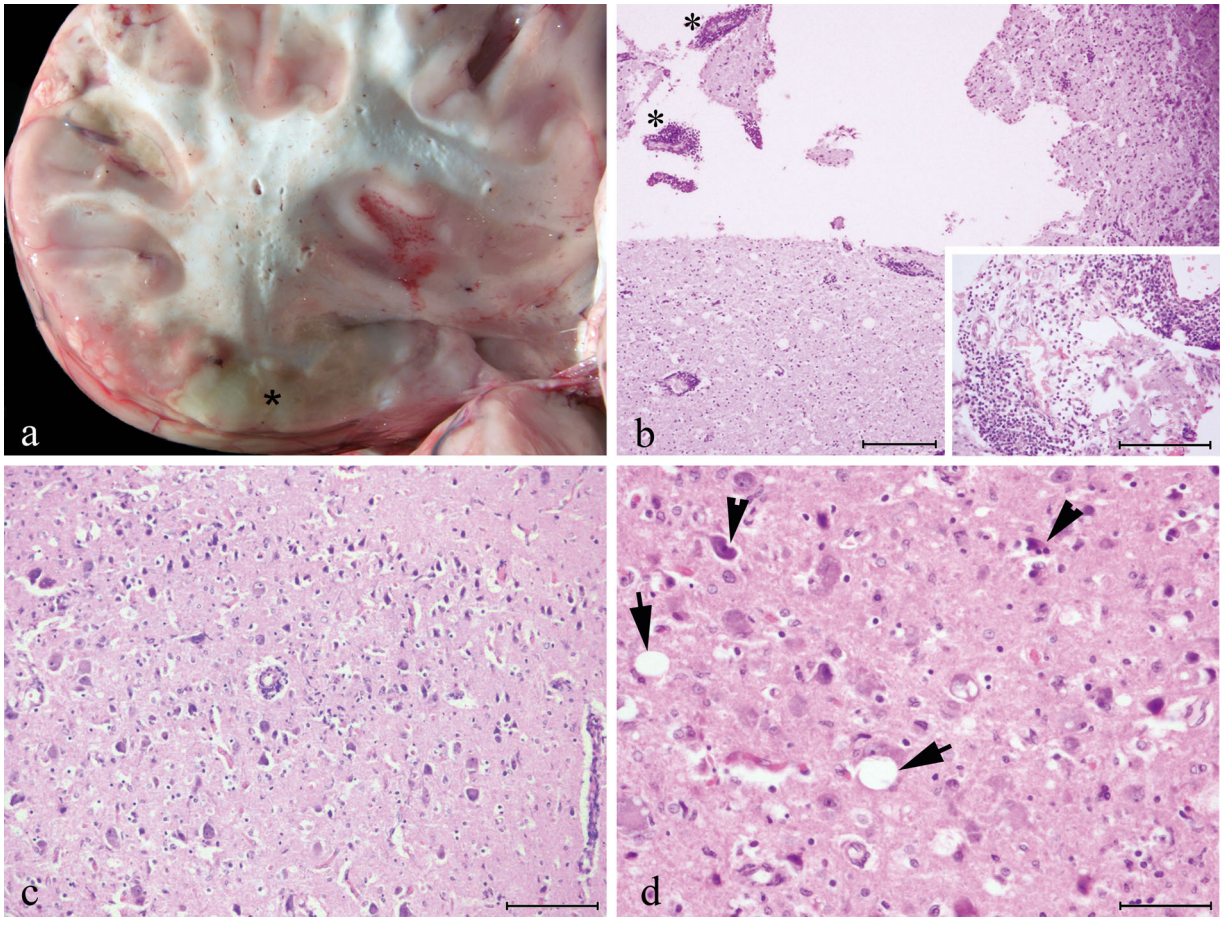

Fig. 2. Gross and histologic brain lesions in Risso's dolphin with cetacean morbillivirus (CeMV). (a) Cross-section of the brain shows a focal area of cortex malacia (asterisk). (b) Necrotic foci left empty spaces after specimen processing, but lymphoplasmacytic inflammatory cells were still present surrounding these foci (asterisks) and in the adjacent meninges (inset). Haematoxylin and eosin stain (HE). Scale bar = 20 µm (inset = 5 µm). (c) Extensive areas of necrotic neurons (hypereosinophilic and shrunken cytoplasm and pyknotic nucleus) were present in the neuropil. HE. Scale bar = 5 µm. (d) Detail of necrotic (arrow heads) and degenerated (vacuolated cytoplasm) (arrows) neurons. Scale bar = 2 µm

malacia were observed within the grey matter of the nervous system (Fig. 2a).

Histologically, mild broncho-interstitial and suppurative pneumonia and oedema were present. Mild centrofollicular lymphocytolysis was seen in all lymph nodes, whereas lymphoid depletion was only observed in the mesenteric lymph node. Severe diffuse hyperplasia and hyperkeratosis of the keratinized stomach were observed as well as multifocal areas of ulcerative and pyogranulomatous gastritis. More damaged areas of the brain (necrotic foci) were lost during sample processing, although moderate multifocal meningitis and perivascular lymphocytic cuffing were still present (Fig. 2b). Degeneration and necrosis of neurons with gliosis was observed in the neuropil adjacent to the necrotic areas (Fig. 2c,d). Moreover, the IHC tests showed intense staining in neurons, glial cells and neuronal processes in the grey matter of the cerebral cortex (Fig. 3).

CeMV cDNA was detected exclusively in the brain by the 3 different PCR methods. The amplicon identities were confirmed by a BLAST search. The brain sample was sequenced for the 3 amplified partial viral proteins (GenBank accession nos. KX512307, KX512308 and KX512309 for the partial P, F and N genes, respectively). P gene fragment analysis (344 bp) showed that the sequence in this specimen was highly similar (99% sequence identity) to DMV sequences from striped dolphins stranded in the Mediterranean Sea in the early 1990s (GenBank accession nos. AJ608288 and Z47758; Bolt et al. 1995, Rima et al. 2005). This sequence was also highly homologous (98%) to samples from striped and common dolphins affected by DMV, both in the Mediterranean and in the Atlantic, from 2007–2013 (GenBank accession nos. KP835983, KP835987, KP835995, KP835999, KP836003, KJ139454, KC572861; Sierra et al. 2014a, Bento et al. 2016) and with a DMV strain identified in long-finned pilot whales and striped dolphins that died during the 2006–2007 epidemic in the Mediterranean (GenBank accession no. HQ829972 and HQ829973, respectively; Bellière et al. 2011b). Similar results were observed for the F gene fragment (206 bp). The N gene fragment (228 bp), however, was less homologous (97% sequence identity) with sequences from striped and common dolphins affected by DMV in the Atlantic from 2012 and 2014 (GenBank accession nos. KT878655, KT878654; Bento et al. 2016), with a DMV strain identified in long-finned pilot whales and striped dolphins that were affected by the 2006–2007 epidemic in the Mediterranean (GenBank accession nos. HQ829972 and HQ829973, respectively; Bellière et al. 2011b), and with the sequence obtained from a white-beaked dolphin *Lagenorhynchus albirostris* in Germany in 2007 (GenBank accession no. EF469546; Wohlsein et al. 2007).

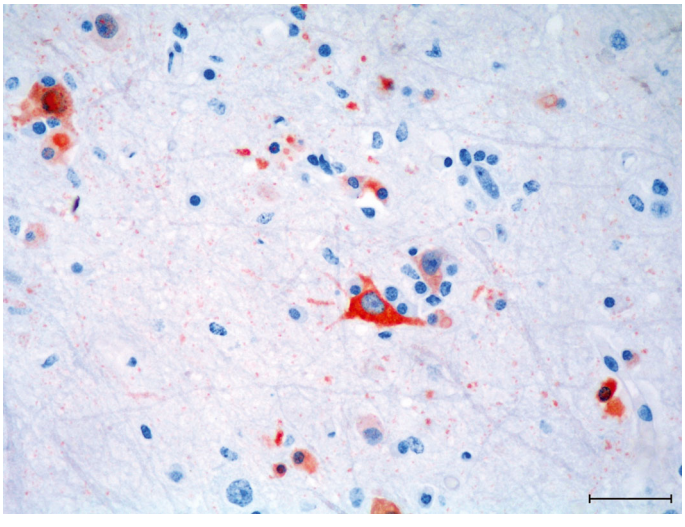

Fig. 3. Immunohistochemical findings in Risso's dolphin with cetacean morbillivirus (CeMV), Specimen 1. Canine distemper virus (CDV)-immunopositive glial cells and neurons were scattered within the tissue section. Scale bar = 2 µm

Specimen 2

Grossly, Specimen 2 was in good body condition and presented an ulcerative lesion of approximately 1 cm in diameter at the tongue apex, which microscopically displayed the occasional presence of amphophilic intracytoplasmic inclusions within keratinocytes, as well as mild lymphoplasmacytic exudates within the submucosa. Other findings included a moderate intestinal helminth infestation, with cestodes in the duodenum (compatible with *Tetrabothrius* sp. or *Trigonocotyle* sp.) and metacestodes within the anal crypts (histologically compatible with tetraphyllidean plerocercoids). Additionally, a severe pyogranulomatous pterygoid air sinusitis with intralesional nematodes, grossly and histologically identified as *Crassicauda* sp. and *Stenurus* sp., and thickened leptomeninges were also observed. Histopathological lesions consistent with CeMV infection could have been partially obscured by the advanced autolysis of the carcasses (code 4) at the time of necropsy, although sufficient histological details were still present to confirm or exclude pathology in most of the slides. In this sense, there was

no infiltration of inflammatory cells in the pulmonary or brain system. Moreover, the IHC tests did not show any positive result in any of the tissues.

The presence of CeMV cDNA was detected in the brain and mesenteric lymph nodes by 2 of the 3 employed PCR methods. The mesenteric lymph nodes were sequenced for the 2 amplified partial viral proteins. Only 1 of the samples was submitted to GenBank (accession no. KY886370 for the partial P gene) since the other did not contain the minimum number of base pairs requested by the repository. P gene fragment analysis (207 bp) showed that the sequence was identical (100% sequence identity over 70–100% cover) to DMV sequences from striped and common dolphins from the northeastern Atlantic and the Mediterranean, from 2007–2013 (GenBank accession nos. KP835983, KP835987, KP835999, KP836003, KT878658, KT878656, KT878660, KT878661, KP835991, KJ139454, KC572861; Sierra et al. 2014a, Bento et al. 2016). Similar results were observed for the F gene fragment (193 bp).

For both specimens, the generated phylogenetic trees of the virus strains were compared with morbillivirus sequences described in GenBank. As such, the sequences from Specimen 1 were located closest to the root of the DMV and PWMV, forming a separate branch within the DMV cluster in the phosphoprotein (Fig. 4) and nucleoprotein (Fig. 5) trees. The sequences from Specimen 2 were placed in a cluster together with sequences detected in striped and common dolphins between 2007 and 2013, most of which came from the northeastern Atlantic (only 1 sequence was detected in the Mediterranean Sea: GenBank accession. no. KC572861) in the 3 generated trees (Figs. 4–6).

DISCUSSION

CeMV infection has been prevalent among odontocetes in the northeastern (Bento et al. 2016) and northwestern (Duignan et al. 1995a,b) Atlantic since the late 1980s (Lipscomb et al. 1994, Duignan et al. 1995a, Krafft et al. 1995, Van Bressem et al. 2014, Bento et al. 2016), as well as in the CE Atlantic (Bellière et al. 2011a, Sierra et al. 2014a,b, 2016). Furthermore, CeMV has been previously detected in Risso's dolphins by serology and PCR (Duignan et al. 1995a, Van Bressem et al. 2001, CREDIMA 2015, Jacob et al.

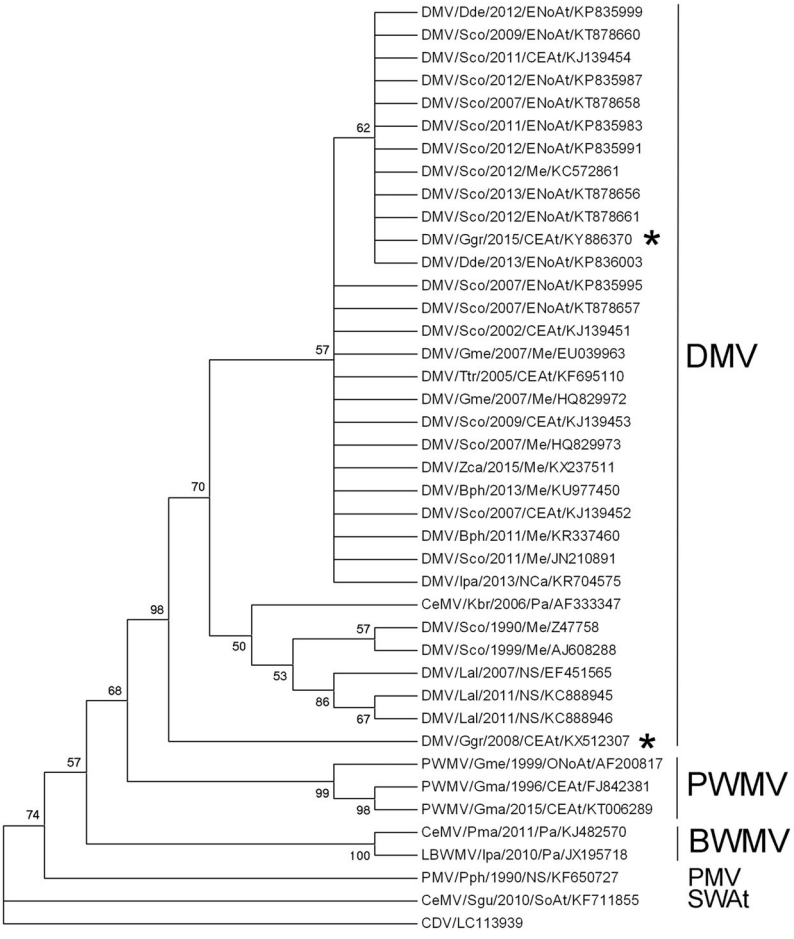

Fig. 4. Phylogram of morbillivirus phosphoprotein (P) gene sequences. Tamura-Nei substitution model and a bootstrap resampling (1000 replicates) were used to assess the reliability of the trees. Bootstrapping values are indicated as percentages next to the bifurcations, with bootstrap values of >50 being condensed. The new isolates from this study are indicated by an asterisk. The name of each sequence includes the virus name (DMV: dolphin morbillivirus; CeMV: cetacean morbillivirus; PWMV: pilot whale morbillivirus; LBWMV: Longman's beaked whale morbillivirus; SWAt: Southwest Atlantic Brazilian regional isolate; PMV: porpoise morbillivirus; CDV: canine distemper virus), the cetacean species (Sco: *Stenella coeruleoalba*; Dde: *Delphinus delphis*; Ttr: *Tursiops truncatus*; Gme: *Globicephala melas*; Bph: *Balaenoptera physalus*; Kb: *Kogia breviceps*; La: *Lagenorhynchus albirostris*; Gg: *Grampus griseus*; Gma: *Globicephala macrorhynchus*; Ipa: *Indopacetus pacificus*; Pma: *Physeter macrocephalus*; Sgu: *Sotalia guianensis*; Pph: *Phocoena phocoena*; Zca: *Ziphius cavirostris*), the year and the geographic area of the stranding (CEAt: central eastern Atlantic Ocean; Me: Mediterranean Sea; ENoAT: eastern North Atlantic Ocean; Pa: Pacific Ocean; NS: North Sea; SoAT: south Atlantic Ocean) and the GenBank accession number

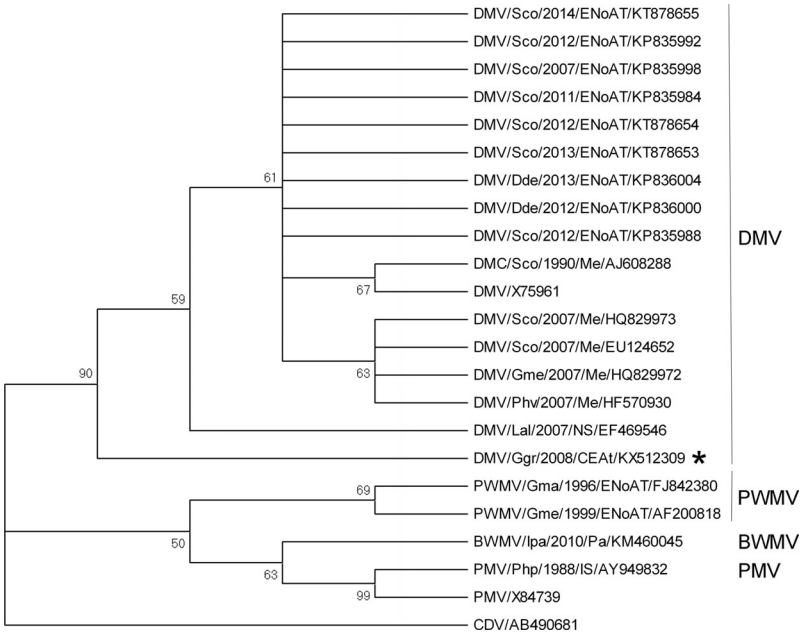

Fig. 5. As in Fig. 4, for morbillivirus nucleoprotein (N) gene sequences

as a subclinical infection since no typical morbillivirus lesions were observed. However, these lesions could have been partially indiscernible due to the carcass autolysis, in which case it could also have been classified as a chronic systemic infection.

Although 8 out of 10 PCR CeMV-negative specimens from our study showed pulmonary alterations that could be attributable to the morbillivirus infection, there was a lack of more morbillivirus-specific lesions such as pneumocyte type II hyperplasia, syncytial cells and/or viral inclusions, which would suggest that these were related to other processes, as previously proposed (Bossart et al. 2010, Sierra et al. 2014a). Similarly, 4/10 PCR CeMV-negative specimens displayed lymphoid depletion, which could be attributable to other factors such as contaminants (Jepson et al. 1999). N-S ME is a featured CeMV lesion in subacute and chronic systemic infections as well as in chronic localized CeMV encephalitis. Four of the 12 specimens in our study showed this type of brain lesion, although only 1 was positive for CeMV. The other 3 specimens (75%) were negative for CeMV as well as for other common pathogens associated with these lesions in cetaceans. Similar results were obtained in a previous study in the Canary Islands, where the etiological agent responsible for 63.2% of the cases presenting N-S ME remained undetermined (Sierra et al. 2014a).

2016), indicating that this species is susceptible to CeMV infection. However, no information exists on CeMV prevalence in Risso's dolphins of the CE Atlantic, despite these dolphins being a resident species in Canary waters (García et al. 2002). In addition, no pathological descriptions exist on CeMV infection in this species.

PCR results revealed a 16.6% DMV prevalence in Risso's dolphins stranded in the Canary Islands (n = 12) over a 12 yr period. However, pathological lesions attributable to the CeMV infection were only detected in Specimen 1 and included neuronal necrosis and degeneration and N-S ME. Because morbillivirus was exclusively detected in the brain (IHC and PCR), the disease presentation in Specimen 1 was classified as chronic localized CeMV encephalitis, as described in the recent cetacean morbillivirus review by Van Bressem et al. (2014). Despite the brain lesions, the cause of death was probably multifactorial. The presence of foreign bodies and gastric nematodes (*Anisakis* sp.) in the first stomach could have aggravated the poor body condition, decreasing the specimen's survival chances, as has been previously described in other cetaceans with CeMV infection (Yang et al. 2006, Sierra et al. 2014b, Stephens et al. 2014).

According to a recent morbillivirus review (Van Bressem et al. 2014), the case of Specimen 2 could be classified

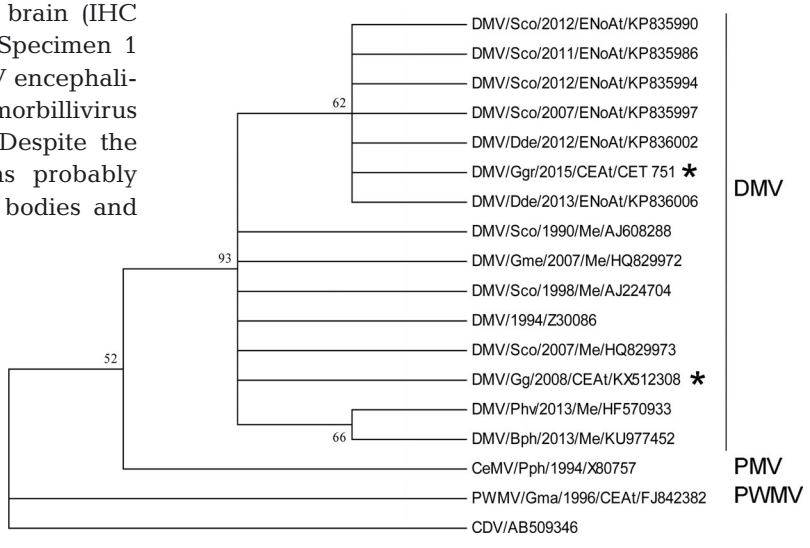

Fig. 6. As in Fig. 4, for cetacean morbillivirus fusion protein (F) gene sequences

Two CeMV strains (i.e. DMV and PWMV) have been detected in the CE Atlantic over a 19 yr period. PWMV has been reported exclusively in short-finned pilot whales (1996 and 2015) (Bellière et al. 2011a, Sierra et al. 2016). The DMV strain has been detected in 3 different cetacean species over 13 yr: bottlenose dolphin (2005) (Sierra et al. 2014b), common dolphin (2007) (Sierra et al. 2014a) and striped dolphin (2002, 2007, 2008, 2009 and 2011) (Sierra et al. 2014a). Some of these cases coincided in time with outbreaks and/or unusual mortality events that occurred in the Mediterranean in 2006–2008 and 2011 (Fernandez et al. 2008, Raga et al. 2008, Rubio-Guerri et al. 2013). This study adds a new CeMV-positive species to the list within the family Delphinidae in the Canary Islands. The infection of Risso's dolphins may have occurred following contact with an enzootically infected species, as has been previously suggested for *Globicephala* sp. in the northwestern Atlantic (Duignan et al. 1995a,b). The 2 positive specimens from this study were stranded 7 yr apart (2008 and 2015), and the partial virus sequences are phylogenetically quite distant. As such, the sequences from Specimen 1 are located very close to the DMV strain node, while the sequences from Specimen 2 are situated in a cluster detected mainly in the striped dolphin species in the northeastern Atlantic since 2007 (supported by a bootstrap value of 62%). Isolates from this cluster are mainly found in waters from Portugal and Galicia (Bento et al. 2016), except for 2 sequences from the Canary Islands and 1 from the Mediterranean, indicating that even if DMV strains from the Atlantic and Mediterranean are relatively isolated (Bento et al. 2016), similar strains are circulating between these 3 proximal ocean provinces. Our results prove that at least 2 different strains can infect Risso's dolphins and that the strain detected in one of the specimens has been circulating in the northeastern Atlantic since 2007, during the second Mediterranean epizootic.

There is a growing concern for animal health and the impact that infectious diseases can have on wildlife, especially morbilliviruses, which are among the most important pathogens in their respective hosts. The appearance, disappearance and re-emergence of this pathogen in cetaceans, with both epidemic potential and high mortality rates, have threatened the health status and cetacean population for the last 3 decades, primarily in the northwestern Atlantic and the Mediterranean. One example is the population of long-finned pilot whales in the Strait of Gibraltar, which decreased by 26.2% over 5 yr after the morbillivirus epizootic outbreak (Verborgh et al. 2016).

CONCLUSIONS

The Canary Islands are strategically located between 3 continents: Europe, North America and Africa. DMV strains detected in the CE Atlantic (previously and in this study) are very similar to strains from both the Mediterranean and the northeastern Atlantic, indicating that this ocean region plays an important role in the epidemiology of the virus. Similarly, it was previously suggested that a strain circulating in the CE Atlantic was introduced into the Mediterranean in 2006 (Van Bressem et al. 2014). Thus, regular monitoring for the presence of CeMV in free-ranging cetaceans in this part of the central Atlantic is essential for detecting the occurrence and distribution of this infectious disease and for predicting novel epidemics worldwide.

Acknowledgements. We thank the members of the Canary Islands Cetacean Stranding network, the Society for the Study of Cetaceans in the Canarian Archipelago and the Canarias Conservación. This work was supported by the Institute of Animal Health and the University of Córdoba through the following grants: ULPGC2013-21, CGL2015-71498 and IJCI-2014-20945. Publication costs were covered by the 'Cabildo de Fuerteventura' through the workshop 'Advances in technology and research on beaked whales and antisubmarine sonar'.

LITERATURE CITED

Barrett T, Visser IK, Mamaev L, Goatley L, van Bressem MF, Osterhaust AD (1993) Dolphin and porpoise morbilliviruses are genetically distinct from phocine distemper virus. Virology 193:1010–1012

Bellière EN, Esperon F, Fernandez A, Arbelo M, Munoz MJ, Sanchez-Vizcaino JM (2011a) Phylogenetic analysis of a new cetacean morbillivirus from a short-finned pilot whale stranded in the Canary Islands. Res Vet Sci 90: 324–328

Bellière EN, Esperon F, Sanchez-Vizcaino JM (2011b) Genetic comparison among dolphin morbillivirus in the 1990–1992 and 2006–2008 Mediterranean outbreaks. Infect Genet Evol 11:1913–1920

Bento MC, Eira CI, Vingada JV, Marcalo AL and others (2016) New insight into dolphin morbillivirus phylogeny and epidemiology in the northeast Atlantic: opportunistic study in cetaceans stranded along the Portuguese and Galician coasts. BMC Vet Res 12:176

Bolt G, Blixenkrone-Moller M, Gottschalck E, Wishaupt RG, Welsh MJ, Earle JA, Rima BK (1994) Nucleotide and deduced amino acid sequences of the matrix (M) and fusion (F) protein genes of cetacean morbilliviruses isolated from a porpoise and a dolphin. Virus Res 34: 291–304

Bolt G, Alexandersen S, Blixenkrone-Moller M (1995) The phosphoprotein gene of a dolphin morbillivirus isolate exhibits genomic variation at the editing site. J Gen Virol 76:3051–3058

Bossart GD, Reif JS, Schaefer AM, Goldstein J, Fair PA, Saliki JT (2010) Morbillivirus infection in free-ranging Atlantic bottlenose dolphins (*Tursiops truncatus*) from the Southeastern United States: seroepidemiologic and pathologic evidence of subclinical infection. Vet Microbiol 143:160–166

Carwardine M (1995) Whales, dolphins and porpoises. Dorling Kindersley, London

Centelleghe C, Beffagna G, Palmisano G, Franzo G and others (2017) Dolphin morbillivirus in a Cuvier's beaked whale (*Ziphius cavirostris*), Italy. Front Microbiol 8:111

CREDIMA (Centro di Referenza Nazionale per le Indagini Diagnostiche sui Mammiferi Marini spiaggiati) (2015) Italian diagnostic report on stranded cetaceans. www.izsto.it/images/CREDIMA/Report_spiaggiamenti_2015.pdf

Domingo M, Visa J, Pumarola M, Marco AJ, Ferrer L, Rabanal R, Kennedy S (1992) Pathologic and immunocytochemical studies of morbillivirus infection in striped dolphins (*Stenella coeruleoalba*). Vet Pathol 29:1–10

Duignan PJ, Geraci JR, Raga JA, Calzada N (1992) Pathology of morbillivirus infection in striped dolphins (*Stenella coeruleoalba*) from Valencia and Murcia, Spain. Can J Vet Res 56:242–248

Duignan PJ, House C, Geraci JR, Duffy N and others (1995a) Morbillivirus infection in cetaceans of the western Atlantic. Vet Microbiol 44:241–249

Duignan PJ, House C, Geraci JR, Early G and others (1995b) Morbillivirus infection in two species of pilot whales (*Globicephala* sp.) from the western Atlantic. Mar Mamm Sci 11:150–162

Fernandez A, Esperon F, Herraez P, de Los Monteros AE and others (2008) Morbillivirus and pilot whale deaths, Mediterranean Sea. Emerg Infect Dis 14:792–794

García S, Martin V, Hildebrandt S, Servidio A (2002) Risso's dolphin, *Grampus griseus*, in the eastern of the Canary Islands. 16th Annual Conference of the European Cetacean Society, Liege, Belgium

Geraci JR, Lounsbury VJ (1993) Marine mammals ashore, a field guide for strandings. Texas A&M Sea Grant Publication, Galveston, TX

Groch KR, Colosio AC, Marcondes MC, Zucca D and others (2014) Novel cetacean morbillivirus in Guiana dolphin, Brazil. Emerg Infect Dis 20:511–513

Jacob JM, West KL, Levine G, Sanchez S, Jensen BA (2016) Initial characterization of novel beaked whale morbillivirus in Hawaiian cetaceans. Dis Aquat Org 117: 215–227

Jepson PD, Bennett PM, Allchin CR, Law RJ and others (1999) Investigating potential associations between chronic exposure to polychlorinated biphenyls and infectious disease mortality in harbour porpoises from England and Wales. Sci Total Environ 243-244:339–348

Kennedy S (1998) Morbillivirus infections in aquatic mammals. J Comp Pathol 119:201–225

Kemper CM, Tomo I, Bingham J, Bastianello SS and others (2016) Morbillivirus-associated unusual mortality event in South Australian bottlenose dolphins is largest reported for the Southern Hemisphere. R Soc Open Sci 3: 160838

Krafft A, Lichy JH, Lipscomb TP, Klaunberg BA, Kennedy S, Taubenberger JK (1995) Postmortem diagnosis of morbillivirus infection in bottlenose dolphins (*Tursiops truncatus*) in the Atlantic and Gulf of Mexico epizootics by polymerase chain reaction-based assay. J Wildl Dis 31: 410–415

Kuiken T, García Hartmann M (1993) Cetacean pathology: dissection techniques and tissue sampling. In: Proceedings of the First ECS Workshop on Cetacean Pathology. ECS Newsletter 17. European Cetacean Society, Leiden

Lipscomb TP, Schulman FY, Moffett D, Kennedy S (1994) Morbilliviral disease in Atlantic bottlenose dolphins (*Tursiops truncatus*) from the 1987–1988 epizootic. J Wildl Dis 30:567–571

Lipscomb TP, Kennedy S, Moffett D, Krafft A and others (1996) Morbilliviral epizootic in bottlenose dolphins of the Gulf of Mexico. J Vet Diagn Invest 8:283–290

Mazzariol S, Centelleghe C, Beffagna G, Povinelli M and others (2016) Mediterranean fin whales (*Balaenoptera physalus*) threatened by dolphin morbillivirus. Emerg Infect Dis 22:302–305

Mazzariol S, Centelleghe C, Di Provvido A, Di Renzo L and others (2017) Dolphin morbillivirus associated with a mass stranding of sperm whales, Italy. Emerg Infect Dis 23:144–146

Morris SE, Zelner JL, Fauquier DA, Rowles TK, Rosel PE, Gulland F, Grenfell BT (2015) Partially observed epidemics in wildlife hosts: modelling an outbreak of dolphin morbillivirus in the northwestern Atlantic, June 2013-2014. J R Soc Interface 12:20150676

Peters IR, Helps CR, Batt RM, Day MJ, Hall EJ (2003) Quantitative real-time RT-PCR measurement of mRNA encoding alpha-chain, pIgR and J-chain from canine duodenal mucosa. J Immunol Methods 275:213–222

Raga JA, Banyard A, Domingo M, Corteyn M and others (2008) Dolphin morbillivirus epizootic resurgence, Mediterranean Sea. Emerg Infect Dis 14:471–473

Ramos-Vara JA, Kiupel M, Baszler T, Bliven L and others (2008) Suggested guidelines for immunohistochemical techniques in veterinary diagnostic laboratories. J Vet Diagn Invest 20:393–413

Reidarson TH, McBain J, House C, King DP and others (1998) Morbillivirus infection in stranded common dolphins from the Pacific Ocean. J Wildl Dis 34:771–776

Rima BK, Collin AM, Earle JA (2005) Completion of the sequence of a cetacean morbillivirus and comparative analysis of the complete genome sequences of four morbilliviruses. Virus Genes 30:113–119

Rubio-Guerri C, Melero M, Esperon F, Belliere EN and others (2013) Unusual striped dolphin mass mortality episode related to cetacean morbillivirus in the Spanish Mediterranean Sea. BMC Vet Res 9:106

Sacristan C, Carballo M, Munoz MJ, Belliere EN, Neves E, Nogal V, Esperon F (2015) Diagnosis of cetacean morbillivirus: a sensitive one step real time RT fast-PCR method based on SYBR(®) Green. J Virol Methods 226:25–30

Sierra E, Sanchez S, Saliki JT, Blas-Machado U, Arbelo M, Zucca D, Fernandez A (2014a) Retrospective study of etiologic agents associated with nonsuppurative meningoencephalitis in stranded cetaceans in the canary islands. J Clin Microbiol 52:2390–2397

Sierra E, Zucca D, Arbelo M, Garcia-Alvarez N, Andrada M, Deniz S, Fernandez A (2014b) Fatal systemic morbillivirus infection in bottlenose dolphin, Canary Islands, Spain. Emerg Infect Dis 20:269–271

Sierra E, Fernández A, Suárez-Santana C, Xuriach A and others (2016) Morbillivirus and pilot whale deaths, Canary Islands, Spain, 2015. Emerg Infect Dis 22: 740–742

Stephens N, Duignan PJ, Wang J, Bingham J and others (2014) Cetacean morbillivirus in coastal Indo-Pacific bot-

tlenose dolphins, Western Australia. Emerg Infect Dis 20: 666–670

Taubenberger JK, Tsai MM, Atkin TJ, Fanning TG and others (2000) Molecular genetic evidence of a novel morbillivirus in a long-finned pilot whale (*Globicephalus melas*). Emerg Infect Dis 6:42–45

Tobeña M, Escánez A, Rodríguez Y, López C, Aguilar de Soto N (2011) Inter-island movements of bottlenose dolphins (*Tursiops truncatus*) in the Canary Islands: implications for conservation and SAC design. European Cetacean Society's 25th Annual Conference, March 2011, Cádiz

Toussaint JF, Sailleau C, Breard E, Zientara S, De Clercq K (2007) Bluetongue virus detection by two real-time RT-qPCRs targeting two different genomic segments. J Virol Methods 140:115–123

Van Bressem MF, Visser IK, Van de Bildt MW, Teppema JS, Raga JA, Osterhaus AD (1991) Morbillivirus infection in Mediterranean striped dolphins (*Stenella coeruleoalba*). Vet Rec 129:471–472

Van Bressem M, Waerebeek KV, Jepson PD, Raga JA and others (2001) An insight into the epidemiology of dolphin morbillivirus worldwide. Vet Microbiol 81:287–304

Van Bressem MF, Duignan PJ, Banyard A, Barbieri M and others (2014) Cetacean morbillivirus: current knowledge and future directions. Viruses 6:5145–5181

van Elk CE, van de Bildt MW, Jauniaux T, Hiemstra S and others (2014) Is dolphin morbillivirus virulent for white-beaked dolphins (*Lagenorhynchus albirostris*)? Vet Pathol 51:1174–1182

VanDevanter DR, Warrener P, Bennett L, Schultz ER, Coulter S, Garber RL, Rose TM (1996) Detection and analysis of diverse herpesviral species by consensus primer PCR. J Clin Microbiol 34:1666–1671

Verborgh P, Gauffier P, Esteban R, Gimenez J, Canadas A, Salazar-Sierra JM, de Stephanis R (2016) Conservation status of long-finned pilot whales, *Globicephala melas*, in the Mediterranean Sea. Adv Mar Biol 75: 173–203

Visser IK, Van Bressem MF, de Swart RL, van de Bildt MW and others (1993) Characterization of morbilliviruses isolated from dolphins and porpoises in Europe. J Gen Virol 74:631–641

Vonk R, Martel VM (1988) First list of Odontocetes from the Canary Islands, 1980-1987. In: Evans PGC (ed) Proc 2nd Annu Conf European Cetacean Society, Troia, Portugal. ECS, Oxford, p 31–36

West KL, Sanchez S, Rotstein D, Robertson KM and others (2013) A Longman's beaked whale (*Indopacetus pacificus*) strands in Maui, Hawaii, with first case of morbillivirus in the central Pacific. Mar Mamm Sci 29: 767–776

Wohlsein P, Puff C, Kreutzer M, Siebert U, Baumgärtner W (2007) Distemper in a dolphin. Emerg Infect Dis 13: 1959–1961

Wu Q, McFee WE, Goldstein T, Tiller RV, Schwacke L (2014) Real-time PCR assays for detection of *Brucella* spp. and the identification of genotype ST27 in bottlenose dolphins (*Tursiops truncatus*). J Microbiol Methods 100: 99–104

Yang WC, Pang VF, Jeng CR, Chou LS, Chueh LL (2006) Morbilliviral infection in a pygmy sperm whale (*Kogia breviceps*) from Taiwanese waters. Vet Microbiol 116: 69–76

Editorial responsibility: Stephen Raverty, Abbotsford, British Columbia, Canada

Submitted: May 4, 2017; Accepted: May 15, 2018
Proofs received from author(s): July 11, 2018