Dolphin Morbillivirus Infection in a Captive Harbor Seal (Phoca vitulina)

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During the second morbillivirus epidemic (2007 to 2011) in cetaceans along the Italian coastline, dolphin morbillivirus (DMV) was detected by molecular analyses in a captive harbor seal (Phoca vitulina), with pathological findings consistent with morbillivirus infection. This report confirms interspecies DMV transmission from cetaceans to pinnipeds.

CASE REPORT

During the second dolphin morbillivirus (DMV) epidemic in the Mediterranean Sea (2006 to 2011), several cetaceans stranded on the Tyrrenian coast of Italy were recovered by the national stranding network between 2010 and 2011. In the summer of 2011, one live bottlenose dolphin (Tursiops truncatus) (no. 48258; Istituto Zooprofilattico Sperimentale del Piemonte, della Liguria e della Val d’Aosta [IZSPLVA], Turin, Italy) and one dead striped dolphin (Stenella coeruleoalba) (no. 48203; IZSPLVA) tested positive for morbillivirus by reverse transcription (RT)-PCR only in the brain, without immunohistochemical evidence of morbillivirus antigen in any other tissue, as reported elsewhere (1). The field operations had been supported by personnel of a zoo for 1 week or animal facilities for 3 weeks after either standing event. Furthermore, procedures were already set up to prevent disease transmission to the zoo animals, disposable material was regularly used and incinerated as medical wastes, with durable equipment systematically disinfected by means of a commercial mixture based on ethyl alcohol and formalin. The personnel were not usually involved in zoological activities and had not entered the zoo for 1 week or animal facilities for 3 weeks after either standing event. Furthermore, personnel had been enabled to prevent disease transmission to the zoo animals, disposable material was regularly used and incinerated as medical wastes, with durable equipment systematically disinfected by means of a commercial mixture based on ethyl alcohol and formalin. The personnel were not usually involved in zoological activities and had not entered the zoo for 1 week or animal facilities for 3 weeks after either standing event.

Postmortem examination showed extensive, multifocal to coalescent colliquative renal necrosis and focal cribiform lobular liver necrosis, in addition to mild gastritis, necrotic enteritis, hemorrhagic foci in the liver, spleen, and lungs, and severe mediastinal emphysema. Pathological findings were consistent with gross alterations: necrotic lesions occasionally associated with mild hemorrhages and, rarely, with mild fibrinous effusions. Neutrophil and fibrin clots were evident within the vascular lumina in all major organs, with hyperplasia/hyper trophy of pulmonary intravascular macrophages and splenic hemosiderophages. Gram-negative coccal bacteria adherent to epithelial cells of intestinal glands and within the luminal contents were seen at microscopic examination. On microbiological investigation, Aeromonas hydrophila was isolated from the spleen and the intestinal tract and presumed responsible for the septic shock and subsequent death of the animal.

A detailed necropsy was carried out within 24 h after death; tissue samples were preserved in 10% neutral buffered formalin for histopathology, refrigerated for microbiology and parasitology, and frozen for biomolecular investigations.

Pathological findings were suggestive of morbillivirus infection, which was subsequently confirmed by RT-PCR (2) with DMV-specific genome sequences amplified from the lungs, inguinal lymph nodes, and brain (3). Biomolecular investigation for canine distemper virus (CDV) and phocine distemper virus (PDV), performed according to the protocol described by Stanton et al. (4), was negative. Partial nucleoprotein (N1), fusion protein (F), and hemagglutinin (H) gene regions were amplified (Table 1) using cetacean morbillivirus (CeMV)-specific primers (5). The incongruence-length-difference (ILD) test (WinClada version 4.2) performed on the nucleotide sequences amplified from the lungs, inguinal lymph nodes, and brain (3) failed to detect significant incongruence, which was subsequently confirmed by ILD test (WinClada version 4.2) performed on the nucleotide sequences amplified from the lungs, inguinal lymph nodes, and brain (3) failed to detect significant incongruence.

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demonstrated that the three data sets were not significantly incongruent \((P = 0.2467)\). Therefore, an approach combining the N1, F, and H gene regions was selected for phylogenetic analysis. The sequences were concatenated into a multigene alignment to increase the discriminatory power. Phylogeny inference according to the maximum likelihood criterion was performed using MEGA version 5. The nucleotide substitution model was general time reversible (GTR), with four substitution rate categories. The robustness of the hypothesis was tested in 1,000 nonparametric bootstrap analyses.

The resulting phylogenetic tree showed a well-separated cluster corresponding to the available DMV sequences (Fig. 2). Even though the analysis, conducted at the same time on the three animals, indicated that the overall genetic diversity of the known circulating DMV strains was low, which is consistent with previous findings (6), our multigene approach allowed us to discriminate a distinct subclade, supported by a 66% bootstrap value and including the DMV sequences obtained from the harbor seal and the two dolphins stranded in 2011. The sequences showed a 100% similarity among them but shared unique nucleotide differences compared to the DMV reference sequences, strongly supporting the hypothesis for DMV transmission from the stranded dolphins to the captive seal.

Over the last 25 years, morbillivirus infections have caused dramatic mortalities among aquatic mammal species and populations worldwide. DMV poses a major threat for free-ranging cetaceans. It has been responsible for two epidemics in the Mediterranean Sea (6): the first outbreak (1990 to 1992) affected striped dolphins (Stenella coeruleoalba) (7, 8), while the second (2006 to 2011) involved several other species besides striped dolphins, including pilot whales (Globicephala melas), bottlenose dolphins (Tursiops truncatus), Risso’s dolphins (Grampus griseus), and fin whales (Balaenoptera physalus) (1, 8–11). Among pinnipeds, mortality events worldwide have involved harbor seals (Phoca vitulina), gray seals (Halichoerus grypus), Baikal seals (Pusa sibirica), and Caspian seals (Pusa caspica) by either CDV or PDV, an agent closely related to CDV (7).

Although members of the Morbillivirus genus identified in cetaceans and pinnipeds belong to separate phylogenetic clusters (12), a virus closely related, but not identical, to DMV was isolated from Mediterranean monk seals (Monachus monachus) found dead during an outbreak which had resulted in mass mortality off the coast of Mauritania (13). The tentative link between the mass die-off dolphins in the coastal waters of Mauritania and the preceding monk seal event in the same area suggested that interspecies transmission of the virus could occur between cetaceans and pinnipeds. However, the lack of specimens from the dead dolphins precluded the notion of interspecies viral transmission.

**TABLE 1 Morbillivirus names and accession numbers**

<table>
<thead>
<tr>
<th>Morbillivirus name( ^a )</th>
<th>Accession no.(^ b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMV 2011 Phoca vitulina ID 204</td>
<td>HF570930 HF570933 HF570936</td>
</tr>
<tr>
<td>DMV 2011 Tursiops truncatus ID 48258</td>
<td>HF570931 HF570934 HF570937</td>
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<tr>
<td>DMV 2011 Stenella coeruleoalba ID 48203</td>
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<td>MeV</td>
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</table>

\(^a\) DMV, dolphin morbillivirus; CeMV, cetacean morbillivirus; CDV, canine distemper virus; PDD, phocine distemper virus; PPRV, peste-des-petits-ruminants virus; RPV, rinderpest virus; MeV, measles virus.

\(^b\) The entries for the DMV 2011 isolates are EMBL accession numbers; all other entries are GenBank accession numbers.

**FIG 1** Microscopic findings in the harbor seal with DMV infection. Note the lymphoid cell depletion and multinucleate syncytia in the prescapular lymph nodes (arrows) (hematoxylin-eosin stain [HE]; magnification, \( \times 10 \); bar, 20 \( \mu \)m) (A) and the mononuclear cell perivascular cuffing around a small capillary in the brain (HE; magnification, \( \times 100 \); bar, 10 \( \mu \)m) (B), with an intracytoplasmic viral inclusion body inside a glial cell (arrowhead) (HE; magnification, \( \times 100 \); bar, 10 \( \mu \)m) (C).
This report describes a spontaneous DMV infection that caused disease in a harbor seal, with epidemiological, molecular, phylogenetic, and pathological data strongly supporting the assumption that interspecies DMV transmission from cetaceans to pinnipeds may occur, as previously suggested for monk seals (13). That morbillivirus can switch from one host species to another has been already demonstrated for CDV infection. Indeed, the repeated independent emergence of CDV in novel species appears to be associated with its adaptation to receptor-binding regions determining virus-host specificity (14). Furthermore, throughout the 2006 to 2011 morbillivirus epidemic in the Mediterranean Sea, DMV infection was repeatedly reported in several species (1, 8–11). Another unprecedented finding of our study is that DMV may not only infect but also cause severe disease and subsequent mortality in harbor seals, whereas DMV-like infection could not be established as the primary cause of mortality in the monk seals from Mauritania (7).

Seropositivity to morbillivirus in captive marine mammals has been previously assessed, although an infectious source in the wild was suspected for these animals (15).

The two stranded cetaceans, particularly the live bottlenose dolphin, might be hypothesized as the presumptive source of DMV infection in the captive seal on the basis of the overlap of biomolecular characterization; however, the exact route by which the virus entered the zoo facility remains unknown. This uncertainty is additionally underscored by the rapid inactivation of Paramyxoviridae under normal environmental conditions (16). Nevertheless, morbillivirus infection has been reported among captive carnivores in zoological facilities, with a direct contact between CDV-infected and uninfected live animals considered a necessary means for viral transmission (17). In our case, however, no morbillivirus-positive wild animals were known to have ever entered the zoo, reasonably excluding the possibility of indirect infection from water or food contamination by aerosols, secretions, or excretions. Furthermore, the quarantine the zoo administrators had imposed following the two wild dolphin strandings reasonably excludes zoo staff or equipment as a potential source of viral contamination, although a role of indirect carriers cannot be completely ruled out due to common biologic and epidemiologic features of morbilliviruses, such as their high transmission rate and low minimum infectious dose (18).

Morbillivirus infections have long been known to be associated with increased immunosuppression in their natural hosts (19). DMV has been frequently reported in association with secondary infections from opportunistic bacteria or protozoa such as Toxoplasma gondii (1, 7, 8, 11). In seals, Aeromonas spp. has been suggested as a possible opportunistic pathogen in morbillivirus-infected individuals (20, 21); likewise, the Aeromonas hydrophila strain recovered from the spleen and the intestinal tract of our DMV-infected captive seal should be considered a secondary pathogen responsible for the acute septic shock and subsequent death in this animal. Aeromonas spp. have been frequently reported in human and veterinary medicine as a cause of acute gastroenteritis, wound infection, and septicemia due to the multiple virulence factors the bacteria produce (21).

In conclusion, the present case of DMV infection in a captive harbor seal, which occurred without direct contact with two DMV-infected dolphins, calls for new guidelines to enforce and extend quarantine protocols for zoological parks housing marine mammals and rehabilitating animals, considering the biologic features of the DMV isolates reported here and the potential for interspecies viral transmission (15, 17).
ACKNOWLEDGMENTS

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We declare no competing interests.

REFERENCES


