

Pilot whales (*G. melas*) mortality due to Morbillivirus in Mediterranean Sea

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Introduction: Morbilliviruses have emerged as significant pathogens of cetaceans and pinnipeds worldwide. Two cetacean morbilliviruses have been identified and named porpoise morbillivirus (PMV) and dolphin morbillivirus (DMV). Although, morbillivirus outbreaks have not been previously reported in pilot whales, antibodies to morbilliviruses have been reported in 86% of two species of pilot whales (*Globicephala melas* and *macrorhynchus*) in the western Atlantic. Interestingly, molecular evidences from one stranded pilot whale suggested that the long-finned pilot whale is host of a different, novel type of cetacean morbillivirus (called pilot whale morbillivirus or PWMV), and distinct from both PMV and DMV.

Material and methods: During a period of six months (November, 2006 to April, 2007) more than 25 long finned pilot whales (*G. melas*) died along the southern Spanish Mediterranean coast and Balearic Islands. Nine pilot whales were fresh or moderate autolytic and they were completely or partially necropsied and sampled. A histological, immunohistochemical and virological study was performed on frozen and formalin fixed tissues. RT-PCR detection of cetacean morbillivirus (CetMV) was carried out on samples of brain, lung, spleen, lymph nodes, liver and kidney, from 7 pilot whales.

Results: Main macroscopical findings detected during the necropsy were related to moderate to severe cachexia. No content was found in the stomachs. Internally, a marked subcutaneous yellowish edema (icterus), enlarged edematous lymph nodes which showed parenchymal multifocal necrosis (especially, digestive tract lymph nodes), erosive stomatitis and erosive to ulcerative necrotizing esophagitis was observed. Microscopically, the main lesions were found in the lymph nodes which showed a multifocal necrotizing lymphadenitis with the presence of multinuclear syncytial cells. A non-purulent encephalitis with syncytial cells and intranuclear and/or intracytoplasmic inclusions bodies were detected in animals from which nervous samples analyzed microscopically. Mild to severe erosive to ulcerative necrotizing esophagitis was detected microscopically in most of the analyzed whales. An immunohistochemical staining, using a polyclonal antibody, demonstrated morbilliviral antigen in bronchiolar epithelium, in syncytial cells, monocytic-like cells and cell debris of affected lymph nodes and brain, often containing positive intracytoplasmic globular and/or granular positive immunoreaction. A morbillivirus was detected by RT-PCR in brain, spleen, kidney, lymph nodes, liver and lung from 7 pilot whales virologically analyzed. Sequencing revealed the same sequence in all positive samples. The novel sequence obtained was closely related to DMV and more divergent to PWM was observed.

Discussion: This represents the first morbillivirus infection inducing high mortality of long-finned pilot whales (*M. melas*). The first morbillivirus epizootic described in cetaceans involved striped dolphins in the Mediterranean Sea in the 1990s when a DMV was described by the first time. Both the pilot whale and the striped dolphin mortalities share a very closely related virus phylogenetically and the potential for interespecific transmission must be considered. Although it has been reported