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Title: Molecular analysis of dolphin morbillivirus: a new sensitive detection method based on nested RT-PCR

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1 **Molecular analysis of dolphin morbillivirus: a new sensitive detection method based on nested**  
2 **RT-PCR**

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15 **Highlights**

- 16 • DMV isolation and identification are challenging in stranded whales.  
17 • The study describes a new nested RT-PCR amplifying small amounts of DMV RNA.  
18 • The nested RT-PCR technique improves DMV detection in badly preserved tissues.  
19 • Nested RT-PCR could be useful when low DMV amounts are present in tissues.

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22 **Abstract**

23 *Cetacean Morbillivirus* (CeMV) has been identified as the most pathogenic virus for cetaceans.  
24 Over the past three decades, this RNA virus has caused several outbreaks of lethal disease in  
25 odontocetes and mysticetes worldwide. Isolation and identification of CeMV RNA is very  
26 challenging in whales because of the poor preservation *status* frequently shown by tissues from  
27 stranded animals. Nested reverse transcription polymerase chain reaction (nested RT-PCR) is used  
28 instead of conventional RT-PCR when it is necessary to increase the sensitivity and the specificity  
29 of the reaction. This study describes a new nested RT-PCR technique useful to amplify small  
30 amounts of the cDNA copy of *Cetacean morbillivirus* (CeMV) when it is present in scant quantity  
31 in whales' biological specimens. This technique was used to analyze different tissues (lung, brain,  
32 spleen and other lymphoid tissues) from one under human care seal and seven cetaceans stranded  
33 along the Italian coastline between October 2011 and September 2015. A well-characterized, 200  
34 base pair (bp) fragment of the dolphin Morbillivirus (DMV) haemagglutinin (H) gene, obtained by

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