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# Exposure occurred, but *Morbillivirus* was not the likely cause of striped dolphin deaths in the Ligurian Sea during 2007

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## ABSTRACT

We report herein the results of *post-mortem*, parasitological, microbiological, histopathological, immunohistochemical (IHC), indirect immunofluorescence (IIF), biomolecular (RT-PCR) and serological investigations carried out on 8 (1 male calf, 1 subadult female, 5 adult females, 1 adult male) striped dolphins (*S. coeruleoalba*) found stranded from August to November 2007 on the Ligurian Sea coast of Italy.

A (broncho-interstitial) pneumonia occurred in 6 animals, with numerous parasitic bodies in both pulmonary and extra-pulmonary locations. Histologically, a multifocal, subacute to chronic, non-purulent meningo-encephalitis occurred in 1 adult male and 3 adult females, with more or less prominent perivascular cuffs of inflammatory mononuclear cells and peri-paravascular macrophage accumulations. A mild choroid plexus lymphocytic-plasmacytic infiltration was also present, along with moderate neuronal damage, although no viral inclusions were seen in any brain cell (nor in any other cell type). The blood sera from 4 dolphins (1 calf, 1 subadult, 2 adults) had anti-*Morbillivirus* neutralizing antibodies, with positive titres ranging from 1:10 to 1:40. Nevertheless, IHC, IIF and RT-PCR investigations gave negative results in all animals.

On the basis of the above findings, we conclude that one or more factors other than *Morbillivirus* likely were the primary cause of these dolphin mortality episodes.

## INTRODUCTION

The impact of *Morbillivirus* infections (MI) on the health status of sea mammals is well established since a long time. Indeed, since 1987, at least ten different MI epidemics have caused mass mortality within several free-living pinniped and cetacean populations around the world (Di Guardo *et al*., 2005).

Among the still debated, or even controversial issues regarding MI in sea mammals, the one related to the origin of their causative agents is of particular concern, while another intriguing question refers to the synergistic effects, if any, associated with chronic exposure to a number of environmental pollutants, such as organochlorines and heavy metals. In fact, it is unknown whether and how these chemicals play a role in modulating the pathogenic and pathogenetic activity primarily displayed by sea mammal morbilliviruses (Kennedy, 1998; Di Guardo *et al*., 2005).

Among MI epidemics, the one which affected Mediterranean striped dolphins (*Stenella coeruleoalba*) throughout 1990-1992 deserves special concern, considering that additional die-off episodes have recently occurred in the Mediterranean Sea basin among at least two different cetacean species, namely pilot-whales (*Globicephala melas*) around Gibraltar (Fernández *et al*., 2008) and striped dolphins along the Spanish coast (Raga *et al*., 2008). More in detail, it has been pointed out that the 2006-2007 Mediterranean Sea epidemic shares many similarities with the mass mortality episodes in the same area during 1990-1992, including also the unusual die-offs recorded in striped dolphins from the Ligurian Sea (Raga *et al*., 2008).

We think this statement is correct considering that, since August 2007, an unusually high rate of strandings was observed along a short coastline tract of the Western Ligurian Sea (North-Western Italy), in an area contiguous to the French borderline. As a matter of fact, within 3 months (from August to November) 8 striped dolphins were found stranded lifeless (mean annual value for the same area within the last 10 years was 4 animals/year).

## MATERIALS AND METHODS

A detailed *post-mortem* examination was carried out on the above 8 dolphins (1 less than 2- weeks-old calf, 1 subadult female, 5 adult females, and 1 adult male), followed by parasitological, microbiological, toxicological (for Hg, Pb, and Cd), histopathological, immunohistochemical (IHC), indirect immunofluorescence (IIF), biomolecular, and serological investigations. More in detail, an anti-*Canine Distemper Virus* (CDV) nucleoprotein (N) antigen monoclonal antibody (MoAb) was utilized for both IHC and IIF studies, with a reverse-transcription polymerase chain reaction (RT-PCR) technique detecting specific *Morbillivirus* [by the universal *Morbillivirus* N gene primers: **P1** (ACAGGATTGCTGAGGACCTAT) and **P2** (GCACCGTACATGGTTATCTTG) (Frisk *et al*., 1999)] and *Dolphin Morbillivirus* [(DMV) by the DMV N gene-specific primers: **DMV_REV** (AGATGGGCGAGACTGCACC) and **DMV_FW** (ATCAGGGCTCACTTTTGCATCCAGA) (*GeneBank Access Number: NC005283*)] genomic sequences being also applied on tissue extracts from different brain areas of the 8 cetaceans under study. Additionally, on their blood sera a virus neutralization assay to detect anti-*Morbillivirus* antibodies was performed using the Onderstepoort CDV strain.

## RESULTS

Macro-microscopic evidence of (broncho-interstitial) pneumonia was found in 6 dolphins, with large numbers of parasitic bodies being detected in both pulmonary and extra-pulmonary locations.
Histologically, a multifocal, subacute to chronic, non purulent meningo-encephalitis occurred in 1 adult male and 3 adult females, with lesions being characterized by a more or less prominent perivascular edema with perivascular cuffs of inflammatory mononuclear cells and peri-paravascular macrophage accumulations. Furthermore, evidence of angiopathy/microangiopathy occurred in several blood vessels, the walls of which often showed an "*onion-like*" appearance, with a more or less pronounced astrogliosis/astrocytosis and a mild choroid plexus infiltration of lymphocytes and plasmacells being also observed. Mild to moderate neuronal damage could be occasionally noted, with no viral inclusions being detected in any brain cell (nor in any other body cell type).
Serological examinations yielded presence of anti-*Morbillivirus* (CDV) neutralizing antibodies in the blood sera from 4 out of the 8 dolphins, with positive titres of 1:10 (1 newborn), 1:20 (1 subadult and 1 adult female), and 1:40 (1 adult female), respectively.
Nevertheless, IHC, IIF, and RT-PCR investigations gave negative results in all 8 cetaceans under study.

## DISCUSSION AND CONCLUSIONS

On the basis of the above results, it seems more than realistic to believe that exposure to a morbilliviral agent occurred in at least 4 dolphins, which may hypothetically argue in favour of their recent exposure to the DMV strain(s) currently circulating in the Mediterranean Sea basin (considering also that such animals were of different ages, including a newborn dolphin, whose 1:10 seropositive titre could have likely resulted from passive colostral transfer of maternal immunity to the virus).
Nevertheless, IHC, IIF, and RT-PCR investigations against *Morbillivirus* yielded negative results in all 8 dolphins, including the 4 adult ones affected with inflammatory brain tissue lesions partially compatible with a morbilliviral etiology (Domingo *et al*., 1992; Kennedy, 1998; Di Guardo *et al*., 2005).
Interestingly, mercury (Hg) levels in the liver tissue were above the immunotoxic threshold (Camara-Pellisso *et al*., 2008), so that a certain immunodeficiency degree was likely present in all 8 cetaceans under study (Zaccaroni *et al*., 2008).
In conclusion, albeit (recent or past) morbilliviral (DMV) exposure occurred in at least 4 dolphins, one or more factors other than *Morbillivirus* could have been involved in both lesions determinism and subsequent animals death.
This may especially be the case of non purulent meningo-encephalitis lesions, showing similarities with those reported in *Brucella* spp. infection among striped dolphins (González *et al*., 2002).
In this respect, the immunotoxic effects displayed by mercury and, possibly, also by other environmental contaminants may have exacerbated the pathogenic activity of the biological disease agent(s) involved in the striped dolphin mortality episode investigated herein.

Further and more-in-depth studies are needed, however, to better characterize, in general, the adverse effects of pollutants on sea mammal health and conservation and, more specifically, their role in the intriguing context of the past (1990-1992) and the recent (2006-2007) Mediterranean Sea morbilliviral epidemics.

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## REFERENCES

Camara-Pellisso, S., Munoz, M.J., Carballo, M. and Sanchez-Vizcaino, J.M. 2008. Determination of the immunotoxic potential of heavy metals on the functional activity of bottlenose dolphin leukocytes *in vitro*. *Vet. Immunol. Immunopathol.* 121:189-98.

Di Guardo, G., Marruchella, G., Agrimi, U. and Kennedy, S. 2005. *Morbillivirus* infections in aquatic mammals: A brief overview. *J. Vet. Med. Series A* 52:88-93.

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

Fernández, A., Esperón, F., Herráez, P., Espinoza de Los Monteros, A., Clavel, C., Bernabé, A., Sánchez-Vizcaino, J.M., Verborgh, P., DeStefanis, R., Toledano, F. and Bayón, A. 2008. *Morbillivirus* and pilot whale deaths, Mediterranean Sea. *Emerg. Infect. Dis.* 14:471-3.

Frisk, A.L., König, M., Moritz, A. and Baumgärtner, W. 1999. Detection of distemper nucleoprotein RNA by reverse transcription-PCR using serum, whole blood, and cerebrospinal fluid from dogs with distemper. *J. Clin. Microbiol.* 37:3634-43.

González, L., Patterson, I.A., Reid, R.J., Foster, G., Barberan, M., Blasco, J.M., Kennedy, S., Howie, F.E., Godfroid, J., MacMillan, A.P., Shock, A. and Buxton, D. 2002. Chronic meningoencephalitis associated with *Brucella* sp. infection in live-stranded striped dolphins (*Stenella coeruleoalba*). *J. Comp. Pathol.* 126:147-52.

Kennedy, S. 1998. *Morbillivirus* infections in aquatic mammals. *J. Comp. Pathol.* 119:201-25.

Raga, J.-A., Banyard, A., Domingo, M., Corteyn, M., Van Bressem, M.-F., Fernández, M., Aznar, F.-J. And Barrett, T. 2008. Dolphin morbillivirus epizootic resurgence, Mediterranean Sea. *Emerg. Infect. Dis.* 14:471-3.

Zaccaroni, A., Scaravelli, D., Proietto, U., Marsilio, F., Mignone, W., Vivaldi, B., Caroggio, P., Casalone, C., Bozzetta, E., Garibaldi, F., Di Francesco, C.E. and Di Guardo, G. 2008. *Morbillivirus* could not be the major factor impacting the health of striped dolphins along the Ligurian Sea coast

of Italy. In: *Proceedings of the 39th Annual Meeting of the International Association for Aquatic Animal Medicine (IAAAM)*, Pomezia (Rome), Italy, 10-14. May. 2008.