

Short Communications

Epizootic of dolphin morbillivirus on the Catalanian Mediterranean coast in 2007

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BETWEEN 1990 and 1992, thousands of striped dolphins (*Stenella coeruleoalba*) stranded along the Mediterranean coast due to a newly described virus, the dolphin morbillivirus (DMV) (Domingo and others 1990, 1992). DMV is one of the several morbilliviruses that have killed marine mammals worldwide since 1987 (Di Guardo and others 2005). A new DMV epizootic has been recently confirmed from the Mediterranean Spanish and French coasts during 2007 to 2008 (Fernández and others 2008, Raga and others 2008, Keck and others 2010). This short communication describes the pathological findings associated with DMV infection and secondary infections, observed during this epizootic on the Mediterranean coast of Catalonia (north-east Spain). This report also provides further evidence of the absence of DMV circulation on the Catalanian Mediterranean coast during the interepizootic period.

From July 2007 to December 2007, 40 cetaceans were found stranded along the Catalanian Mediterranean coast: 36 striped dolphins, two Risso's dolphins (*Grampus griseus*) and two bottlenose dolphins (*Tursiops truncatus*). Ten well-preserved animals (nine striped dolphins and one Risso's dolphin) were submitted to the authors' laboratory for postmortem examination. A complete set of organs was collected, fixed in 10 per cent neutral buffered formalin and routinely processed for histopathology. Immunohistochemistry was performed on selected samples of lung, lymphoid and brain tissues, as described previously by Raga and others (2008). A monoclonal

Veterinary Record (2011) 169, 101a

doi: 10.1136/vr.d1686

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Provenance: not commissioned; externally peer reviewed

Published Online First: 22 June 2011

TABLE 1: Biological data, results of immunohistochemistry (IHC) for dolphin morbillivirus (DMV) and presence of other infections in nine striped dolphins (*Stenella coeruleoalba*) and a Risso's dolphin (*Grampus griseus*) found stranded on the Catalanian Mediterranean coast in 2007

| Case | Sex | Bodyweight (kg) | Body length (cm) | DMV IHC | Other systemic infections |
|------|-----|-----------------|------------------|---------|---------------------------|
| Sc 1 | M | 26.0 | 148 | + | Herpesvirus |
| Sc 2 | F | 49.0 | 180 | + | <i>Toxoplasma gondii</i> |
| Sc 3 | M | 44.8 | 170 | + | <i>Toxoplasma gondii</i> |
| Sc 4 | F | 38.3 | 155 | + | <i>Aspergillus</i> -like |
| Sc 5 | F | 62.4 | 178 | + | <i>Toxoplasma gondii</i> |
| Sc 6 | M | NR | 205 | - | <i>Toxoplasma gondii</i> |
| Sc 7 | M | 67.8 | 195 | - | None |
| Sc 8 | M | 75.0 | 185 | - | None |
| Sc 9 | F | 9.8 | 104 | - | None |
| Gg 1 | F | NR | 290 | - | None |

+ Positive, - Negative, F Female, Gg *Grampus griseus*, M Male, NR Not reported, Sc *Stenella coeruleoalba*

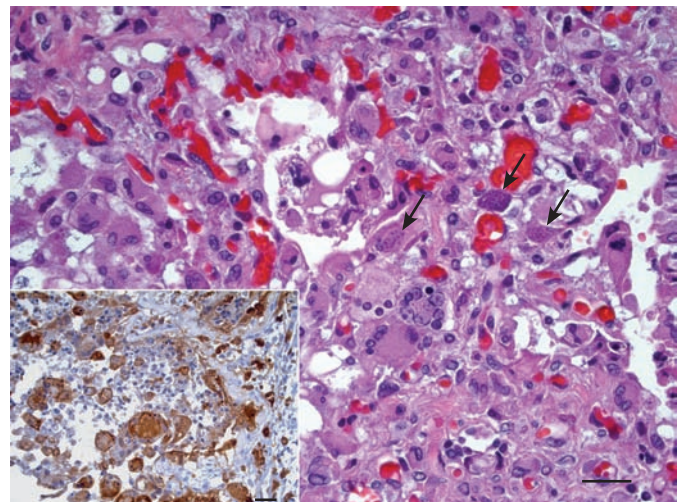


FIG 1: Lung of a striped dolphin (*Stenella coeruleoalba*) (case Sc 3) showing severe pneumonia with formation of syncytial cells, characteristic of dolphin morbillivirus (DMV) infection, and with associated presence of *Toxoplasma gondii* cysts (arrows). Haematoxylin and eosin. Bar=25 µm. Inset: the same lung showing strong immunopositivity associated with the lesion. Immunohistochemistry for DMV. Bar=25 µm

antibody (mAb) raised against the nucleoprotein of canine distemper virus (CDV) (NP 050505; VMRD) known to react with DMV was used. Alternatively, another mAb raised against the nucleoprotein of CDV (clone DD11; INGENASA), also recognising DMV, was also used. Immunohistochemistry for the detection of *Toxoplasma gondii* (Resendes and others 2002) was performed in selected tissues from animals showing lesions compatible with this parasitic infection.

Five of nine striped dolphins that stranded between July 2007 and December 2007 inclusive were found by immunohistochemistry to be infected with DMV. In addition, lesions of systemic coinfection with an *Aspergillus*-like fungus (one case), herpesvirus (one case) (Soto and others 2011) and *T. gondii* (three cases) were found (Table 1, Fig 1). One further case of toxoplasmosis without histopathological or immunohistochemical evidence of DMV infection was also recorded. One investigated Risso's dolphin was negative for DMV.

The five DMV-infected dolphins and the case of toxoplasmosis with no evidence of DMV were all found live-stranded. All the dolphins died within minutes or a few hours of stranding, except for one dolphin, which was euthanased. Clinical signs of convulsions were observed in four of the five DMV-infected dolphins.

Macroscopic and microscopic lesions attributable to DMV infection – bronchointerstitial pneumonia, lymphoid depletion and/or encephalitis – were similar to the lesions observed in the 1990 epizootic (Domingo and others 1992). Macroscopic lesions, when evident, were mainly due to secondary infections such as toxoplasmosis and mycotic pneumonia and encephalitis. Histological lesions attributable to toxoplasmosis were similar to those recorded previously (Inskeep and others 1990, Migaki and others 1990). There is a striking association between systemic toxoplasmosis and DMV epizootics in Mediterranean striped dolphins. In spite of a seroprevalence of *T gondii* infection of 11 per cent in the striped dolphin population in Spanish Mediterranean waters (Cabezón and others 2004), systemic toxoplasmosis in striped dolphins has been recorded only by Di Guardo and others (2010) and by the authors of the present report in the frame of DMV epizootics. Toxoplasmosis cases are usually coinfecting with DMV, although individual DMV-negative cases may also be found. These cases could represent animals that overcame DMV but succumbed to the *T gondii* secondary infection. A similar temporal association has been found between DMV epizootics and mycotic pneumonia and encephalitis (Domingo and others 1992). The occurrence of these secondary infections along with the DMV epizootics, and not in the interepizootic period, strongly reflects the deep immunosuppression caused by DMV in striped dolphins.

Between the two Mediterranean DMV epizootics occurring specifically on the Catalan coast, 17 years have elapsed. In the interepizootic period, a total of 105 routine postmortem examinations of cetaceans stranded on the Catalan Mediterranean coast have been performed by the authors' group, and no evidence of DMV-associated lesions was found. To assess the possibility of unnoticed DMV circulation during the interepizootic period, these cases were reviewed retrospectively and a total of 58 well-preserved cases showing some kind of microscopic inflammatory lesions in the lungs or brain (50 striped dolphins, four Risso's dolphins, three bottlenose dolphins and two common dolphins [*Delphinus delphis*]) were investigated by immunohistochemistry. All cases were immunohistochemically negative, supporting the hypothesis that DMV was not circulating during the interepizootic period in the striped dolphin population in the Mediterranean. A similar conclusion was drawn by Van Bressem and others (2001) following serological investigations for DMV. Another possibility, which cannot be fully excluded, would be that DMV has circulated without evidence of disease and mortality. The apparently smaller impact of the 2007 epizootic compared with the epizootic in the early 1990s is probably related to the partial residual protection of the population after the first epizootic (Van Bressem and others 2001). Observations from the Mediterranean coasts of France and Valencia (Spain), based on comparison of mean body length measurements, indicate that stranded dolphins in the 2007 epizootic were younger than those that stranded during the 1990 epizootic (Raga and others 2008, Keck and others 2010), further supporting this interpretation. The mean length of the five DMV-affected dolphins examined in 2007 and 33 DMV-affected dolphins from 1990 was compared by a Student's *t* test. Mean (sd) values were 193.6 (16.5) cm for the cases from 1990 and 166.2 (14.14) cm for the cases from 2007. The difference was significant assuming either that the variances of both samples were the same ($P=0.001$) or dissimilar ($P=0.003$). Therefore, it was concluded that also on the Catalan coast, affected dolphins in the 2007 epizootic were shorter in length (that is, younger) than those affected in 1990, which suggests that older animals may have been protected from DMV by previous exposure in 1990.

During the 2007 epizootic, the most severely affected cetacean species was the striped dolphin, as was the case in the 1990 epizootic (Di Guardo and others 2005). However, a distinctive feature of the 2007 epizootic was the early detection of DMV-infected long-finned pilot whales (*Globicephala melas*) on the southern Mediterranean coasts and Balearic Islands in the months immediately preceding the epizootic in striped dolphins (Fernández and others 2008). A pilot whale and a bottlenose dolphin were also found to be affected on the French Mediterranean coast (Keck and others 2010).

The reappearance of DMV affecting the Mediterranean cetacean population reinforces the importance of continuous monitoring of cetacean strandings and of performing pathological and virological studies to understand the dynamics of DMV infection and disease in these cetacean populations.

Acknowledgements

The authors thank Ana Alba (CRESA) for support during the statistical analysis of the length data of the dolphins, and Mónica Pérez (CRESA) and Blanca Pérez (Departament de Sanitat i d'Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona) for technical support. JAR was supported by a PROMETEO2011/40 project (Conselleria de Educació, Generalitat Valenciana).

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Veterinary Record 2011 169: 102 originally published online June 22, 2011

doi: 10.1136/vr.d1686

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