

DOLPHIN MORBILLIVIRAL INFECTION FROM THE MEDITERRANEAN SEA DID NOT SPREAD INTO THE ADRIATIC SEA

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In July of 1990, a mass mortality of striped dolphins due to morbillivirus infection had begun in the western Mediterranean. By 1992, the infection had spread to the eastern Mediterranean and the Aegean Sea. Other dolphin species in the Mediterranean were not found to have died due to this infection, although it is possible for many species of marine mammals to be infected. In 1994, it was published that morbillivirus infection had caused Atlantic bottlenose dolphin mortality in the USA. Although striped dolphins are not residents of the Adriatic Sea, it was hypothesised that the infection could have spread from them to Adriatic bottlenose dolphins. From October 1990 through April 1997, 16 dolphin carcasses found along the Croatian Adriatic coast were examined. Tissues were examined by light microscopy for syncytia and inclusion bodies, histopathologic lesions characteristic of dolphin morbillivirus infection, and by detection of morbilliviral RNA by a reverse transcriptase polymerase chain reaction (RT-PCR). No signs of morbillivirus infection were found in the examined animals. It was concluded that this infection had not spread to dolphins of the Adriatic Sea up until that date.

Key words: Bottlenose dolphin (*Tursiops truncatus*), morbillivirus, epizootic, survey, Adriatic Sea, Croatia

At the present time, the bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821) is the only dolphin species resident in the Adriatic Sea (Gomerčić et al., 1994; Notarbartolo di Sciara et al., 1994). At the same time, the most abundant dolphin species in the Mediterranean Sea has been the striped dolphin, *Stenella coeruleoalba* (Aguilar and Raga, 1993), which is not resident in the Adriatic. Only one striped dolphin has been found in the Croatian Adriatic Sea (Gomerčić et al., 1994) and it was included in this study. Another marine mammal species

in the Adriatic Sea is a very small population of Mediterranean monk seal, *Monachus monachus* (Gomercic et al., 1984; Antica et al., 1994).

Morbilliviruses are extremely contagious, causing measles in humans, rinderpest in bovines, canine distemper and "peste des petits ruminants" in goats and sheep. Phocid distemper virus, dolphin morbillivirus and porpoise morbillivirus cause infections in marine mammals (Haas and Barrett, 1996). Modified morbilliviruses — phocid distemper virus-2 (probably a strain of, or identical with, canine distemper virus) and phocid distemper virus-1 (Visser et al., 1993), which caused an infection among Baikal seals, *Phoca sibirica* (Grachev et al., 1989; Cvetnić, 1991; Visser et al., 1993) and harbour seals, *Phoca vitulina* (Kennedy et al., 1988; Cvetnić, 1991; Visser et al., 1993) — were recently detected. Many seals have died in Baikal Lake and in Northern Europe due to being infected by that virus. Morbillivirus infection in Northern Europe has also been detected in harbour porpoises, *Phocoena phocoena* (Kennedy et al., 1991; Kennedy et al., 1992). Van Bresseem et al. (1993) detected morbillivirus neutralising serum antibodies in a common dolphin (*Delphinus delphis*) beached on the Sardinian coast (Cagliari) in Italy in July 1990, and Di Guardo et al. (1995) in an adult minke whale (*Balaenoptera acutorostrata*) beached on the Tyrrhenian coast of Tuscany in Italy in 1993. In July 1990, mass deaths of striped dolphins began in the western Mediterranean due to morbillivirus infection (Domingo et al., 1990; Van Bresseem et al., 1991; Gomerčić and Huber, 1991; Di Guardo et al., 1992; Aguilar and Raga, 1993; Visser et al., 1993). Morbillivirus infection in Mediterranean striped dolphins began spreading eastwards, and by 1991 it had spread to the central Mediterranean, and by 1992 to the eastern Mediterranean and the Aegean Sea (Aguilar and Raga, 1993; Cebrian, 1995), where it caused the death of numerous striped dolphins. By July 1991 the infection had spread to the whole of the Ionian Sea (Cebrian, 1995). In the Mediterranean Sea, morbillivirus infection did not spread to other dolphin species and there are no data relating to its spread to Mediterranean monk seals (Cebrian, 1995)*. As late as the end of 1994 it was published that morbillivirus infection was proved to have spread to Atlantic bottlenose dolphins (*Tursiops truncatus*) on the Atlantic coast of the USA in 1987/88 (Lipscomb et al., 1994a). In 1993 and 1994, morbillivirus infection was diagnosed in dead bottlenose dolphins in the Gulf of Mexico (Lipscomb et al., 1994b, 1996). In 1995, the infection was discovered among long-finned (*Globicephala melas*) and short-finned (*Globicephala macrorhyn-*

* In the summer of 1997 about half the population of this highly endangered species, which inhabits the Mauritanian coast, was reported to have been killed by morbillivirus [Osterhaus, A., Groen, J., Niesters, H., van de Bildt, M., Martina, B., Vedder, L., Vos, J., van Egmond, H., Sidi, B. A. and Barham, M. E. O. (1997): Morbillivirus in monk seal mass mortality. *Nature* **388**, 838–839.].

chus) pilot whales, and even among Florida manatees, *Trichechus manatus latirostris* (Duignan et al., 1995a, 1995b).

The most significant histologic evidence of dolphin morbilliviral infection are multinucleated syncytia of lung epithelial cells and intranuclear and intracytoplasmic inclusion bodies in cells, particularly of lymph nodes, although morbilliviruses very often also affect an animal's brain (Domingo et al., 1990).

Although striped dolphins are not resident in the Adriatic Sea, it was feared that the infection could spread from them to Adriatic bottlenose dolphins (*Tursiops truncatus*), and even to Mediterranean monk seals (*Monachus monachus*).

Materials and methods

From October 1990 to April 1997, 15 bottlenose dolphins, *Tursiops truncatus* (Montagu, 1821) and 1 striped dolphin, *Stenella coeruleoalba* (Meyen, 1833) found dead in the Croatian part of the Adriatic Sea (between about 42°15'N and 45°35'N latitude) were examined. The dolphins were measured according to Perrin (1975) and aged by growth-layer groups in the dentine according to Slooten (1991). All carcasses were autopsied. Samples of lung, bronchial, mediastinal and parapharyngeal lymph nodes, pharyngeal mucous membrane, livers, kidneys, spleens, gonads, brains and muscles, stained by haematoxylin and eosin, were studied by light microscopy. Eighteen 10% formalin-fixed samples of lungs, bronchial lymph nodes, parapharyngeal lymph node, pharyngeal mucous membrane, livers, kidneys and muscles from 7 animals (Table 1) were examined for presence of morbillivirus, using the reverse transcriptase polymerase chain reaction (RT-PCR) technique (Krafft et al., 1995), at the Department of Veterinary Pathology, and the Department of Cellular Pathology, Armed Forces Institute of Pathology, Washington DC, USA. The dolphins which were partly autolysed were primarily selected for RT-PCR analyses. The RT-PCR was performed using the following primers: the first primer used for reverse transcription was 5'-ATTGGGTTGCACCACTTGTC-3', and the second added for PCR was 5'-ATTAAAAAGGG(GC)ACAGGAGAGAGATCAGCC-3'. The primers were designed to amplify the conserved region of the morbillivirus phosphoprotein gene. As a control for amplifiable RNA, RT-PCR for beta-actin was performed in each case.

Table 1

Dolphins (*Tursiops truncatus* and *Stenella coeruleoalba*) found dead in the Croatian part of the Adriatic Sea and examined for morbillivirus infection

Species	Found month/year	Sex	Age year	Location north/east	Body length cm	Body mass kg	Morbilliviral RNA molecules	Morbilliviral syncytia or inclusion bodies
<i>T. truncatus</i>	10/90	F	4	45°03'N, 13°35'E	250	204	N ^{2,3}	N
<i>T. truncatus</i>	11/90	F	< 1	45°05'40"N, 13°38'40"E	164	52	N ^{4,5}	N
<i>S. coeruleoalba</i>	10/91	?	adult	42°55'N, 17°11'25"E	?	?	–	–
<i>T. truncatus</i>	6/92	M	7	45°04'N, 13°34'E	263	240	N ^{2,*}	N
<i>T. truncatus</i>	summer/93	?	?	44°23'30"N, 14°40'E	?	?	–	N
<i>T. truncatus</i>	9/93	M	3–4?	44°33'40"N, 14°23'E	242	?	N ^{1,2,3}	N
<i>T. truncatus</i>	3/94	F	6	45°04'25"N, 13°38'20"E	219	120	N ^{1,2,6}	–
<i>T. truncatus</i>	5/94	F	6	44°52'05"N, 14°E	265	?	N ^{1,2,6}	–
<i>T. truncatus</i>	5/94	F	fetus	44°52'05"N, 14°E	107	9,1	N ^{1,2,3,7}	–
<i>T. truncatus</i>	9/94	M	< 1	42°43'45"N, 16°53'40"E	163	75	–	N
<i>T. truncatus</i>	4/95	F	adult	45°18'N, 14°33'E	?	?	–	N
<i>T. truncatus</i>	12/95	M	9	43°43'40"N, 15°53'30"E	278	237	–	N
<i>T. truncatus</i>	4/96	F	5	44°49'30"N, 13°51'05"E	240	?	–	N
<i>T. truncatus</i>	8/96	?	young	43°12'30"N, 16°33'20"E	210	?	–	N
<i>T. truncatus</i>	10/96	M	12	45°06'05"N, 13°37'50"E	290	279	–	N
<i>T. truncatus</i>	4/97	F	7	44°46'20"N, 14°19'15"E	?	?	–	N

F = female; M = male; N = negative; – = not examined; RT-PCR examined organs and tissues: ¹ = liver; ² = kidney; ³ = lungs; ⁴ = parapharyngeal lymph node; ⁵ = mucous membrane of pharynx; ⁶ = muscle; ⁷ = bronchial lymph node; * = result uncertain

Results

The only striped dolphin, stranded in October 1991 at Lumbarda beach on Korčula Island, was a physically mature animal and, according to interviews with witnesses, it had displayed some neurological symptoms, i.e., before stranding the animal rolled and swam in small circles. Neither histological nor RT-PCR-technique study of this striped dolphin was done as there was no soft tissue remaining of this animal by the time the remains were reached by us.

The examined dolphins were of both sexes (8 female, 5 male, 3 undetermined), aged from preterm fetus and cub (< 1 year old) to 12-year-old animals, a body mass of between 9 and 279 kg, and a total body length of between 107 and 290 cm (Table 1).

Autopsies and histological studies revealed the following changes in dead dolphins from the Croatian part of the Adriatic Sea: pneumonia, chronic fibrous bilateral arthritis (*Aeromonas* sp.) of shoulder joints, spondylosis, moderate to strong diffuse fatty changes in liver with extensive subcutaneous (between blubber and muscles) oedema and moderate hydrops of body cavities, splenic atrophy, mild multifocal subacute pharyngitis with superficial fungal hyphae, moderate multifocal acute pneumonia with gram-negative bacilli, moderate multifocal granulomatous and eosinophilic pneumonia, minimal multifocal interstitial lymphoplasmacytic nephritis, and nematodes in the lungs, intestine and urethra.

Light-microscopic histological examination of the lungs, bronchial, mediastinal and parapharyngeal lymph nodes, pharyngeal mucous membrane, livers, kidneys, spleens, gonads, brains and muscles of all unautolysed bottlenose dolphins revealed not a single instance of morbilliviral infection. Neither syncytia nor inclusion bodies were found in any of those organs of twelve dead bottlenose dolphins taken from the Adriatic Sea.

All examined specimens of lungs, bronchial lymph nodes, parapharyngeal lymph nodes, pharyngeal mucous membrane, livers, kidneys and muscles of all seven studied bottlenose dolphins were negative for morbillivirus by the RT-PCR technique.

Discussion

The presented findings suggest that dolphin morbillivirus infection has not spread throughout the Croatian part — nor, very probably, throughout the whole — of the Adriatic Sea up until this moment in time. This conclusion is supported by Di Guardo et al. (1995) who wrote: “Despite the presence of lesions characteristic of morbilliviral pneumonia in two other striped dolphins stranded on the coast of Apulia in 1991, no morbillivirus antigen was detected in the tissues of these animals.” The Apulian coast is the most southerly part of the

Italian Adriatic coast. The results of this study are in contrast to the situation presented in Fig. 2 of the review article by Dhermain et al. (1994), in which it was shown that the infection had spread over almost the entire Adriatic Sea. That claim is not supported by the cited references nor by other studies of dolphin morbillivirus infection in the Mediterranean Sea. Between 1990 and 1992 the infection spread throughout the whole of the Mediterranean Sea (Aguilar and Raga, 1993; Visser et al., 1993; Cebrian, 1995). The infection spread south-east from the Ionian Sea to the Aegean Sea (Cebrian, 1995). According to Cebrian (1995) the average speed of progression was 4.6 km/day for the whole Mediterranean Sea, and 16.3 km/day for the western Mediterranean Sea. If the infection had the same tendency to spread in a north-westerly direction, i.e., in the Adriatic Sea, as it did in the Mediterranean Sea, it should have affected dolphins in all parts of the Adriatic Sea by the spring of 1992. The distance between Zakynthos Island in the Ionian Sea, where the first reported striped dolphin was stranded in July 1991 (Cebrian, 1995), and the north-west part of the Adriatic Sea is about 1,100 km. Why then did the dolphin morbillivirus infection not spread in the Adriatic Sea? Possible explanations are: (1) markedly pelagic striped dolphins, which can travel long distances and hence spread infection, do not live in the Adriatic Sea, and (2) only bottlenose dolphins live in the Adriatic Sea, their absolute number being relatively small and their density low (Notarbartolo di Sciara and Bearzi, 1992; Gomerčić et al., 1994; Notarbartolo di Sciara et al., 1994). Furthermore, schools of Adriatic bottlenose dolphins are probably isolated and their home ranges are relatively small (Bearzi et al., 1993). It can be speculated that the moribund striped dolphin found on Korčula Island died of morbillivirus infection but that, fortunately, it did not infect resident Adriatic bottlenose dolphins. Its moribund status was probably the very reason that it was found in the Adriatic Sea, in which, as we have already stressed, striped dolphins are not resident.

Finally, it can be concluded that there is no evidence that Mediterranean dolphin morbillivirus epizootic from the beginning of the 1990s, has affected the only dolphin species — the bottlenose dolphin — resident in the Adriatic Sea, although bottlenose dolphins in the Atlantic Ocean and the Gulf of Mexico have been so affected.

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