Arch Virol (1993) 129: 235–242

Archives of Virology

Dolphin morbillivirus infection in different parts of the Mediterranean Sea

Brief Report

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Accepted August 28, 1992

Summary. Morbillivirus were isolated from Mediterranean striped dolphins (*Stenella coeruleoalba*) dying along the coasts of Italy and Greece in 1991. They were antigenically identical to the morbilliviruses isolated from striped dolphins in Spain in 1990.

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Within the genus *Morbillivirus* of the family *Paramyxoviridae,* five different viruses have been recognised, which infect members of different mammalian orders under natural conditions: measles virus (MV) of humans, canine distemper virus (CDV) of dogs and other carnivores, rinderpest virus (RPV) and peste des petits ruminants virus (PPRV) of artiodactyls [4, 7]. The fifth member of the genus, phocid distemper virus-1 (PDV-1) [21], caused a devastating epizootic among the harbour seals (*Phoca vitulina*) of North Western Europe in 1988 [18, 19], PDV-1 proved to be antigenically and genetically closely related to CDV [13, 15, 24]. Since this die-off, morbillivirus infections have been described in other pinniped species and cetaceans [11].

In ceteceans, morbilliviruses were isolated from harbour porpoises (*Phocoena*

phocoena) stranded on the Irish [12] and Dutch coasts [20]. In the summer of 1990, a morbillivirus associated die-off occurred among the striped dolphins (*Stenella coeruleoalba*) of the western part of the Mediterranean Sea [1, 5, 8, 22]. From June to August 1991, a high mortality was observed among striped dolphins along the southern coast of Italy [6]. From September 1991 to January 1992, a similar die-off was noticed among striped dolphins in the Greek waters. On the basis of epizootiological data, the mortality was suspected to be the result of an extension of the morbillivirus-associated disease outbreak which previously occurred in the western part of the Mediterranean Sea.

We now document the presence of morbillivirus antigen and morbillivirus-specific serum antibodies in samples from several striped dolphins stranded during this recent die-off. We also report the results of the preliminary antigenic characterization of the morbilliviruses isolated from the lungs of three of these animals.

From May to December 1991 organ material was collected from 23 striped dolphins, two common dolphins (*Delphinus delphis*), one bottlenose dolphin (*Tursiops truncatus*) and one Cuvier's beaked whale (*Ziphius cavirostris*) stranded on the coasts of southern Italy and Greece as indicated in Table 1.

Morbillivirus antigen was detected by a previously described antigen capture ELISA [22] in lung suspensions of four out of the eleven striped dolphins stranded in Italy, of four out of the eight striped dolphins beached in Greece, and not in organ materials of the bottlenose dolphin and the Cuvier's beaked whale. Morbilliviruses were isolated from the lungs of one of the Greek (D 6) and two of the Italian (D 2, D 4) animals positive in the antigen detection assay.

Virus isolation was performed in peripheral blood mononuclear cell (PBMC) cultures of a bottlenose dolphin (TT. PBMC). TT. PBMC were inoculated with 10% suspension prepared from the lungs of four striped dolphins stranded in Italy and two dolphins beached in Greece during the die-off of 1991 (Table 1). They were co-cultivated with Vero cells after 7 days. TT. PBMC and Vero cell monolayers were checked at regular intervals for the development of cytopathic changes. Syncytia were detected in the TT. PBMC cultures inoculated with the lung suspensions of these dolphins within seven days. In the Vero cells co-cultivated with these cultures, a cytopathic effect was subsequently observed within the following ten days. Morbillivirus antigen was detected in cell-free supernatants of all of these cultures, using the antigen capture ELISA. Nucleocapsids typical for the members of the family *Paramyxoviridae* were observed in infected cell-lysates by negative contrast electron microscopy (not shown).

In order to assess whether an epizootiological link had existed between the outbreaks in the eastern and western parts of the Mediterranean Sea, the reactivities of CDV and PDV-1-specific MoAbs with the morbillivirus isolates were tested in an indirect ELISA [16, 17]. For preparation of ELISA antigens the isolates from Italy (DMV/Lucca/0691, DMV/Trapani/0891) and Greece (DMV/Zakinthos/0991) were passaged at least four times in Vero cell monolayer cul-

Table 1. Data gathered on materials collected from cetaceans stranded in Italy and Greece

Organ material nr.	Serum nr.	Species	Sex	SL (cm)	Site of stranding	Date (month/year)	Ag detection	Virus isolation	ELISA Ab titer	VN Ab titer
I-D 3	1935	*S. coeruleoalba*	F		Lucca, Italy	05/91	−	−	20	160
I-D 2	NA	*S. coeruleoalba*	F		Lucca, Italy	06/91	+	+	NT	NT
NA	1951	*S. coeruleoalba*			Reggio Calabria, Italy	08/91	NT	NT	−	160
I-D 4	2086	*S. coeruleoalba*	M		Trapani, Italy	08/91	+	+	160	160
NA	2087	*S. coeruleoalba*	F		Italy	08/91	NT	NT	−	−
I-D 5	NA	*S. coeruleoalba*	M	94	Brindisi, Italy	08/91	−	−	NT	NT
I-D 6	NA	*S. coeruleoalba*	M	201	Lecce, Italy	08/91	−	NT	NT	NT
I-D 28	NA	*S. coeruleoalba*	M	193	Brindisi, Italy	08/91	+	NT	NT	NT
I-D 29	NA	*S. coeruleoalba*	M	198	Brindisi, Italy	08/91	−	NT	NT	NT
I-D 30	NA	*S. coeruleoalba*	M	214	Monopoli, Italy	08/91	−	NT	NT	NT
I-D 032	NA	*S. coeruleoalba*	M	199	Molfetta, Italy	08/91	−	NT	NT	NT
I-D 03	NA	*S. coeruleoalba*	F		Lecce, Italy	08/91	+	NT	NT	NT
I-D 04	NA	*S. coeruleoalba*	F		Lecce, Italy	08/91	−	NT	NT	NT
NA	2117	*S. coeruleoalba*	F		Ravenna, Italy	04/90	NT	NT	−	−
I-D 31	NA	*T. truncatus*	M	237	Foggia, Italy	08/91	−	NT	NT	NT
NA	2116	*D. delphis*			Cagliari, Italy	07/90	NT	NT	80	640
G-D 1	NA	*S. coeruleoalba*	F	88	Zakinthos, Greece	08/91	−	−	NT	NT
G-D 6	2124	*S. coeruleoalba*	M	164	Zakinthos, Greece	09/91	+	+	80	640
G-D 7	2125	*S. coeruleoalba*	M	204	Zakinthos, Greece	09/91	NT	NT	80	40
G-D 9	2198	*S. coeruleoalba*				11/91	−	NT	160	320
G-D 10	2199	*S. coeruleoalba*	M		Aegina, Greece	11/91	+	NT	320	2560
G-D 11	NA	*S. coeruleoalba*		200	Crete, Greece	12/91	−	NT	NT	NT
G-D 12	NA	*S. coeruleoalba*	F	140	Crete, Greece	12/91	+	NT	NT	NT
G-D 13	2227	*S. coeruleoalba*	M	180	Crete, Greece	12/91	+	NT	80	160
G-D 14	NA	*S. coeruleoalba*		122	Crete, Greece	12/91	−	NT	NT	NT
G-BW 1	2126	*Z. cavirostris*	F	~ 500	Alonyssos, Greece	09/91	−	NT	−	< 80*
G-D 8	NA	*D. delphis*			Crete, Greece	09/91	−	NT	NT	NT

NA Not available, *NT* not tested, *SL* standard length, + positive result in the assay, − negative result in the assay, * sample toxic to the cells at lower dilutions, *F* female, *M* male

tures. No differences were observed between the reactivity patterns of these viruses with the panel of MoAbs used. The reactivity patterns of the Italian and Greek isolates were also identical to the previously described patterns of the Spanish isolates DMV-16 A and DMV-MUC [20] (Table 2).

Morbillivirus neutralizing antibodies present in the serum samples were detected in a micro-neutralization assay [23] using about 100 $TCID_{50}$ of DMV 16 A and serial two fold dilutions of the serum samples (starting at 1:40). Neutralization titers were expressed as the reciprocals of the dilution still giving complete reduction of cytopathic changes. Morbillivirus neutralizing serum antibodies were detected in three out of four striped dolphins which died in Italy during the 1991 outbreak, in five out of five striped dolphins stranded in Greece and in a common dolphin beached in Italy in July 1990. Virus neutralization titers ranged from 40 to 2560 (Table 1). With the exception of one serum sample (1951), all samples positive in the VN test were also positive in an ELISA detecting morbillivirus specific antibodies [23] on a coat of DMV 16 A antigen. Morbillivirus-specific antibodies were not detected on the serum from a Cuvier's beaked whale and a striped dolphin stranded in Italy in April 1990 (Table 1).

Table 2. Reaction of monoclonal antibodies raised against CDV with the Spanish (DMV 16 A and DMV MUC) and the Italian (DMV/Lucca/0691, DMV/Trapani/0891) and Greek (DMV/Zakinthos/0991) isolates of DMV

Designation of clone	Specificity	CDV	DMV Spain (n = 2)	DMV Italy (n = 2)	DMV Greece (n = 1)
3.662	NP 1	+	−	−	−
3.958	NP 3	+	+	+	NT
3.991	NP 4	+	−	−	−
4.100	NP 5	+	−	−	−
3.568	P 2	+	+	+	NT
3.695	P 3	+	−	−	−
4.051	P 5	+	−	−	−
4.088	P 6	+	+	+	+
1.347	H 1	+	−	−	−
2.267	H 2	+	−	−	−
3.734	H 3	+	−	−	−
3.775	H 4	+	−	−	−
4.074	H 5	+	−	−	−
4.275	H 6	+	−	−	−
4.941	H 7	+	−	−	−
3.633	F 1	+	+	+	+
3.697	F 2	+	−	−	−
5.148	F 3	+	−	−	−

+ Reactivity in the assay, − no reactivity in the assay; *NT* not tested

The detection of morbillivirus antigen in the lungs of several affected striped dolphins and the subsequent isolation of a morbillivirus from capture ELISA positive lung suspensions indicated the role of a morbillivirus infection during the die-off of 1991. Although the DMV isolates originated from striped dolphins stranded in different regions, they exhibited the same reaction patterns in the indirect ELISA with a set of MoAbs raised against CDV and PDV-1 (Tables 2 and 3). These patterns were also identical to those of the two morbilliviruses isolated during the outbreak among striped dolphins in the western part of the Mediterranean Sea [20]. This indicates the existence of an epizootiological link between this outbreak and a similar morbillivirus-related die-off which occurred in the same species, in the western part of the Mediterranean Sea in 1990.

The detection of morbillivirus-specific antibodies in serum samples from several striped dolphins further confirmed the circulation of a morbillivirus in these populations. The presence of VN serum antibodies in absence of ELISA antibodies in one of the sera, may be explained by the fact that the morbillivirus-specific IgM antibodies produced in the acute stage of the infection, were probably not recognized by protein-A used in the ELISA [14]. Furthermore,

Table 3. Reaction of monoclonal antibodies raised against PDV-1 with the Spanish (DMV 16 A and DMV MUC) and the Italian (DMV/Lucca/0691, DMV/Trapani/0891) and Greek (DMV/Zakinthos/0991) isolates of DMV

Designation of clone	Specificity	PDV-1	DMV Spain (n = 2)	DMV Italy (n = 2)	DMV Greece (n = 1)
1.064 C 5	NP 1	+	−	−	−
1.071 E 2	NP 1	+	−	−	−
1.069 G 2	NP 2	+	−	−	−
1.062 G 5	H 1	+	−	−	−
1.063 C 3	H 1	+	−	−	−
1.063 E 9	H 1	+	−	−	−
1.069 D 9	H 1	+	−	−	−
1.071 E 5	H 1	+	−	−	−
1.067 E 5	H 2	+	−	−	−
1.068 F 2	H 3	+	−	−	−
1.070 B 5	H 4	+	−	−	−
1.072 C 4	H 4	+	−	−	−
1.085 C 4	H 5	+	−	−	−
1.122 D 11	H 6	+	−	−	−
1.062 E 2	F 1	+	−	−	−
1.068 B 2	F 1	+	−	−	−
1.067 D 2	F 2	+	+	+	+
1.073 G 10	F 3	+	+	+	+
1.092 C 3	F 3	+	+	+	+

+ Reactivity in the assay, − no reactivity in the assay

the relatively low levels of antibodies demonstrated in the ELISA vs. VN antibodies may have been caused by a limited reactivity of protein A for Ig of cetacean origin. The presence of morbillivirus neutralizing and ELISA-reactive antibodies in a common dolphin indicated that this animal had also suffered from a morbillivirus infection. Unfortunately, from this animal no materials were available for antigen detection or virus isolation. The limited number of samples from other cetacean species makes it difficult to conclude whether they are also susceptible to DMV infection. Our success in isolating DMV in lymphocytes from a bottlenose dolphin suggests that other cetacean species could be infected by DMV. However, although the striped dolphin can be seen swimming together with other cetaceans like the common dolphin [9] and the short-finned pilot whale (*Globicephala macrorhynchus*) [10], the die-off was so far only reported to affect the endangered Mediterranean striped dolphin [3]. Besides a possible higher susceptibility to DMV infection and DMV associated disease development, the tendency of the striped dolphin to form groups of dozens of animals [2] may have favoured the spread of the infection in this species. The high mortality rate during the die-off indicates that DMV had entered a non-immune population [4].

Speculations that the recent morbillivirus related mortality in striped dolphins was the result of transmission of PDV-1 from seals to dolphins can now be contradicted on the basis of the antigenic differences between these two viruses. Also the natural tendency of the morbillivirus to be confined to one mammalian order, and the biological differences shown between PDV-1 and DMV [20] indicate that there has been no etiological link between the PDV-1 and DMV related outbreaks.

The identification of at least two new members of the genus *Morbillivirus* causing devastating outbreaks among aquatic mammals raises questions about the origin of these viruses, the reason for their sudden appearance and their antigenic or genetic relationships with the other members of the genus. These questions are subject of our present investigations in this field.

Acknowledgements

We kindly acknowledge J. S. Teppema for the electron microscopy, R. Basso, A. Bortolotto, L. Casini, A. Di Natale, G. Gnone, M. G. Hartmann, the Italian Centre for Cetacean Studies, the Italian Merchant Ministry, V. Kouroutos, L. Lino, C. Meotti, E. Tounta, L. Vedder and V. Zavras, for their help in collecting the samples, Dr. P. J. H. Van Bree for the identification of the Cuvier's beaked whale, A. Vecchione for her appreciated assistance during the processing of the samples, and C. Kruyssen for help in preparing the manuscript.

References

1. Aguilar A (1990) Dolphin die-off in the Western Mediterranean. ECS Newslett 9: 4
2. Anonymous (1990) Technical report on the situation of small cetaceans in the mediterranean and the impact of fishing gear and fishery practices on these animals. Greenpeace International, November 1990
3. Anonymous (1991) Report on the workshop on the mortality in passive fishing nets

and traps, La Jolla, California, USA, 22–25 October 1990. International Whaling Commission Report
4. Black F (1991) Epidemiology of paramyxoviruses. In: Kingsburry D (ed) The paramyxoviruses. Plenum, New York, pp 509–536
5. Bompart JM, Dhermain F, Poitevin F, Cheylan M (1991) Les dauphins méditerranées victimes d'un virus mortel. La Recherche 231: 506–508
6. Bortolotto A, Casini L, Stanzani LA (1992) Dolphin mortality along the Southern Italian coast. Aquatic Mammals (in press)
7. De Vries P, Osterhaus ADME (1992) Vaccines against morbillivirus infections. In: Höglund S, Pandey R (eds) Progress in vaccinology, vol 4: veterinary vaccines. Springer, Berlin Heidelberg New York Tokyo (in press)
8. Domingo M, Ferrer L, Pumarola M, Marco A, Plana J, Kennedy S, McAliskey M, Rima BK (1990) Morbillivirus in dolphins. Nature 348: 21
9. Ellis R (1989) Dolphins and porpoises. AA Knopf, New York
10. Heimlich-Boran JR, Heimlich-Boran SL (1990) Occurrence and group structure of the short-finned pilot whale *Globicephala macrorhynchus* off the Western coast of Teneriffe, Canary Island. In: Evans PGH, Aguilar A, Mink C (eds) Proceedings of the fourth annual conference of the European Cetacean Society, Palma de Majorca 1990. European Cetacean Society, Cambridge, England, pp 102–103
11. Kennedy S (1991) A review of the 1991 European seal morbillivirus epizootic. Vet Rec 8: 563–567
12. Kennedy S, Smyth JA, Cush PF, McCullogh SJ, Allan GM, McQuaid S (1988) Viral distemper now found in porpoises. Nature 336: 21
13. Kovamees J, Blixenkrone-Möller M, Sharma B, Örvell C, Norrby E (1991) The nucleotide sequence and deduced amino acid composition of the haemaglutinin and fusion proteins of the morbillivirus phocid distemper virus. J Gen Virol 72: 2959–2966
14. Lindmark R, Thoren-Tholling K, Sjoquist J (1983) Binding of immunoglobulins to Protein A and immunoglobulin levels in mammalian sera. J Immunol Methods 62: 1–13
15. Mahy BWJ, Barrett T, Evans SA, Anderson EC, Bostock CJ (1988) Characterization of a seal morbillivirus. Nature 336: 115
16. Örvell C, Blixenkrone-Möller M, Svansson V, Have P (1990) Immunological relationships between phocid and canine distemper virus studied with monoclonal antibodies. J Gen Virol 71: 2085–2092
17. Örvell C, Shesheberadaran H, Norrby E (1985) Preparation and characterization of monoclonal antibodies directed against four structural components of canine distemper virus. J Gen Virol 66: 443–456
18. Osterhaus ADME, Groen J, Spijkers HEM, Broeders HWJ, UtydeHaag FGCM, De Vries P, Teppema JS, Visser IKG, Van de Bildt MWG, Vedder EJ (1990) Mass mortality in seals caused by a newly discovered morbillivirus. J Vet Microbiol 23: 343–350
19. Osterhaus ADME, Vedder EJ (1988) Identification of virus causing recent seal deaths. Nature 335: 20
20. Osterhaus ADME, Visser IKG, De Swart RL, Van Bressem MF, Van de Bildt MWG, Örvell C, Barrett T, Raga JA (1992) Morbillivirus threat to Mediterranean monk seals? Vet Rec 130: 141–142
21. Pringle CR (1991) Paramyxoviridae. In: Francki RIB, Fauquet CM, Knudson DL, Brown F (eds) Classification and nomenclature of viruses. Fifth report of the international committee on taxonomy of viruses. Springer, Wien New York (Arch Virol [Suppl] 2: 242–246)
22. Van Bressem MF, Visser IKG, Van de Bildt MWG, Teppema JS, Raga JA, Osterhaus ADME (1991) Morbillivirus infection in Mediterranean striped dolphins (*Stenella coeruleoalba*). Vet Rec 129: 471–472

23. Visser IKG, Kumarev VP, Örvell C, De Vries P, Broeders HWJ, Van de Bildt MWG, Groen J, Teppema JS, Burger MC, UytdeHaag FGCM, Osterhaus ADME (1990) Comparison of two morbilliviruses isolated from seals during outbreaks of distemper in North West Europe and Siberia. Arch Virol 111: 149–164
24. Visser IKG, Van de Bildt MWG, Brugge HN, Reijnders PJH, Vedder EJ, Kuiper J, De Vries P, Groen J, Walvoort HC, UytdeHaag FGCM, Osterhaus ADME (1989) Vaccination of harbour seals (*Phoca vitulina*) against phocid distemper with two different inactivated canine distemper virus (CDV) vaccines. Vaccine 7: 521–526

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Received May 15, 1992