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An insight into the epidemiology of dolphin morbillivirus worldwide

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Abstract

Serum samples from 288 cetaceans representing 25 species and originating from 11 different countries were collected between 1995 and 1999 and examined for the presence of dolphin morbillivirus (DMV)-specific antibodies by an indirect ELISA (iELISA) ($N = 267$) or a plaque reduction assay ($N = 21$). A total of 35 odontocetes were seropositive: three harbour porpoises (*Phocoena phocoena*) and a common dolphin (*Delphinus delphis*) from the Northeastern (NE) Atlantic, a bottlenose dolphin (*Tursiops truncatus*) from Kent (England), three striped dolphins (*Stenella coeruleoalba*), two Risso's dolphins (*Grampus griseus*) and a bottlenose dolphin from the Mediterranean Sea, one common dolphin from the Southwest (SW) Indian Ocean, three Fraser's dolphins (*Lagenodelphis hosei*) from the SW Atlantic, 18 long-finned pilot whales (*Globicephala melas*) and a bottlenose dolphin from the SW Pacific as well as a captive bottlenose dolphin (*Tursiops aduncus*) originally from Taiwan. The presence of morbillivirus antibodies in 17 of these animals was further examined in other iELISAs and virus neutralization tests. Our results indicate that DMV infects cetaceans worldwide. This is the first report of DMV-seropositive animals from the SW Indian, SW Atlantic and West Pacific Oceans. Prevalence of DMV-seropositives was 85.7% in 21 pilot whales from the SW Pacific and both sexually mature and immature individuals were infected. This indicates that DMV is endemic in these animals. The same situation may occur among Fraser's dolphins from the SW Atlantic. The prevalence of DMV-seropositives was 5.26% and 5.36% in 19 common dolphins and 56 harbour porpoise from the NE Atlantic, respectively, and 18.75% in 16 striped dolphins from the Mediterranean. Prevalence varied significantly with sexual maturity in harbour porpoises and striped dolphins; all DMV-seropositives being mature animals. The prevalence of seropositive harbour porpoise and striped dolphins appeared to have decreased since previous studies. These data suggest that DMV is not endemic within these populations, that they are losing their humoral immunity against the virus and that they may be vulnerable to new epidemics.

Keywords: Dolphin morbillivirus; Cetaceans; Epidemiology; Serology; Conservation

1. Introduction

The dolphin and porpoise morbilliviruses (DMV and PMV) are strains of a recently recognized member of the genus *Morbillivirus* (family *Paramyxoviridae*) (Barrett et al., 1993a) which was called cetacean morbillivirus (CeMV) (Bolt et al., 1994; Blixenkrone-Møller et al., 1996). Other morbilliviruses and their respective natural hosts are measles virus (MV) in humans, rinderpest virus (RPV) and peste des petits ruminants virus (PPRV) in artiodactyls, and canine and phocine distemper viruses (CDV and PDV) in carnivores. DMV and PMV are more closely related to the ruminant morbilliviruses and MV than to the distemper viruses (Visser et al., 1993; Barrett et al., 1993a; Blixenkrone-Møller et al., 1994, 1996; Haffar et al., 1999). All members of this genus require large populations of individuals (e.g. 300 000 for measles virus in humans) to be maintained endemically (Black, 1991).

The dolphin and porpoise morbilliviruses can cause serious, potentially lethal diseases in cetaceans. DMV was responsible for the deaths of thousands of striped dolphins (*Stenella coeruleoalba*) in the Mediterranean Sea in 1990–1992 (Domingo et al., 1990;

Van Bressem et al., 1991, 1993). PMV caused mortalities in harbour porpoises (*Phocoena phocoena*) along the coasts of Ireland and The Netherlands in 1988–1990 (McCullough et al., 1991; Visser et al., 1993). Both PMV and DMV gave rise to fatal epidemics in bottlenose dolphins (*Tursiops truncatus*) from the Northwestern (NW) Atlantic in the periods 1987–1988 and 1993–1994 (Lipscomb et al., 1994; Krafft et al., 1995; Duignan et al., 1996; Taubenberger et al., 1996). In addition, DMV, PMV or antigenically closely related viruses are probably endemic in several species of cetaceans from the NW Atlantic and the East Pacific (Peru and California) (Duignan et al., 1995a,b; Van Bressem et al., 1998a; Reidarson et al., 1998). Some species are likely to be reservoirs of infection and may act as vectors to spread the viruses to others (Duignan et al., 1995a; Van Bressem et al., 1998b).

Further information about geographic and host population distribution of cetacean morbillivirus worldwide as well as about its current epidemiological status in cetacean populations may be crucial for the management of populations suffering high additional, non-natural, mortalities in fisheries or under pressure from other human activities. Indeed, it has recently been suggested that the synergistic interactions between mortalities in fisheries and morbillivirus epizootics could significantly reduce the numbers of individuals of some populations and increase their risk of extinction (Van Bressem et al., 1999). High levels of organochlorine contaminants in marine mammal populations may reduce host immune resistance and contribute to the severity of morbillivirus outbreaks (Aguilar and Borrell, 1994; Ross et al., 1996).

In this context, we have conducted a serological survey among 288 cetaceans originating from the Northeast (NE) and Southwest (SW) Atlantic Oceans, the North and Mediterranean Seas, the NE and West Pacific and the SW Indian Oceans.

2. Material and methods

2.1. Samples

Blood samples were collected in the period 1995–1999 from 288 cetaceans belonging to 25 species caught off, or stranded along the coasts of the British Isles, the Iberian Peninsula, British Columbia (Canada), Brazil, Argentina, South Africa, Indonesia, Taiwan, Japan, Australia and New Zealand (Table 1). The animals from Taiwan, Indonesia and Japan were in captivity at the time of sampling. They had been caught in 1978 (two bottlenose dolphins (*Tursiops aduncus*)), 1987 (four *T. aduncus* and one false killer whale (*Pseudorca crassidens*)) and 1997 (five *T. aduncus*). A striped dolphin from the Mediterranean Sea was alive at the time of sampling. The condition of the other animals varied from fresh to decomposed, and all blood samples obtained from them were hemolyzed.

As harbour porpoises from the North Sea are considered a population distinct from porpoises in NW Scottish, Irish and western British waters and the Celtic shelf (Donovan and Bjørge, 1995), we divided porpoises from the UK in two ocean provinces: the North Sea and NE Atlantic. The latter includes the English Channel, the Irish and Celtic Seas. The common dolphins sampled along the coasts of Europe were assumed to be *Delphinus*

Table 1
Locality of origin and year of sampling as well as species and number of cetaceans examined for the presence of morbillivirus serum antibodies

Country	Species	Year						Total
		1995	1996	1997	1998	1999	Unknown	
British Isles (NE Atlantic and North Sea)								
	Balaenoptera acutorostrata		1			1		2
	Delphinus delphis	1	2	4	8	4		19
	Globicephala melas			1				1
	Grampus griseus					1		1
	Lagenorhynchus acutus				1			1
	L. albirostris		1		1			2
	Mesoplodon bidens				1			1
	Phocoena phocoena	3 [1[a]/2[b]]	23 [13[a]/10[b]]	36 [13[a]/23[b]]	32 [19[a]/13[b]]	22 [10[a]/12[b]]		116
	Stenella coeruleoalba	1	2		1	1		5
	Tursiops truncatus					2		2
Iberian Peninsula (NE Atlantic)								
	B. acutorostrata					1		1
	D. delphis.					2		2
	G. melas					1		1
	G. griseus					1		1
	P. phocoena					5		5
	T. truncatus					1		1
Iberian Peninsula (Mediterranean)								
	Balaenoptera physalus			1				1
	D. delphis				1			1
	G. griseus			1	1	2		4
	S. coeruleoalba			6	8	2		16
	T. truncatus			1	1			2
British Columbia (NE Pacific)								
	Lagenorhynchus obliquidens		1			4		5
	P. phocoena		2		1		1	4
	Phocoenoides dalli		1					1
	Orcinus orca				1			1

South Africa (SW Indian Ocean)						
	D. delphis				8	8
	Lagenodelphis hosei				1	1
	Sousa chinensis				4	4
	Stenella attenuata				1	1
	S. coeruleoalba				1	1
	T. truncatus				2	2
	G. griseus				3	3
	Kogia breviceps			1		1
	Mesoplodon mirus				1	1
Brazil (SW Atlantic)						
	L. hosei		1		1	2
	Pontoporia blainvillei	1	6	6	1	14
	Sotalia fluviatilis		1	4		5
	Stenella frontalis	1	4		1	6
Argentina (SW Atlantic)						
	L. hosei				2	2
{Indonesia (W Pacific)}[c]						
	Tursiops aduncus				9	9
{Taiwan (NW Pacific)}[c]						
	T. aduncus				2	2
{Japan (NW Pacific)}[c]						
	Pseudorca crassidens				1	1
New Zealand (SW Pacific)						
	G. melas		21			21
Australia (SW Pacific)						
	T. truncatus		5	1		6
	O. orca		1			1
	Phocoena dioptrica			1		1

[*] In the case of harbour porpoises from the British isles, [a] Indicate the number of animals originating from the NE Atlantic, [b] Indicate the number of animals originating from the North Sea and [c] The brackets { } indicate that the dolphins were in captivity at the time of sampling.

delphis with the caveat that specimens whose specific identity was not positively determined could also be long-beaked common dolphins (*Delphinus capensis*). Common dolphins from the SW Indian Ocean are designated as *D. delphis* following Ross (1984) and Peddemors (1999). Sexual maturity was determined directly from an examination of gonads and lactation or was inferred from standard body length (SL) and known life history parameters for these populations (Collet and Saint Girons, 1984; Lockyer, 1995; Calzada, 1995). Sexual maturity of two Risso's dolphins (*Grampus griseus*) and a bottlenose dolphin from the Mediterranean Sea was inferred from Raga's unpublished observations. Sexually mature harbour porpoises from the UK are regarded as being more than 3 to 4 years old (Lockyer, 1995). Sexually mature female and male striped dolphins from the Mediterranean Sea are considered to be more than 12 and 11 years old (Calzada, 1995). The age of some animals was determined by standard techniques (Perrin and Myrick, 1980; Hohn et al., 1989; Crespo et al., 1994).

2.2. Serological tests

With the exception of 21 serum samples from New Zealand, all samples were tested for the presence of DMV-specific antibodies on a coat of DMV antigen in an indirect enzyme-linked immunosorbent assay (iELISA) previously described (Van Bressem et al., 1998a). This assay allows the detection of morbillivirus specific-antibodies in hemolyzed sera which may be cytotoxic and as such might prevent the detection of antibodies at low dilutions in virus neutralization tests (Van Bressem et al., 1998a,b). In summary, Vero cell antigen was used as a negative control for each individual serum to overcome high background values. Cetacean immunoglobulins were detected by horseradish-peroxidase conjugated protein A (Sigma, England), a cell wall constituent of *Staphylococcus aureus* which binds non-specifically the immunoglobulins of several species of vertebrates including cetaceans (Lindmark et al., 1983; Van Bressem et al., 1993, 1998a,b). After addition of the chromogen substrate and the development of color, the reaction was stopped by the addition of a sulphuric acid solution (2 M) and the resulting optical density (OD) was read at 492 nm. Serum samples were considered positive when their OD on morbillivirus antigen equalled or exceeded twice their own OD on the negative control antigen at dilutions equal or greater than 20. To further confirm that the DMV–iELISA seropositive cetaceans had been infected by a morbillivirus, their sera were tested on a coat of PPRV and RPV antigen in the iELISA and examined for the presence of DMV and PPRV neutralizing antibodies in a neutralization assay using approximately 100 tissue culture infective dose (TCID) per well and serial two-fold dilutions (starting at 1:20) of the sera (Visser et al., 1993; Van Bressem et al., 1993). Sera from 21 long-finned pilot whales (*Globicephala melas*) stranded in New Zealand in 1997 (Table 1) were independently tested for the presence of DMV antibodies by a modified plaque reduction (PR) assay previously described (Duignan et al., 1997; Nielsen et al., 2000). This test has been shown to be an acceptable method for detecting morbillivirus antibodies in hemolyzed blood (Duignan et al., 1997) and, though it appears to be less sensitive than the VN test, does provide a means of detecting antibodies in samples otherwise unsuitable for the VN assay (Duignan et al., 1997; Nielsen et al., 2000). Briefly, 100 μl of each serum dilution was mixed with the same volume of a DMV suspension containing

approximately 200 plaque forming units (PFU) and incubated for 1 h at 37°C. One hundred μl of these aliquots were subsequently inoculated onto duplicate confluent Vero cell monolayers grown in tissue culture dishes (60mm × 15 mm). Plaque reduction was evaluated after 8 days and titres were expressed as the reciprocal of the highest dilution of sample that gave an 80% reduction in the number of plaques compared to the control plates (virus alone) (Habel, 1969). Antibody titres greater than 16 were considered positive.

2.3. Statistics

Samples size was sufficiently large for statistical analysis for five groups: harbour porpoises from the North Sea and NE Atlantic, common dolphins from Great Britain, striped dolphins from the Mediterranean Sea and pilot whales from New Zealand. To determine whether the prevalence of DMV-seropositives was related to the age of the animals, we divided them into sexually immature (calves and juveniles) and mature individuals. To examine temporal trends in the prevalence of DMV-seropositive individuals in these populations, we compared our results with those obtained in previous studies (Van Bressem, 1997; Van Bressem et al., 1998b). However, results of these comparisons should be regarded with caution as the techniques used to detect morbillivirus-specific antibodies or to determine sexual maturity differed between studies (see below). Significance of differences in prevalence ($\alpha = 0.05$ level) was verified with chi-square contingency tests or one-tailed Fisher's exact tests (Swinscow, 1981). Sexual variation in prevalence was verified independently for immature and mature subsamples.

3. Results

3.1. Northeast Atlantic and North Sea

Three harbour porpoises, a common dolphin and a bottlenose dolphin, all mature animals stranded along the coasts of Great Britain in 1996–1999, had specific antibodies against the dolphin morbillivirus with titres ranging from 20 to 80 (Table 2) in the iELISA. Two of the positive porpoises also had antibodies against PPRV but all animals were negative on a coat of RPV antigen. DMV and PPRV neutralizing antibodies were detected in the sera of three and two porpoises, respectively (Table 2). Sera were often toxic at dilutions less than 40, preventing the detection of low titres of virus neutralizing (VN) antibodies (Table 2).

The prevalence of iELISA DMV-antibodies seropositives was 5.26% (1/19) in the common dolphins and 5.36% (3/56) in porpoises from the NE Atlantic. None of the 60 porpoises from the North Sea had antibodies against DMV (Table 3). DMV-seropositives were only found among sexually mature animals even though immature dolphins and porpoises represented 52.6% (common dolphin) and 67.9% (harbour porpoises from the NE Atlantic) of the sample (Table 3). For subsamples of mature animals, there was no significant variation in prevalence of seropositives based on gender (porpoises from the NE Atlantic: $\chi^2 = 0.4$, $df = 1$, $P = 0.52$; common dolphins: $\chi^2 = 0.32$, $df = 1$,

Table 2
Presence of morbillivirus-specific antibodies in the sera of small cetaceans kept in captivity (C), or stranded (S) in eight countries in 1996–1999 as detected by iELISA on a coat of dolphin morbillivirus (DMV), peste des petits ruminants (PPRV) and rinderpest virus (RPV) as well as by virus neutralization (VN) tests against DMV and PPR or by a plaque reduction (PR) assay using DMV

Country	Code	Species	Sex	SL (cm)	SM	Age	D	M	Y	Locality	Ocean province	C/S	iELISA antibody titer on			VN antibody titre against		PR titre against
													DMV ag	PPRV ag	RPV ag	DMV	PPRV	DMV
United Kingdom																		
	SW1998/104	*Delphinus delphis*	F	208	Mat		10	6	1998	Dyfed	Northeast Atlantic	S	40	0	0	<40[c]	0	–
	SW1996/107	*Phocoena phocoena*	F	171	Mat		24	6	1996	Dyfed	Northeast Atlantic	S	80	40	0	40	20	–
	SW1997/55	*P. phocoena*	F	168	Mat		3	3	1997	Gwynedd	Northeast Atlantic	S	40	0	0	40	<40[c]	–
	SW1997/116	*P. phocoena*	M	160	Mat		23	7	1997	Pembrokeshire	Northeast Atlantic	S	40	20	0	40	20	–
	SW1999/197	*Tursiops truncatus*	F	320	Mat		15	11	1999	Kent	Channel/North Sea	S	20	0	0	<80[c]	<40[c]	–
Spain																		
	Gg 970121	*Grampus griseus*	F	304	[Mat][a]		21	1	1997	Valencia	Western Mediterranean	S	20	0	0	20	0	–
	Gg 990512	*G. griseus*	M	308	[Mat][a]		12	5	1999	Valencia	Western Mediterranean	S	80	20	0	<40[c]	<40[c]	–
	Sc 970130	*Stenella coeruleoalba*	F	198	Mat		30	1	1997	Valencia	Western Mediterranean	S	40	0	0	0	<40[c]	–
	Sc 970308	*S. coeruleoalba*	F	188	[Mat][a]		8	3	1997	Alicante	Western Mediterranean	S	20	20	0	<40[c]	0	–
	Sc 980305	*S. coeruleoalba*	M	196	[Mat][a]		5	3	1998	Alicante	Western Mediterranean	S	40	320	20	40	0	–
	Tt 970115	*T. truncatus*	F	298	[Mat][a]		15	1	1997	Alicante	Western Mediterranean	S	80	≥640	0	≥160	40	–
Brazil																		
	#3	*Lagenodelphis hosei*	M	220			3	9	1999	Arraial do Cabo	Southwest Atlantic	S	160	1280	80	80	0	–
Argentina																		
	LH1	*L. hosei*	F	231		~20	18	3	1999	Puerto Madryn	Southwest Atlantic	S	80	20	0	160	40	–
	LH2	*L. hosei*	M	237.5	Mat	~18	27	4	1999	Puerto Madryn	Southwest Atlantic	S	80	20	20	80	20	–
South Africa																		
	2784	*Delphinus delphis*	F	215	Mat		28	7	1999	East London	Southwest Indian Ocean	S	80	0	0	0	0	–
Taiwan																		
	Gordina	*Tursiops aduncus*	F	246	Mat	~35	11	11	1999	{Penghu}[b]	Northwest Pacific	C	640	160	80	160	80	–
Australia																		
	DBO-7	*T. truncatus*	M	248	Mat		15	4	1997	Tasmania	Southwest Pacific	S	80	≥2560	160	80	20	–
New Zealand																		
	WS97-27Gm	*Globicephala melas*	F	430	Mat		9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	32
	WS97-28Gm	*G. melas*	F	440	Mat		9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	32
	WS97-29Gm	*G. melas*	M	325	Imm		9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	256
	WS97-30Gm	*G. melas*	F	233	Imm		9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	256

WS97-31Gm	*G. melas*	M	321	Imm	9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	256
WS97-32Gm	*G. melas*	F	379	Mat	9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	128
WS97-33Gm	*G. melas*	F	269	Imm	9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	128
WS97-34Gm	*G. melas*	F	328	Imm	9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	256
WS97-37Gm	*G. melas*	M	310	Imm	9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	256
WS97-38Gm	*G. melas*	M	290	Imm	9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	128
WS97-39Gm	*G. melas*	M	510	Mat	9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	64
WS97-40Gm	*G. melas*	M	430	Imm	9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	256
WS97-41Gm	*G. melas*	F	414	Mat	9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	32
WS97-42Gm	*G. melas*	F	320	Imm	9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	32
WS97-43Gm	*G. melas*	F	430	Mat	9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	64
WS97-44Gm	*G. melas*	F	435	Mat	9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	32
WS97-45Gm	*G. melas*	M	430	Imm	9	10	1997	Northland	Southwest Pacific	S	–	–	–	–	–	> 16
WS97-48Gm	*G. melas*	M		Imm	30	10	1997	Firth of Thames	Southwest Pacific	S	–	–	–	–	–	128

Acronyms used are: SL = standard body length, SM = sexual maturity, Mat = mature, Imm = immature.

[a] The brackets [ ] indicate that sexual maturity was inferred from SL.

[b] The brackets { } indicate that the animal was in captivity at the time of sampling.

[c] Indicates that the sera were toxic at lower dilutions.

Table 3
Number (*N*) of sexually immature and mature striped dolphins (*S. coeruleoalba*) from the Mediterranean Sea (Iberian Peninsula), harbour porpoises (*P. phocoena*) and common dolphins (*D. delphis*) from the North Sea and NE Atlantic (British Isles) as well as long-finned pilot whales (*G. melas*) from the SW Pacific (New Zealand) tested and/or positive (*N* positive) for the presence of dolphin morbillivirus-specific serum antibodies in the iELISA or PR assay

Species	Mediterranean Sea						North Sea						Northeast Atlantic						Southwest Pacific					
	Immatures			Matures			Immatures			Matures			Immatures			Matures			Immatures			Matures		
	N	*N* positive	% positive	*N*	*N* positive	% positive	*N*	*N* positive	% positive	*N*	*N* positive	% positive	*N*	*N* positive	% positive	*N*	*N* positive	% positive	*N*	*N* positive	% positive	*N*	*N* positive	% positive
S. coeruleoalba	10	0	0	6	3	50																		
D. delphis							0	0	0	0	0	0	10	0	0	9	1	11.1						
P. phocoena							35	0	0	25	0	0	38	0	0	18	3	16.7						
G. melas																			14	11	78.6	7	7	100

$P = 0.57$), with the caveat of small sample sizes. Thus, the sexes were pooled for subsequent analyses. Variation in seroprevalence between sexually mature and immature individuals was significant in the porpoises (Fisher's exact test, $P = 0.029$) but not in the common dolphins (Fisher's exact test, $P = 0.47$). Mature porpoises from the Atlantic were more likely to be seropositive (16.7%) than those from the North Sea (0%) but statistical significance was borderline (Fisher's exact test, $P = 0.066$; $\chi^2 = 4.48$, d$f = 1$, $P = 0.034$). Prevalence of DMV–iELISA seropositive, mature porpoises from the North Sea decreased from 10% (1/10) between the period April 1991–April 1996[1] (Van Bressem, Jepson and Barrett, unpublished data) to 0% ($N = 21$[2]) in June 1996–March 1999. Similarly, the prevalence of DMV–iELISA seropositive, mature porpoises from the NE Atlantic declined from 25% (7/28) in the period February 1991–January 1996 (Van Bressem, Jepson and Barrett, unpublished data) to 16.7% in June 1996–August 1999. However, these apparent declines were not significant (North Sea: Fisher's exact test, $P = 0.32$; NE Atlantic: $P = 0.38$).

None of the 16 individuals belonging to seven other species sampled around the British Isles and the Iberian Peninsula (Table 1), were seropositive in the DMV–iELISA.

3.2. Mediterranean Sea

Three striped dolphins, two Risso's dolphins and a bottlenose dolphin, all mature animals stranded along the coasts of Valencia and Alicante in 1997–1999, had specific-antibodies against the dolphin morbillivirus in the iELISA with titres ranging from 20 to 80 (Table 2). Four animals had antibodies against PPRV and one striped dolphin also had antibodies against RPV in this assay. DMV and PPRV neutralizing antibodies were observed in three and one dolphin, respectively (Table 2).

Overall prevalence of seropositive striped dolphins was 18.75% (3/16). Only mature striped dolphins were positive, although more immature animals were examined (Table 3). There was no sexual variation in prevalence of seropositives in the mature striped dolphin subsample ($\chi^2 = 1.2$, d$f = 1$, $P = 0.27$), with the caveat of small samples. Hence, sexes were pooled for subsequent analysis. The variation in seroprevalence between sexually mature and immature striped dolphins was significant (Fisher's exact test, $P = 0.036$). Prevalence of DMV-seropositive, mature striped dolphins appeared to have decreased from 100% ($N = 8$[3]) during the 1990–1992 epidemic (Van Bressem, 1997) to 50% ($N = 6$) in 1997–1999 ($P = 0.055$).

A fin whale (*Balaenoptera physalus*) and a common dolphin found beached on the coasts of Alicante and Castellón in 1997 and 1998, respectively, were negative in the DMV–iELISA.

[1] The sexual maturity of the porpoises from the North Sea and NE Atlantic stranded in the period February 1991–April 1996 was inferred from standard body length (SBL) and known life history parameters (Lockyer, 1995; Van Bressem et al., 1998a) while that of all but one porpoises of this study was determined directly.

[2] Four mature porpoises stranded before April 1996 were excluded from this sample explaining the difference with Table 3.

[3] The sera of these dolphins were screened for DMV-specific antibodies in a neutralization test.

3.3. Southwestern Atlantic

Three of four Fraser's dolphins (*Lagenodelphis hosei*), that had stranded in 1997 and 1999 along the coasts of Argentina and Brazil had antibodies against DMV in the iELISA with titres ranging from 80 to 160. These sera also reacted with PPRV antigen and, except for one individual, with RPV antigen. All had DMV neutralizing antibodies and the two dolphins from Argentina (older than 15 years) also had PPRV neutralizing antibodies (Table 2).

None of the 14 franciscanas (*Pontoporia blainvillei*), five tucuxis (*Sotalia fluviatilis*) or six Atlantic spotted dolphins (*Stenella frontalis*) incidentally caught in fisheries off Brazil (Table 1) were positive in the DMV–iELISA.

3.4. Indian Ocean

Of the 22 individuals of nine species tested from the SW Indian Ocean (Table 1), only one, a mature common dolphin, had DMV-specific serum antibodies (Table 2) in the iELISA. This serum was negative in the other tests. However, it showed some neutralizing activity against PPRV at dilution 1:20. The serum of her calf was negative in all tests but showed the same incomplete neutralizing activity against PPRV at 1:20 (Table 2).

3.5. Western Pacific

In all, 18 of 21 (85.7%) long-finned pilot whales from New Zealand had antibodies against DMV in the PR assay with titres ranging from 32 to 256 (Table 2). Adults of both sexes were all positive. There was no sexual variation in prevalence of DMV-seropositives among immature whales (Fisher's exact test, $P = 0.39$) and hence, sexes were pooled for ensuing analysis. Variation in seroprevalence between sexually mature and immature individuals was not significant (Fisher's exact test, $P = 0.27$). The three negative whales were calves, one of them a neonate. DMV-antibodies were not detected in serum of a foetus from a positive whale. All animals but the negative neonate and a positive immature male were from a single mass stranding that included 102 animals in total (P.J. Duignan, unpublished data).

Two mature bottlenose dolphins from Taiwan and Tasmania, respectively, had serum antibody titres against DMV in the iELISA. They were also positive on a coat of PPRV and RPV antigen and had neutralizing antibodies against DMV and PPRV (Table 2). One of them, caught off Taiwan in 1978 and kept in captivity since then, already had a high (320) DMV neutralization titre in 1994 (Duignan, J.R. Geraci and Kinoshita, unpublished data). Apart from an inflammatory blood profile in the early 1980s and episodes of elevated liver enzymes and anorexia, to the best of our knowledge, it had not experienced any serious disease during its captivity. None of 11 other dolphins (10 bottlenose dolphins and a false killer whale) from the same facility had antibodies against DMV in the iELISA test though all of them could have had some contact with this dolphin, at some point in time, through sharing of the same pool enclosure. The second bottlenose dolphin with DMV-specific serum antibodies had stranded in Marion Bay, Tasmania in 1997

together with 24 other members of a pod of offshore bottlenose dolphins chased ashore by killer whales (*Orcinus orca*). This animal was apparently healthy at the time of death (K. Evans, unpublished data). The other four dolphins which died during that incident as well as three other cetaceans which stranded in this region in the period 1997–1998 (Table 1) were negative in the DMV–iELISA.

3.6. Northeastern Pacific

None of 11 odontocetes belonging to four species and sampled along the coast of British Columbia (Table 1) were seropositive in the DMV–iELISA.

4. Discussion

The iELISA and the plaque reduction assay used in this study were developed to detect morbillivirus-specific antibodies in poor quality sera which may be cytotoxic and as such prevent the detection of antibodies at low dilutions in VN tests (Duignan et al., 1997; Van Bressem et al., 1998a). Reactivity of the DMV positive sera with PPRV and RPV antigen has been previously observed (Van Bressem et al., 1998a,b) and is most likely due to the close antigenic relationship between those viruses (Barrett et al., 1993a; Visser et al., 1993; Haffar et al., 1999). The higher titres against PPRV than against DMV in the iELISA in four of 17 animals are difficult to explain. They may reflect different reactivities to particular epitopes in some members of those outbred populations to an epitope not present, or hidden, in the DMV strain used for the iELISA. On the other hand, these results may reflect the existence of different strains of cetacean morbillivirus some of which may be antigenically closer to PPRV than others. Characterization of virus isolates from two outbreaks of mortalities in Mediterranean striped dolphins and harbour porpoises from the NE Atlantic and North Sea has shown that they represent two strains of cetacean morbillivirus, i.e. PMV and DMV (McCullough et al., 1991; Van Bressem et al., 1991, 1993; Barrett et al., 1993a; Visser et al., 1993; Bolt et al., 1994). Sequence data obtained from the tissues of bottlenose dolphins and a striped dolphin that died during two episodes of mortalities along the Atlantic coasts of the United States indicated that the same strains had also infected these animals (Taubenberger et al., 1996). Analysis of RPV from more than 30 different outbreaks showed that they form three distinct lineage groups (Barrett et al., 1998). Similarly, sequencing of viruses from more than 15 outbreaks of PPRV revealed that they group into four distinct lineages (Shaila et al., 1996). It is possible, therefore, that multiple lineages of cetacean morbillivirus also circulate in odontocetes and mysticetes. The recent recovery of sequence data from another morbillivirus, related to, but distinct from, PMV and DMV, from the tissues of a long-finned pilot whale from the NW Atlantic (Taubenberger et al., 2000) argues in favor of this hypothesis. The iELISA allows the detection of antibodies directed against the N, P, F and H morbillivirus proteins whereas only antibodies to the surface glycoproteins (F and H) are detected by the VN assays (Barrett et al., 1993b). This may explain why some sera positive in the iELISAs were negative in the VN tests.

DMV-specific antibodies were detected in the sera of eight species of odontocetes from the NE Atlantic (including North Sea), Mediterranean Sea, SW Indian Ocean, SW Atlantic and West Pacific but not the NE Pacific. Cetacean morbillivirus infection has also been previously detected in the NW Atlantic and East Pacific (Duignan et al., 1995a,b, 1996; Reidarson et al., 1998; Van Bressem et al., 1998a). Collectively, these data demonstrate that DMV or other strains of cetacean morbillivirus infect cetaceans worldwide. It is the first time that cetaceans with DMV antibodies have been found in the Indian Ocean, South Atlantic and West Pacific. A morbillivirus probably caused a non-purulent meningo-encephalomyelitis in a Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) from Japan but the presence of serum DMV-specific antibodies in this animal was not examined (Uchida et al., 1999). Five seropositive dolphins from the Southern Hemisphere had high titres ($\geq$80) against DMV in the iELISA and two of them had also high titres against PPRV and RPV in the same test. High titres ($\geq$64) against DMV were also observed in 12 pilot whales from New Zealand as determined by the plaque reduction assay.

The presence of DMV-specific antibodies in three of four Fraser's dolphins from the SW Atlantic suggests that the virus is endemic in that population. Similarly, 48% of 23 Fraser's dolphins which mass-stranded on the east coast of the USA in 1994 had PMV neutralizing antibodies (Duignan et al., 1995b). Fraser's dolphins are highly gregarious, may form groups of a hundred to a thousand and associate with several other species of odontocetes (Leatherwood and Reeves, 1983), a behavior which likely facilitates virus transmission and maintenance. The high prevalence of DMV seropositive individuals among pilot whales from New Zealand and the presence of DMV-antibodies in both sexually mature and immature individuals in the absence of outbreaks of severe disease indicate that the virus is endemic in this 'population' like it is in *Globicephala* spp. from the NW Atlantic (Duignan et al., 1995a). Pilot whales are gregarious and associate with other cetaceans (Leatherwood and Reeves, 1983). They may be vectors and reservoirs of the infection to other species, such as offshore bottlenose dolphins in the SW Pacific, as suggested for their NW Atlantic counterparts (Duignan et al., 1995a). The absence of DMV-specific antibodies in the sera of a foetus from a seropositive mother and a neonate indicates that morbillivirus-specific antibodies are not transmitted to the foetus during pregnancy. Cetacean placenta is of the epitheliochorial type (Harrison, 1969) and it is likely that maternal immunoglobulins are transmitted to the offspring through colostrum as in cattle and other ungulates.

The positive captive bottlenose dolphin from Taiwan (*Gordina*) may have been infected in the wild or in captivity between 1978–1994. Interestingly, another bottlenose dolphin (*Victor*) from Ocean Park Corporation, caught in Indonesian waters in 1987, euthanized in July 1999 because of a progressive neurological disease and not tested during this study, had serum antibodies against DMV (titre = 640) in 1994 (P.J. Duignan, J.R. Geraci and R. Kinoshita, unpublished data). Though *Victor* and *Gordina* did not share the same pool and were unlikely to have physical contacts, their pools were only separated by stainless steel bar gates and theoretically, aerosolized virus could have been transmitted between them. However, there is no record of any severe generalized disease during their time in captivity and none of their pool mates examined in this study had

antibodies against DMV. Since morbilliviruses are highly transmissible (Black, 1991), it might be expected that some of their pool mates would have been infected and have developed antibodies against the virus if *Gordina* and *Victor* had been infected during captivity. It is therefore possible that the DMV-specific antibodies detected in these animal were produced when they were in the wild, as morbilliviruses may induce a long-lasting humoral immunity (Black, 1991). All the cetaceans of Ocean Park caught in the wild, originated from the West Pacific.

The possible decrease in prevalence of seropositive porpoises from the North Sea and NE Atlantic as well as the significant absence of seropositives among immature individuals suggest that DMV is not endemic in these populations. Porpoises, as well as common dolphins, white-beaked dolphins (*Lagenorhynchus albirostris*; Visser et al., 1993; Van Bressem et al., 1998b) and bottlenose dolphins from the NE Atlantic and North Sea are probably accidental hosts of the virus. DMV seropositives may have become infected in the late 1980s, early 1990s, at the time when morbillivirus infections were detected in cetaceans from the NE Atlantic and North Sea (Kennedy et al., 1991, 1992; Visser et al., 1993). Morbillivirus-infected porpoises were found along the coasts of the North Sea (England, Scotland and The Netherlands; Kennedy et al., 1992; Visser et al., 1993) and the NE Atlantic (Northern Ireland; Kennedy et al., 1991) during this period. The absence of DMV-seropositive individuals now found in porpoises from the North Sea suggests that only a small proportion of this population was infected during the epidemic. Long-finned pilot whales were suspected to be one of the reservoirs of DMV and vectors to other species in the NE Atlantic (Van Bressem et al., 1998b). However, DMV-specific antibodies could not be demonstrated in two individuals (both mature) from this species that died along the coasts of the British Isles and Iberian Peninsula in 1997 and 1999, respectively.

The apparent decrease in prevalence of DMV seropositive striped dolphins from the Mediterranean since the epidemic in 1990–1992 and the absence of seropositives in immature dolphins suggest that DMV is not endemic in this species. Two of three mature Risso's dolphins and a mature bottlenose dolphin also had antibodies against DMV. Though the epidemic apparently only affected striped dolphins in 1990–1992 (Aguilar and Raga, 1993), it is likely that these animals were infected during that event. A common dolphin stranded in Cagliari (Italy) in 1990 had also been infected by DMV or a closely related virus (Van Bressem et al., 1993).

The new data presented in this study suggest that the populations of harbour porpoises and common dolphins from the NE Atlantic and North Sea as well as of striped dolphins from the Mediterranean Sea are losing their immunity to the dolphin morbillivirus and may be soon at risk from new virus introductions. They have suffered high mortalities in fisheries and have a high burden of contaminants (Aguilar and Borrell, 1994, 1995; Di Natale and Nortobartolo-Di-Sciara, 1994; Kuiken et al., 1994; Donovan and Bjørge, 1995; Silvani et al., 1999). The re-introduction of cetacean morbillivirus into these populations could cause new epidemics which would further deplete their numbers. The presence of cetacean morbillivirus strains throughout the world oceans is an additional risk to cetaceans, particularly to those under pressure from human activities, and should be included in any future attempts to model sustainability of odontocete and mysticete populations.

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References

Aguilar, A., Raga, J.A., 1993. The striped dolphin epizootic in the Mediterranean Sea. Ambio 22, 524–528.

Aguilar, A., Borrell, A., 1994. Abnormally high polychlorinated biphenyl levels in striped dolphins (*Stenella coeruleoalba*) affected by the 1990–1992 Mediterranean epizootic. Sc. Total Environ. 154, 237–247.

Aguilar, A., Borrell, A., 1995. Pollution and harbour porpoises in the eastern North Atlantic: a review, Rep. Int. Whal. Commn. (special issue 16), 231–242.

Barrett, T., Visser, I.K.G., Mamaev, L.V., Goatley, L., Van Bressem, M.-F., Osterhaus, A.D.M.E., 1993a. Dolphin and porpoise morbilliviruses are genetically distinct from phocine distemper virus. Virology 193, 1010–1012.

Barrett, T., Romero, C.H., Baron, M.D., Yamanouchi, K., Diallo, A., Bostock, C.J., Black, D., 1993b. The molecular biology of rinderpest and peste des petits ruminants. Ann. Méd. Vét. 137, 77–85.

Barrett, T., Forsyth, M.A., Inui, K., Wamwayi, H.M., Kock, R., Wambua, J., Rossiter, P.B., 1998. Rediscovery of the second African lineage of rinderpest virus: its epidemiological significance. Vet. Rec. 142, 669–671.

Black, F.L., 1991. Epidemiology of Paramyxoviruses. In: Kingsburry, D.W. (Ed.), The Paramyxoviruses. Plenum Press, New York, NY, pp. 509–536.

Blixenkrone-Møller, M., Bolt, G., Gottschalck, E., Kenter, M., 1994. Comparative analysis of the gene encoding the nucleocapsid protein of dolphin morbillivirus reveals its distant evolutionary relationship to measles virus and ruminant morbilliviruses. J. Gen. Virol. 75, 2829–2834.

Blixenkrone-Møller, M., Bolt, G., Jensen, T.D., Harder, T., Svansson, V., 1996. Comparative analysis of the attachment protein gene (H) of dolphin morbillivirus. Virus Res. 40, 47–55.

Bolt, G., Blixenkrone- Møller, M., Gottschalck, E., Wishaupt, R.G., Welsh, M.J., Earle, J.A., Rima, B.K., 1994. Nucleotide and deduced amino acid sequences of the matrix (M) and fusion (F) protein genes of cetacean morbilliviruses isolated from a porpoise and a dolphin. Virus Res. 34, 291–304.

Calzada, N., 1995. Biología del delfín listado (*Stenella coeruleoalba*) del Mediterraneo occidental. Ph.D. thesis, University of Barcelona, p. 161.

Collet, A., Saint Girons, H., 1984. Preliminary study of the male reproductive cycle in common dolphins, *Delphinus delphis*, in the eastern North Atlantic Rep. Int. Whal. Commn. (special issue 6), 355–360.

Crespo, E.A., Schiavini, A., Pérez Macri, A.G., Reyes, L., Dans, S., 1994. Estudios sobre la determinación de edad en mamíferos marinos del Atlántico Sudoccidental. Anales 4° Reunión de Trabajo de Especialistas en Mamíferos Marinos de América del Sur, 12–15 November 1990, Valdivia, Chile, pp. 31–55.

Di, Natale, A., Nortobartolo-Di-Sciara, G., 1994. A review of the passive fishing nets and trap fisheries in the Mediterranean Sea and of the cetacean bycatch, Rep. Int. Whal. Commn. (special issue 15), 189–202.

Domingo, M., Ferrer, L., Pumarola, M., Marco, A., Plana, J., Kennedy, S., Mcalisky, M., Rima, B.K., 1990. Morbillivirus in dolphins. Nature 348, 21.

Donovan, G.P., Bjørge, A., 1995. Harbour porpoises in the North Atlantic: edited extract from the report of the IWC Scientific Committee, Dublin 1995, Rep. Int. Whal. Commn. (special issue 16), 4–25.

Duignan, P.J., House, C., Geraci, J.R., Early, G., Copland, H., Walsh, M.T., Bossart, G.D., Cray, C., Sadove, S., St Aubin, D.J., Moore, M., 1995a. Morbillivirus infection in two species of pilot whales (*Globicephala* sp.) from the western Atlantic. Mar. Mamm. Sci. 11, 150–162.

Duignan, P.J., House, C., Geraci, J.R., Duffy, N., Rima, B.K., Walsh, M.T., Early, G., St Aubin, D.J., Sadove, S., Koopman, H., Rhinehart, H., 1995b. Morbillivirus infection in cetaceans of the western Atlantic. Vet. Microb. 44, 241–249.

Duignan, P.J., House, C., Odell, D.K., Wells, R.S., Hansen, L.J., Walsh, M.T., St Aubin, D.J., Rima, B.K., Geraci, J.R., 1996. Morbillivirus in bottlenose dolphins: evidence for recurrent epizootics in the western Atlantic and Gulf of Mexico. Mar. Mamm. Sci. 12, 495–515.

Duignan, P.J., Nielsen, O., House, C., Kovacs, K., Duffy, N., Early, G., Sadove, S., St.Aubin, D.J., Rima, B., Geraci, J.R., 1997. Epizootiology of morbillivirus infection in harp, hooded, and ringed seals from the Canadian Arctic and western Atlantic. J. Wildl. Dis. 33, 7–19.

Habel, K., 1969. Virus neutralization test. In: Habel, K., Selzman, N.P. (Eds.), Fundamental Techniques in Virology. Academic Press, New York, NY, pp. 288–296.

Haffar, A., Libeau, G., Moussa, A., Minet, C., Diallo, A., 1999. The matrix protein gene sequence analysis reveals close relationship between peste des petits ruminants (PPRV) and dolphin morbillivirus. Virus Res. 64, 69–75.

Harrison, R.J., 1969. Reproduction and reproductive organs. In: Anderson, H.T. (Ed.), The Biology of Marine Mammals. Academic Press, London, pp. 253–348.

Hohn, A., Scott, M.D., Wells, R.S., Sweeney, J.C., Irvine, A.B., 1989. Growth layers in teeth from known age, free-ranging bottlenose dolphins. Mar. Mamm. Sci. 5, 315–342.

Kennedy, S., Smyth, J.A., Cush, P.F., McAliskey, M., McCullough, S.J., Rima, B.K., 1991. Histopathologic and immunocytochemical studies of distemper in harbor porpoises. Vet. Pathol. 28, 1–7.

Kennedy, S., Kuiken, T., Ross, H.M., McAliskey, M., Moffett, D., McNiven, C.M., Carole, M., 1992. Morbillivirus infection in two common porpoises (*Phocoena phocoena*) from the coasts of England and Scotland. Vet. Rec. 131, 286–290.

Krafft, A., Lichy, J.H., Lipscomb, T.P., Klaunberg, B.A., Kennedy, S., Taubenberger, J.K., 1995. Postmortem diagnosis of morbillivirus infection in bottlenose dolphins (*Tursiops truncatus*) in the Atlantic and Gulf of Mexico epizootics by polymerase chain reaction-based assay. J. Wildl. Dis. 31, 410–415.

Kuiken, T., Simpson, V.R., Allchin, C.R., Bennett, P.M., Codd, G.A., Harris, E.A., Howes, G.J., Kennedy, S., Kirkwood, J.K., Law, R.J., Merrett, N.R., Phillips, S., 1994. Mass mortality of common dolphins (*Delphinus delphis*) in south west England due to incidental capture in fishing gear. Vet. Rec. 134, 81–89.

Lindmark, R., Thorén-Tolling, K., Sjöquist, J., 1983. Binding of immunoglobulins to protein A and immunoglobulin levels in mammalian sera. J. Immunol. Methods 62, 1–13.

Leatherwood, S., Reeves, R.R., 1983. The Sierra Club Handbook of Whales and Dolphins. Sierra Club Books, San Francisco, CA.

Lipscomb, T.P., Schulman, F.Y., Moffett, D., Kennedy, S., 1994. Morbilliviral disease in Atlantic bottlenose dolphins (*Tursiops truncatus*) from the 1987–1988 epizootic. J. Wildl. Dis. 30, 567–571.

Lockyer, C., 1995. Investigation of aspects of the life history of the harbour porpoise, *Phocoena phocoena*, in British waters, Rep. Int. Whal. Commn. (special issue 16), 189–197.

McCullough, S.J., McNeilly, F., Allan, G.M., Kennedy, S., Smyth, J.A., Cosby, S.L., McQuaid, S., Rima, B.K., 1991. Isolation and characterization of a porpoise morbillivirus. Arch. Virol. 118, 247–252.

Nielsen, O., Stewart, R.E.A., Measures, L., Duignan, P.J., House, C., 2000. A morbillivirus antibody survey of Atlantic walrus, narwhal and beluga in Canada. J. Wildl. Dis. 36 (3), 508–517.

Peddemors, V.M., 1999. Delphinids of southern Africa: a review of their distribution, status and life history. J. Cet. Res. Manage. 1, 157–165.

Perrin, W.F., Myrick Jr. A.C. (Eds.), 1980. Age determination of toothed whales and sirenians. Rep. Int. Whal. Commn. (special issue 3), 97–133.

Reidarson, T.H., McBain, J., House, C., King, D.P., Stott, J.L., Krafft, A., Taubenberger, J.K., Heyning, J., Lipscomb, T.P., 1998. Morbillivirus infection in stranded common dolphins from the Pacific Ocean. J. Wildl. Dis. 34, 771–776.

Ross, G.J.B., 1984. The smaller cetaceans of the Southeast coast of Southern Africa. Ann. Cape prov. museums 11, 135–194.

Ross, P.S., De Swart, R.L., Addison, R.F., Van Loveren, H., Vos, J.G., Osterhaus, A.D.M.E., 1996. Contaminant-induced immunotoxicity in harbour seals: wildlife at risk. Toxicology 112, 157–169.

Shaila, M.S., Shamaki, D., Forsyth, M.A., Diallo, A., Goatley, L., Kitching, R.P., Barrett, T., 1996. Geographic distribution and epidemiology of peste des petits ruminants virus. Virus Res. 43, 149–153.

Silvani, L., Gazo, M., Aguilar, A., 1999. Spanish drifnet fishing and incidental catches in the western Mediterranean. Biol. Cons. 90, 79–85.

Swinscow, T.D.V., 1981. Statistics at Square One, 7th Edition. British Medical Association, London.

Taubenberger, J.K., Tsai, M., Krafft, A.E., Lichy, J.H., Reid, A.H., Schulman, F.Y., Lipscomb, T.P., 1996. Two morbilliviruses implicated in bottlenose dolphin epizootics. Emerg. Infect. Dis. 2, 213–216.

Taubenberger, J.K., Tsai, M.M., Atkin, T.J., Fanning, T.G., Krafft, A.E., Moeller, R.B., Kodsi, S.E., Mense, M.G., Lipscomb, T.P., 2000. Molecular genetic evidence of a novel morbillivirus in a long-finned pilot whale (*Globicephalus (sic) melas*). Emerg. Infect. Dis. 6, 42–45.

Uchida, K., Muranaka, M., Horii, Y., Murakami, N., Yamaguchi, R., Tateyama, S., 1999. Non-purulent meningoencephalomyelitis of a Pacific striped dolphin (*Lagenorhynchus obliquidens*): the first evidence of morbillivirus infection in a dolphin at the Pacific Ocean around Japan. J. Vet. Med. Sci. 61, 159–162.

Van Bressem, M.-F., 1997. Natural History of Virus Infections in Cetaceans. Ph.D. thesis, University of Liège.

Van Bressem, M.-F., Visser, I.K.G., Van De Bildt, M.W.G., Teppema, J.S., Raga, J.A., Osterhaus, A.D.M.E., 1991. Morbillivirus infection in Mediterranean striped dolphins (*Stenella coeruleoalba*). Vet. Rec. 129, 471–472.

Van Bressem, M.-F., Visser, I.K.G., De Swart, R.L., Örvell, C., Stanzani, L., Androukaki, E., Siakavara, K., Osterhaus, A.D.M.E., 1993. Dolphin morbillivirus infection in different parts of the Mediterranean Sea. Arch. Virol. 129, 235–242.

Van Bressem, M.-F., Van Waerebeek, K., Fleming, M., Barrett, T., 1998a. Serological evidence of morbillivirus infection in small cetaceans from the Southeast Pacific. Vet. Microb. 59, 89–98.

Van Bressem, M.-F., Jepson, P., Barrett, T., 1998b. Further insight on the epidemiology of cetacean morbillivirus in the Northeastern Atlantic. Mar. Mamm. Sci. 14, 605–613.

Van Bressem, M.-F., Van Waerebeek, K., Raga, J.A., 1999. A review of virus infections of cetaceans and the potential impact of morbilliviruses, poxviruses and papillomaviruses on host population dynamics. Dis. Aquat. Org. 38, 53–65.

Visser, I.K.G., Van Bressem, M.-F., De Swart, R.L., Van De Bildt, M.W.G., Vos, H.W., Van Der Heijden, R.W.J., Saliki, J.T., Örvell, C., Kitching, P., Kuiken, T., Barrett, T., Osterhaus, A.D.M.E., 1993. Characterization of morbilliviruses isolated from dolphins and porpoises in Europe. J. Gen. Virol. 74, 631–641.