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# Morbillivirus infection in free-ranging Atlantic bottlenose dolphins (*Tursiops truncatus*) from the Southeastern United States: Seroepidemiologic and pathologic evidence of subclinical infection

Gregory D. Bossart [a,b,*], John S. Reif [c], Adam M. Schaefer [b], Juli Goldstein [b], Patricia A. Fair [d], Jeremiah T. Saliki [e]

[a] *Georgia Aquarium, 225 Baker Street, NW, Atlanta, GA 30313, USA*
[b] *Harbor Branch Oceanographic Institute at Florida Atlantic University, 5600 US 1 North, Ft. Pierce, FL 34946, USA*
[c] *Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO 80523, USA*
[d] *National Oceanic and Atmospheric Administration, National Ocean Service, Center for Coastal Environmental Health and Biomolecular Research, 219 Fort Johnson Rd, Charleston, SC 29142, USA*
[e] *Athens Veterinary Diagnostic Laboratory, University of Georgia, Athens, GA 30602, USA*

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A B S T R A C T

From 2003 to 2007, sera ($n = 234$) from free-ranging Atlantic bottlenose dolphins (*Tursiops truncatus*) inhabiting two southeast Atlantic estuarine regions, the Indian River Lagoon (IRL), FL and Charleston, SC (CHS) were tested for antibodies to cetacean morbilliviruses as part of a multidisciplinary study of individual and population health. Positive morbillivirus titers were found on initial capture in 12 of 122 (9.8%) IRL dolphins in the absence of an epizootic. All CHS dolphins were seronegative. Positive fluctuating morbillivirus titers and seroconversion were found in IRL dolphins. Seropositivity was detected in dolphins 8–13 years of age as well as in dolphins that were alive during the 1987–1988 epizootic. During the study period, pathologic and immunohistochemical findings from stranded IRL dolphins ($n = 14$) did not demonstrate typical morbillivirus-associated lesions or the presence of morbillivirus antigen. The findings suggest that morbillivirus infections are occurring in the absence of widespread mortality in IRL dolphins.

## 1. Introduction

Between 1987 and 1988 an epizootic of morbillivirus infection characterized by widespread mortality occurred in bottlenose dolphins (*Tursiops truncatus*) along the eastern coast of the United States (Lipscomb et al., 1994a). An estimated 2500 deaths occurred, representing a 10-fold increase in mortality and loss of approximately 50% of the inshore population of bottlenose dolphins (McLellan et al., 2002). Deaths were reported from New Jersey to central Florida (Geraci, 1989). Stranded dolphins were found along the coast adjacent to the Indian River Lagoon (IRL), FL and in inlets connecting the ocean to the estuary (Geraci, 1989). In retrospect, serological testing of archived samples indicates that morbillivirus infections had been present in IRL since at least 1982, as shown by serological testing of archived samples (Duignan et al., 1996).

This epizootic was followed by further die-offs of bottlenose dolphins in the Gulf of Mexico in 1993 and 1994. Morbillivirus antigen and characteristic lesions were detected in dolphin tissues from both outbreaks (Lipscomb et al., 1994a,b) and morbillivirus RNA was demonstrated by reverse transcription polymerase chain reaction (RT-PCR) testing (Krafft et al., 1995; Schulman et al., 1997).

* Corresponding author at: Georgia Aquarium, 225 Baker Street, NW, Atlanta, GA 30313, USA. Tel.: +1 404 581 4304; fax: +1 404 581 4303.
*E-mail address:* gbossart@georgiaaquarium.org (G.D. Bossart).

A major morbillivirus epizootic occurred in striped dolphins (*Stenella coeruleoalba*) along the Spanish Mediterranean coast in 1990 that resulted in several thousand deaths by the end of 1992 (Domingo et al., 1990, 1992; Aguilar and Raga, 1993). This epizootic was shown to be due to dolphin morbillivirus (DMV) (Domingo et al., 1990, 1992). Subsequently, porpoise morbillivirus (PMV) was isolated from harbor porpoises (*Phocoena phocoena*) that had died along the Irish coast of the North Sea in 1990 (McCullough et al., 1991), during a phocine distemper virus (PDV) epizootic in harbor seals. DMV and PMV appear to be closely related strains of a cetacean morbillivirus (Blixenkrone-Moller et al., 1994), distinct from PDV and canine distemper virus (CDV) and more closely related to the ruminant morbilliviruses and measles virus (Visser et al., 1993; Barrett et al., 1993). Bottlenose dolphins from the 1987 to 1988 epizootic were shown to be infected with either DMV or PMV, while only PMV was identified in the later Gulf of Mexico epizootic by gene sequence analysis (Taubenberger et al., 1996). Recently, a novel morbillivirus was identified in a long-finned pilot whale (*Globicephala melas*) that died off the coast of New Jersey and may represent a third member of the cetacean morbillivirus group (Taubenberger et al., 2000).

Morbillivirus infection in the western Atlantic is not confined to bottlenose dolphins, but has occurred in at least 11 other cetacean species since 1986 as shown by the presence of neutralizing antibodies in archived samples (Duignan et al., 1995a). A central role for infection in pilot whales (*Globicephala* sp.) has been suggested on the basis of high seroprevalence in samples obtained during multiple stranding events for both *G. melas* and *G. macrorhynchus* dating back to 1982 (Duignan et al., 1995b). The high rates of seroprevalence and gregarious nature of the species led to the hypothesis that pilot whales may serve as reservoirs for cetacean morbilliviruses and transmit the agent to other cetaceans (Duignan et al., 1995b); however, the nature of enzootic infection in *Globicephala*, if it occurs, remains unresolved. Thus important, unanswered questions regarding the epidemiology of cetacean morbillivirus infection remain, including the maintenance of infection in cetacean populations during inter-epidemic periods and the factors that lead to clinical disease and the development of epizootics. In this paper we present serologic evidence for recurring subclinical morbillivirus infection in IRL dolphins in the post-1987–1988 period in the absence of clinical or histopathologic evidence of disease. Together, the epidemiologic and pathologic data provide important new clues regarding the characteristics of morbillivirus infection in cetaceans.

## 2. Methods

### 2.1. Free-ranging dolphins

Serum samples for evaluation of antibodies to cetacean morbilliviruses were collected as part of the Bottlenose Dolphin Health and Risk Assessment (HERA) project, a multidisciplinary, integrated, collaborative effort to assess individual and population health in two southeast Atlantic estuarine regions (Fair et al., 2006). The estimated population for the Indian River Lagoon (IRL), FL is approximately 1000; the Charleston, SC (CHS) site has approximately 470 dolphins in summer and 275 in winter (Speakman et al., 2006). Dolphins were sampled throughout each estuary. Dolphin health was assessed by comprehensive examinations including a complete physical examination, ultrasound survey and the collection of a suite of traditional and specialized clinicopathologic assays. As part of the health assessment, sera were screened for antibodies to multiple marine mammal and human pathogens, including morbilliviruses.

The IRL is a shallow-water ecosystem that comprises 40% of Florida's central east coast. The lagoon is an aggregate of three estuarine water bodies: the Indian and Banana Rivers and the Mosquito Lagoon that extends 250 km from Ponce De Leon Inlet in the north to Jupiter Inlet in the south. The estuary is connected to the Atlantic ocean through five inlets and one lock. The CHS site is an estuarine environment in the central region of South Carolina's coastal zone that includes the Charleston Harbor, as well as portions of the Ashley River, Cooper River, Wando River, and Stono River estuary. Photo-identification survey data indicate that dolphins at both sites display long-term residency patterns and site fidelity (Speakman et al., 2006; Mazzoil et al., 2008).

All methods used for capture and blood collection were approved under a National Marine Fisheries Service Scientific Research Permit as part of the Bottlenose Dolphin Health and Risk Assessment (HERA) Project and by the Harbor Branch Oceanographic Institutional Animal Care and Use Committee. Dolphins were captured in the IRL during June of 2003 to June of 2007 and in the waters near CHS during August of 2003 to August of 2005. Sampling was conducted in specific areas within each site based on photo-identification survey data. Standard operating protocols and techniques used for capture, sample collection and release of dolphins are described in detail elsewhere (Fair et al., 2006). Health status, classified as clinically normal, possibly diseased or definitely diseased, was determined by a panel of marine mammal veterinarians (Reif et al., 2008). Age was estimated by counting post-natal dentine layers in an extracted tooth (Hohn et al., 1989).

Blood samples were collected by insertion of a 19 gauge, 1.9 cm butterfly catheter (Becton, Dickinson, Franklin Lakes, NJ, USA) into periarterial venous rete in the flukes. Serum for serological testing was collected in 10-ml vacutainer tubes containing a clot activator and serum separator (Becton, Dickinson, and Co., Franklin Lakes, NJ, USA), placed in a cooler for between 20 and 40 min, and centrifuged for 15 min at 1200 rpm. Sera were frozen at −80 °C, stored and then shipped overnight on dry ice to the Oklahoma State Veterinary Diagnostic Laboratory (Stillwater, OK, USA).

### 2.2. Serum neutralization test

The serum neutralization test (SNT) was performed by using DMV and PMV (provided by Dr. Seamus Kennedy, Belfast, UK through Dr. Padraig Duignan). Virus was grown in Vero cells and the test was performed as previously described (Saliki and Lehenbauer, 2001). Briefly, serial

two-fold dilutions of heat-inactivated sera were made in duplicate columns of 96-well plates using Dulbecco's minimum essential medium (DMEM), starting at a 1:2 dilution. An equal volume (25 μl) of virus containing about 100 $TCID_{50}$ was added. Addition of virus yielded a final starting dilution of 1:4. The virus-serum mixtures were incubated at 37 °C for 1 h in 5% $CO_2$ and a Vero cell suspension (150 μl containing $10^4$ cells/well) was added to the plates. The plates were incubated at 37 °C in 5% $CO_2$ for 4 days. The test was read by examining cell monolayers under an inverted microscope for virus-specific cytopathic effects (CPE). Antibody titers were expressed as the reciprocal of the highest dilution of serum that completely neutralized CPE in duplicate wells. Titers ≥8 were considered positive for the presence of morbillivirus neutralizing antibody in the serum.

### 2.3. Stranded IRL dolphins-pathology

Fourteen IRL dolphins (six males, eight females) that stranded from 2002 to 2008 received complete gross and microscopic pathologic analyses. Age categories were estimated from total body length. Tissue sections from the lung, heart, liver, spleen, multiple lymph nodes, thymus (if present), gastrointestinal tract, pancreas, kidney, adrenal gland, brain, reproductive tract, skeletal muscle and skin were collected for histologic examination. Tissues were placed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin.

Immunohistochemical (IHC) staining for DMV antigen was performed in formalin-fixed, paraffin-embedded tissue sections of lung, brain, skin, spleen and pulmonary, mesenteric and subscapular lymph nodes as previously described (Raga et al., 2008). Briefly, a monoclonal antibody to canine distemper virus (MoAb CDV-NP, VMRD, Inc., Pullman, WA, USA), known to react with DMV, was used as primary antiserum at a dilution of 1:200. The secondary antibody, a biotinylated goat anti-mouse immunoglobulin serum, was used at the same dilution. Finally, the avidin biotin peroxidase complex was incubated at a dilution of 1:100. Sections were counterstained with hematoxylin. Lung sections from a DMV infected dolphin were used as a positive control in each test. Duplicate tissue sections were also reacted with an unrelated monoclonal antibody as a negative control.

### 2.4. Statistical analyses

The prevalence of antibody to DMV and PMV was compared by capture sites and year of capture. Among IRL dolphins, prevalence was compared between males and females and between dolphins born during 1988 and before and dolphins born during 1989 or later. Seroconversion was defined as a change in antibody titer from seronegative to ≥1:8 to DMV or PMV. Statistical analyses were conducted in EPI INFO, Version 6.0 (Centers for Disease Control and Prevention, U.S.P.H.S.). Proportions of dolphins that tested positive for antibodies to DMV or PMV were compared using a Chi-square test with Yates' correction applied when a cell contained <5. Fisher's exact test was applied when an expected cell frequency was <5.

## 3. Results

### 3.1. Free-ranging bottlenose dolphins

Sera ($n = 234$) from IRL and CHS dolphins were tested for antibodies to DMV and PMV. Data for the initial capture of each dolphin were used for the calculation of prevalence. A total of 205 samples (122 IRL, 83 CHS) from the first capture of individual dolphins and 29 samples from 23 recaptured dolphins were tested. Titers ≥1:8 were found only in IRL dolphins; 7.4% had antibodies to DMV and 5.2% to PMV on initial capture (Table 1). Most seropositive dolphins were >10 years old. Positive titers to DMV ranged from 1:8 to 1:64, while those to PMV ranged from 1:8 to 1:128 (Table 2). Six of nine dolphins seropositive for DMV on initial capture had antibodies only to DMV, while three of the six dolphins seropositive to PMV were also seropositive for DMV. Among the three dolphins seropositive to both morbilliviruses, titers were higher to DMV than to PMV in two dolphins and of equal magnitude in the third. The highest titer found was 1:128 against PMV in a dolphin negative for antibody to DMV.

The health status of seropositive IRL dolphins was examined by linking the data to those from a previous report (Reif et al., 2008) (Table 2). Three seropositive dolphins had orogenital papillomatosis (Bossart et al., 2005) when captured; two had lacaziosis (Reif et al., 2006). Dolphin 936 had a titer of 1:32 against DMV when caught initially in 2003. In 2004, the titer to DMV remained at 1:32 and it had a titer of 1:8 against PMV. When caught again in 2005 the titer was reported as 1:6 as the result of unequal results in duplicate wells at 1:4 and 1:8. This dolphin showed a persistent, declining titer to DMV with probable cross reaction with PMV. It was classified as possibly diseased in each of the first two capture years and had developed genital papillomatosis when examined in 2005. Four dolphins (900, 906, 927 and 930) were clinically normal when examined; one (964) was classified as

**Table 1**
Prevalence of antibodies against dolphin morbillivirus (DMV) and porpoise morbillivirus (PMV), IRL 2003–2007 and CHS, 2003–2005[a].

| Antigen | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Indian River Lagoon, FL | | | | Charleston, SC | | | |
| | Pos | Neg | Total | Prevalence | Pos | Neg | Total | Prevalence |
| DMV | 9 | 113 | 122 | 7.4% | 0 | 83 | 83 | 0.0% |
| PMV | 6 | 109 | 115 | 5.2% | 0 | 83 | 83 | 0.0% |

[a] Data for first capture of each dolphin.

**Table 2**
Antibody titers to DMV and PMV in seropositive[a] dolphins from the Indian River Lagoon, FL, 2003–2007.

| ID number | Year | Age | Sex | Recapture | DMV titer | PMV titer | Health status |
|---|---|---|---|---|---|---|---|
| 900 | 2003 | 13 | Male | | 8 | 0 | Normal |
| 905 | 2003 | 19 | Female | | 0 | 128 | Possible Disease |
| 906 | 2003 | 17 | Male | | 0 | 8 | Normal |
| 927 | 2003 | NA | Female | | 64 | 32 | Normal |
| 930 | 2003 | 13 | Male | | 64 | 0 | Normal |
| 936 | 2003 | 14 | Male | | 32 | 0 | Possible Disease |
| 936 | 2004 | 15 | Male | Yes | 32 | 8 | Possible Disease |
| 936 | 2005 | 16 | Male | Yes | 6 | 0 | Papillomatosis |
| 961 | 2004 | NA | Female | | 8 | 0 | Lobomycosis |
| 964 | 2004 | 8 | Male | | 16 | 0 | Diseased |
| 977 | 2006 | NA | Female | | 0 | 0 | Normal |
| 977 | 2007 | NA | Female | Yes | 8 | 0 | Papillomatosis |
| 978 | 2004 | 12 | Male | | 0 | 8 | Possible Disease |
| 982 | 2004 | 11 | Male | | 16 | 0 | Papillomatosis |
| 989 | 2007 | NA | Female | | 64 | 48 | Papillomatosis |
| 9D0 | 2006 | 11 | Male | | 0 | 0 | Normal |
| 9D0 | 2007 | 12 | Male | Yes | 8 | 0 | Possible Disease |
| 9V8 | 2007 | NA | Female | | 8 | 8 | Lobomycosis |

[a] Titers ≥8 considered positive.

**Table 3**
Prevalence of antibodies against dolphin morbillivirus (DMV) and porpoise morbillivirus (PMV), by year, Indian River Lagoon, FL[a].

| Year | DMV+ | DMV− | Total | Prevalence | PMV+ | PMV− | Total | Prevalence |
|---|---|---|---|---|---|---|---|---|
| 2003 | 4 | 38 | 42 | 9.5% | 3 | 39 | 42 | 7.1% |
| 2004 | 4 | 35 | 39 | 10.3% | 2 | 37 | 39 | 5.3% |
| 2005 | 0 | 18 | 18 | 0.0% | 0 | 7 | 7 | 0.0% |
| 2006 | 0 | 26 | 26 | 0.0% | 0 | 26 | 26 | 0.0% |
| 2007 | 4 | 14 | 18 | 22.2% | 2 | 16 | 18 | 11.1% |
| Total | 12 | 131 | 143 | 8.4% | 7 | 126 | 133 | 5.3% |

[a] Data for all captures of each dolphin.

definitely diseased and one (978) as possibly diseased on the basis of clinicopathologic findings (Reif et al., 2008).

Two dolphins showed evidence of probable seroconversion to DMV during the study (Table 2). Dolphins 977 and 9D0 were seronegative when sampled initially during 2006 and had titers to DMV of 1:8 when re-sampled 1 year later. Dolphin 9D0 was classified clinically normal in 2006 and had developed an orogenital papilloma in 2007; dolphin 977 was classified as clinically normal in 2006 and as possibly diseased in 2007 when it was seropositive to DMV.

Annual seroprevalence in the IRL was compared for the 5 capture years to evaluate potential changes over time (Table 3). All 143 IRL dolphin samples (including recaptures) were included in the analysis; no clear pattern was found although the prevalence of antibody to DMV was higher in the final year of the study (2007) than in earlier years ($p = 0.46$, Fisher's exact test). The temporal pattern of PMV antibody prevalence followed that for DMV, albeit at lower levels. The prevalence of antibody to DMV and PMV was not significantly different for males and females (7.9% and 6.5% for DMV, $p > 0.05$; 4.2% and 6.8% for PMV, $p > 0.05$, respectively).

The prevalence of antibody to DMV and PMV was compared between dolphins that were alive during the 1987–1988 epizootic, compared to dolphins that were born after the epizootic (Table 4). Despite the fact that 34 CHS dolphins had been present during the 1987–1988 epizootic, while 36 were born in 1989 or later, none of the CHS dolphins were seropositive to either morbillivirus strain. In contrast, among IRL dolphins born during 1988 or before, no DMV seropositive animals were identified but 2 of 20 had antibodies to PMV (10.0%). Among dolphins born during 1989 or later, the prevalence of antibody to DMV and PMV was 10.1% and 3.2%, respectively.

### 3.2. Stranded IRL dolphins-pathology

Fourteen stranded dolphins that died between 2002 and 2008 were necropsied and tissues examined for

**Table 4**
Prevalence of antibodies against dolphin morbillivirus (DMV) and porpoise morbillivirus (PMV), by date of birth and presence during 1987–1988 epizootic, Indian River Lagoon, FL[a,b].

| | DMV+ | DMV− | Total | Prevalence | PMV+ | PMV− | Total | Prevalence |
|---|---|---|---|---|---|---|---|---|
| Born 1988 or before | 0 | 203 | 20 | 0.0% | 2 | 18 | 20 | 10.0% |
| Born after 1988 | 7 | 62 | 69 | 10.1% | 2 | 61 | 63 | 3.2% |

[a] Data for first capture of each dolphin.
[b] Analysis restricted to IRL dolphins of known age.

morbillivirus with IHC. This group consisted of three calves, three subadults and eight adults. Two of the 14 were captured during HERA; both were seronegative. No sera were available from the remainder. Causes of death included trauma (six cases), primary infectious/inflammatory disease (six cases), starvation (one case) and unknown (one case). Microscopically, all dolphins that died of traumatic injuries had secondary associated degenerative lesions of the central nervous system (one case) or infectious disease of the central nervous system, lungs, heart, liver and/or alimentary tract (five cases). The secondary infectious lesions consisted of pneumonia alone (one case); pneumonia and hepatitis with intralesional bacteria (one case); myocarditis, aortitis and pericarditis with intralesional bacteria (one case); esophagitis with widespread bacterial embolism (one case) and pneumonia and meningoencephalitis (one case). Additionally, cutaneous lobomycosis was present in two cases.

Primary infectious and inflammatory diagnoses included pneumonia of bacterial, parasitic and fungal origin (six cases). Less common lesions included gastritis (two cases), bacterial lymphadenitis (one case) and bacterial endometritis and placentitis with cutaneous lobomycosis (one case). Additionally, a genital sessile papilloma was an incidental finding in a single case with pneumonia. All tissues tested with IHC for morbillivirus antigen were negative. We found no evidence of the classical suite of histopathologic lesions described in morbillivirus epizootics (Lipscomb et al., 1994a,b).

## 4. Discussion

The most important finding in this study was the detection of antibodies against DMV and PMV in dolphins from the IRL in the absence of an epizootic and typical morbillivirus-associated pathologic lesions. The 1987–1988 epizootic of morbillivirus along the eastern coast of the United States was characterized by widespread mortality (Lipscomb et al., 1994a). Dolphins experienced a 10-fold increase in mortality with 742 confirmed deaths and an estimated 50% mortality among inshore populations. In 2003, only dolphins 15-years of age and older would have been exposed to these agents during the epizootic. However, we found evidence of more recent morbillivirus activity in the IRL showing that morbillivirus infections are occurring in the absence of widespread mortality although titers were generally low. In particular, there was evidence of DMV infection during the post epizootic period with a 10.1% seroprevalence among dolphins born after 1988. Antibody was detected in IRL dolphins 8–13 years of age at the time of capture during HERA, indicating that morbillivirus transmission and subclinical infections are occurring in the absence of widespread mortality. A recent photo-identification study of 221 dolphins along the east-central coast of Florida found only limited evidence of movement between the estuarine IRL and the adjacent ocean where contact with other cetaceans could occur (Mazzoil et al., in press).

Previous seroepidemiologic investigations on archived samples strongly suggest subclinical morbillivirus infections may have spread to immunologically naïve Atlantic bottlenose dolphin populations, causing severe epizootics (Duignan et al., 1995a; Bossart, 1995; Kennedy, 1998; Van Bressem et al., 1998, 2001). Subclinically infected cetaceans may have played a more important role as reservoirs and sources of transmission than clinically affected animals (Di Guardo et al., 2005). However, endemic subclinical infection has not been previously reported in Atlantic bottlenose dolphins. The current data are consistent with the finding of morbillivirus infection in bottlenose dolphins that stranded in the Gulf of Mexico from 1993 to 1994 (Lipscomb et al., 1996) and with serologic evidence of infection and recurrent epidemics in the Western Atlantic and Gulf of Mexico through the mid-1990s (Duignan et al., 1996). Interestingly, the first evidence of morbillivirus activity occurred in the IRL in 1982 (Duignan et al., 1996) and was followed by the massive epizootic in 1987–1988 and by further outbreaks in the Gulf of Mexico between 1992 and 1994. In retrospect, a 1982 increase in strandings of bottlenose dolphins in the Banana and Indian Rivers may have been due to morbillivirus infection, based on the finding of antibodies in five of six dolphins sampled in that year and the absence of antibody in 24 samples obtained from the same area in 1980 (Duignan et al., 1996). Thus, the IRL dolphin population may serve as an early warning system for morbillivirus epizootics, emphasizing the need for active serological and pathological surveillance of live and stranded dolphins.

Recently, an outbreak of die-offs among striped dolphins (*S. coeruleoalba*) was reported from the Gulf of Valencia along the Spanish coast of the Mediterranean Sea (Raga et al., 2008). Over 100 striped dolphins were found dead and a virus strain closely related to the dolphin morbillivirus isolated during the 1990–1992 epidemic was detected in tissues. The epidemic was similar to the 1990 die-off in several respects: it started in the same location and followed a similar temporal pattern with respect to the stranding rate. However, dolphins in the 2007 die-off were younger than those from the earlier event and included a preponderance of juveniles (Raga et al., 2008). Serologically, only adult striped dolphins in the area had antibodies to DMV and the rate of seropositivity had declined from 100% in 1990–1992 to 50% in 1997 in a small sample (Van Bressem et al., 2001). The authors concluded that DMV infection was not endemic in striped dolphins in the Spanish Mediterranean and that a decline in herd immunity and an increase in population density had rendered the population susceptible to new epizootics. They further suggested that the location of both epizootics near the Straits of Gibraltar could indicate that the agent was introduced through contact with infected cetaceans in the Atlantic Ocean (Raga et al., 2008). However, evidence for chronic infection of striped dolphins in the Mediterranean, consisting of a nonsuppurative encephalitis characterized by inflammatory lesions, gliosis and DMV antigen in the brain in the absence of other systemic involvement was reported in this population (Domingo et al., 1995).

DMV titers and antigen persistence in the brain in the absence of typical morbillivirus lesions or systemic involvement have been observed in captive Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) originating from

the South Pacific further illustrating the complexity of this infection (G.D. Bossart, unpublished data). Similarly, 6 of 18 common dolphins (*Delphinus delphinus*) stranded along the southern California coast tested positive for antibody to DMV. The authors concluded on the basis of histopathologic examination of tissues and other data that DMV, or a closely related morbillivirus, is present in common dolphins in the Pacific Ocean and that infection of common dolphins may not be associated with morbillivirus disease (Reidarson et al., 1998).

The pattern of morbillivirus seroprevalence in younger animals, seroconversions and fluctuating titers combined with absence of typical morbillivirus pathologic lesions in stranded IRL bottlenose dolphins during the same time period supports the hypothesis that subclinical morbillivirus infections occur in this population as well. The most prominent histopathologic changes found in dolphin morbillivirus infection involve the respiratory tract, central nervous system and the lymphoid tissues (Di Guardo et al., 2005). Histologically, common pathologic findings are nonsuppurative encephalitis, severe and generalized lymphoid tissue depletion and nonsuppurative bronchointerstitial pneumonia characterized by type II pneumocyte hyperplasia and by formation of "Warthin–Finkeldey type" syncytia. Intracytoplasmic and intranuclear eosinophilic viral inclusions are often detected within bronchial and bronchiolar epithelial, pulmonary syncytial, neuronal, and other cell types. These inclusions, along with lymphoid and other cellular elements, are often found to be IHC positive for morbillivirus antigen.

We found no evidence of this suite of histopathologic lesions in stranded IRL dolphins. Bronchopneumonia and chronic interstitial pneumonia were observed but these were typically associated with intralesional bacteria, fungi or parasites in the absence of type II pneumocyte hyperplasia, syncytia, viral inclusion bodies and positive morbillivirus IHC staining. Furthermore, the microscopic pneumonias observed are common in stranded IRL dolphins (Bossart et al., 2003).

The sites for viral introduction, replication and initial systemic dissemination in the pathogenesis of morbillivirus infection in dolphins are unknown. In dogs, CDV initially proliferates in pulmonary lymph nodes and within a few days after exposure is distributed throughout the lymphatic tissue, including the bone marrow, spleen, and thymus (Appel, 1970). The infection is primarily confined to the lymphoid tissues until 8–9 days after exposure. In dogs that develop protective neutralizing antibody titers within 2 weeks, the virus does not spread to epithelial tissues and the infection is mild or inapparent. Based on the canine model, dolphins may respond similarly with production of a humoral immune response, neutralizing antibody titers and the development of subclinical infection. Further studies are needed to define the pathogenesis of morbillivirus infection in dolphins.

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