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Update on the dolphin morbillivirus outbreak and the 2013-2014 U.S. Mid-Atlantic bottlenose dolphin (*Tursiops truncatus*) unusual mortality event

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ABSTRACT

Dolphin morbillivirus (DMV) has caused previous outbreaks of disease in bottlenose dolphins (*Tursiops truncatus*) in the Mid-Atlantic region of the United States East Coast (1987-88) and the Gulf of Mexico (1992, 1994. An Unusual Mortality Event (UME) was declared by the U.S. National Marine Fisheries Service on August 8, 2013, due to increased numbers of bottlenose dolphin strandings documented in New York, New Jersey, Delaware, Maryland and Virginia during the months of July and August. Strandings have remained elevated and the geographic scope of the event extends from New York to northern Florida (through Brevard County) with the UME still on-going. From July 1, 2013, to April 27, 2014, >1200 bottlenose dolphins stranded within the UME area with 18% of animals stranding alive or fresh dead, and 82% of the carcasses in moderate to advanced states of decomposition. Gross necropsy findings included dermal, oral, joint, and pulmonary lesions. Consistent histopathologic findings included bronchointerstial pneumonia and/or pulmonary fibrosis, lymphoid depletion, syncytial cells, and viral inclusions. Secondary bacterial, fungal, viral (non-morbillivirus), and protozoal infections were observed. Tissue samples from

dolphins were tested for morbillivirus via polymerase chain reaction (PCR) and/or immunohistochemistry and positive PCR results were confirmed by sequencing as dolphin morbillivirus. Research is ongoing to better understand the impacts of this large scale outbreak on bottlenose dolphin populations and affected coastal stocks.

INTRODUCTION

Morbilliviruses are in the family Paramyxoviridae. Different morbilliviruses cause measles (in people), canine distemper (in dogs, coyotes, wolves, and seals), rinderpest (in cattle), and peste-des-petits-ruminants (goats and sheep). Five types of morbillivirus have been detected in marine mammals in the United States: canine distemper virus (CDV) in seals, phocine distemper virus (PDV) in sea otters and seals, and dolphin morbillivirus (DMV), pilot whale morbillivirus (PWMV), and Longman's beaked whale morbillivirus (LBWMV), which are collectively referred to as cetacean morbillivirus (CMV), that have been found in porpoises, dolphins and whales (Kennedy 1998, DiGuardo *et al.* 2005).

The most common organs affected are the lungs and central nervous system (DiGuardo *et al.* 2005). Sick animals may appear thin, have respiratory difficulties due to pneumonia, and/or exhibit abnormal behavior. However, these signs are also present with other types of illness and are not specific to morbillivirus. When exposed to morbillivirus, some animals mount an antibody response, which may protect them against future infections and severe clinical disease (Van Bressem *et al.* 2009). Other animals may not acquire this protection and can succumb to the disease or to secondary infections that arise as a result of immunosuppression from the infection (Carvalho *et al.* 2012)). Morbilliviruses are usually spread through inhalation of respiratory particles or direct contact between animals (Carvalho *et al.* 2012). Animals can also be exposed to the virus through other entryways such as the eyes, mouth, stomach, skin wounds, and the urogenital tract.

In the United States, there have been morbillivirus mortality events caused by PDV in harbor seals (*Phoca vitulina*) in the northeast (2006) and DMV or PMV in bottlenose dolphins (*Tursiops truncatus*) in the northeast (1987–1988; Lipscomb *et al.* 1994) and Gulf of Mexico (1992 and 1994; Krafft *et al.* 1995, Lipscomb *et al.* 1996). Internationally, there have been outbreaks of morbillivirus in harbor seals in the North Atlantic (1988, 2002; Harkonen *et al*. 2006), in striped dolphins (*Stenella coeruleoalba*) in the Mediterranean (1990-92, 2007-8; Duignan *et al.* 1992, Raga *et al.* 2008) and most recently in bottlenose dolphins in Australia (2009; Stone *et al.* 2011).

The current Unusual Mortality Event (UME) along the Atlantic seaboard of the U.S. was declared by National Marine Fisheries Service on August 8, 2013 due to increased numbers of bottlenose dolphin strandings documented in New York, New Jersey, Delaware, Maryland and Virginia during the months of July and August (NOAA 2013). Since that time strandings have remained elevated and the geographic scope of the event now extends from New York to northern Florida (through Brevard County) with the UME still on-going. In this paper we will provide preliminary findings from this dolphin morbillivirus UME.

MATERIAL AND METHODS

Level A data consisting of species, date, location, carcass condition, sex, and standard length were reported by the U.S. National Marine Mammal Stranding Network (NMMSN) to NMFS' Marine Mammal Health and Stranding Response Program (MMHSRP). Level A stranding records were extracted from the NMFS MMHSRP National Database and the data reported here are the most accurate data available as of April 27, 2014.

Tissue samples were obtained from the NMMSN from cetaceans stranding along the U.S. Atlantic coast. Samples were collected from July, 1, 2014 to present. Representative tissue samples were collected for histopathology, morbillivirus testing, viral isolation, and other diagnostic testing. Tissues for histopathology were processed with routine stains and, if the case dictated, morbillivirus immunohistochemistry (IHC). Additionally, from some live dolphins stranding during the event serum was tested for morbillivirus neutralizing antibody titers.

Morbillivirus testing was performed using the universal morbillivirus primers directed against the phosphoprotein (P) gene (Barret *et al.*1993). Sequencing of the PCR products from the Pan-morbillivirus PCR were compared against DMV sequences in the Genbank database.

Dolphin morbillivirus was isolated in Vero-SLAM, MDCK-SLAM, and Mv1-Lu-SLAM cells (Lednicky and Wyatt, 2012). The presence of cetacean morbillivirus in the cell cultures is indicated by virus-induced cytopathic effects including cell death and/or the formation of syncytia and confirmed by electron microscopy and PCR. New primers were designed based on DMV sequences in GenBank to amplify the entire genome of cetacean morbilliviruses. Infected cetacean tissues were used for amplification whenever possible to eliminate mutations acquired through *in vitro* passaging.

RESULTS

From July 1, 2013, to April 27, 2014, approximately 1242 bottlenose dolphins stranded in the UME area extending from New York to Brevard County in Florida (see map at http://www.nmfs.noaa.gov/pr/health/mmume/midatldolphins2013.html#map). Peak strandings occurred in August and the states with the highest number of strandings were Virginia, North Carolina, and Florida (Figures 1 & 2).

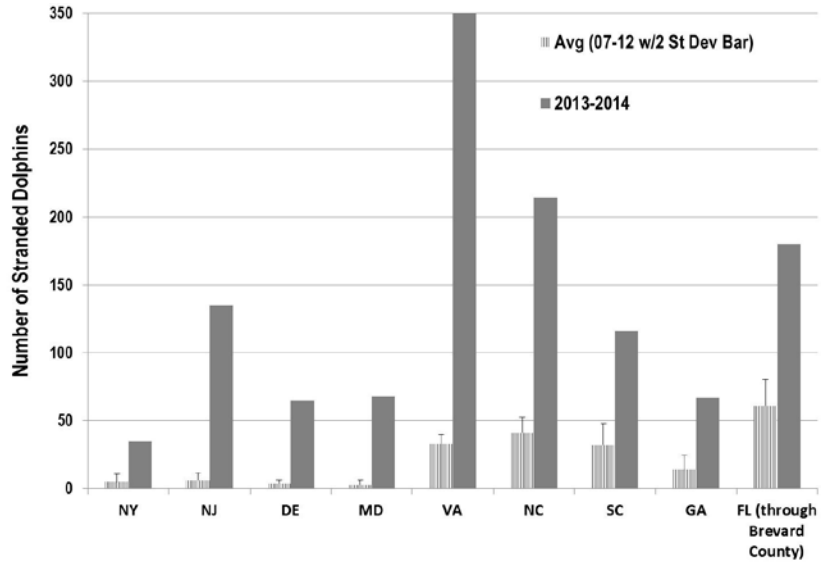

Figure 1: Bottlenose dolphin strandings by state - July 1, 2013-Apr 27, 2014 (n=1242)

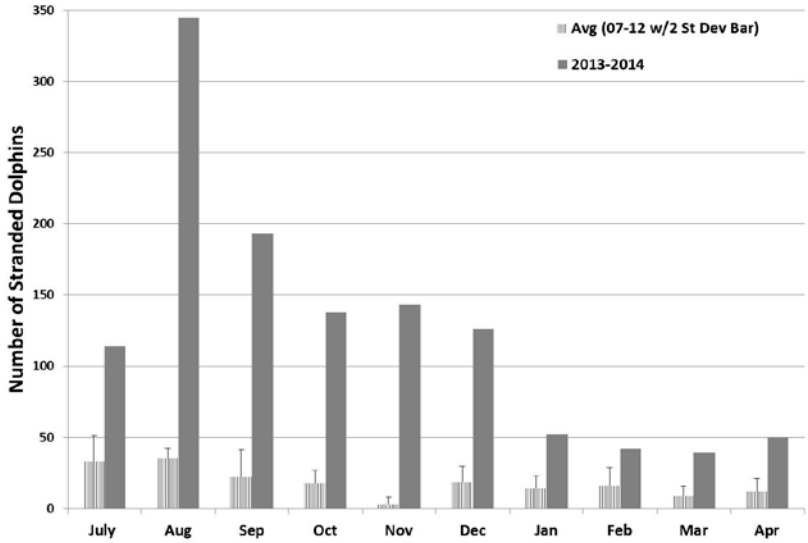

Figure 2: Monthly bottlenose dolphin strandings in all states - July 1, 2013-Apr 27, 2014 (NY to N. FL-Brevard County; n=1242)

Currently over 100 dolphins have had tissues processed for histologic evaluation. The most common histologic lesions observed included bronchointerstitial pneumonia, lymphoid depletion and necrosis, syncytial cells, meningoencephalitis, and skin lesions. Intracytoplasmic and/or intranuclear viral inclusions were less frequently found, but when observed were identified within syncytial cells, skin, mucosa, and urinary bladder epithelium. Lesions secondary to probable immune suppression included bacterial pneumonia, ciliate-associated deep dermatitis, fungal pneumonia, fungal tracheitis, herpesviral infection, and protozoal enteritis. *Brucella* sp. infection was diagnosed in some dolphins with confirmed morbillivirus infections; however, it is not clear whether these infections are truly secondary or whether they may have been chronic, pre-existing infections. Dolphins with chronic morbillivirus infections were often emaciated and had extensive pulmonary fibrosis. Pathologic lesions were similar to the viral lesions noted in the 1987-1988 events (Lipscomb *et al.* 1994, Schulman *et al*. 1997).

Ninety six percent of tissue samples from dolphins from ten states tested for morbillivirus via PCR and/or IHC were positive (213 of 223) and of those, 90% (186 of 207) were confirmed as dolphin morbillivirus by sequencing (Table 1). Serum from 15 DMV-positive bottlenose dolphins was tested for morbillivirus neutralizing antibody titers, which ranged from 32 to 8,192. Virus isolation was successful on 13 animals to date.

State	Suspect/Probable Positive	Confirmed Positive	Negative	Total	% Positive
NY	0	5	0	5	100
NJ	24	22	4	50	92
DE	2	3	0	5	100
MD	10	2	0	12	100
VA	17	30	1	48	98
NC	28	15	1	44	98
SC	6	7	1	14	93
GA	9	0	0	9	100
FL	28	5	3	36	92
Total	**124**	**89**	**10**	**223**	**96%**

Table 1: Summary of bottlenose dolphin morbillivirus PCR/IHC results (as of 4/27/2014; n=223)

Additionally, 72 cetaceans of other species have been tested by PCR for morbillivirus including common dolphins (*Delphinus delphis)*, dwarf sperm whales (*Kogia sima)*, fin whales (*Balaenoptera physalus*), Gervais beaked whales, (*Mesoplodon europaeus)*, harbor porpoises, (*Phocoena phocoena)*, humpback whales (*Megaptera novaeangliae*), pilot whales, (*Globicephala spp.)*, pygmy killer whales (*Feresa attenuate)*, pygmy sperm whales (*Kogia breviceps*), sei whales (*Balaenoptera borealis)*, sperm whales (*Physeter macrocephalus)*, spotted dolphins (*Stenella frontalis)*, striped dolphins, and True's beaked whales (*Mesoplodon mirus).* To date only four species consisting of striped dolphins, pygmy sperm whales, fin whales, and humpback whales have been positive for DMV representing eight individual animals. In only one striped dolphin was DMV confirmed as causing clinical disease based upon compatible histopathological lesions, positive PCR with DMV sequence confirmation, and positive IHC. Preliminary whole genome sequencing of the dolphin morbillivirus genome in several bottlenose dolphins has found the sequences to be 99.9% similar to each other. Additionally, preliminary whole genome sequencing of the DMV genome in two humpback whales, two pygmy killer whales, and one striped dolphin were 99.9% similar to the bottlenose dolphin DMV whole genomes sequenced from this event.

PRELIMINARY CONCLUSIONS

This UME is still ongoing and all results presented here are preliminary. Currently the event appears to be primarily caused by a DMV outbreak that is mostly affecting bottlenose dolphins, although a few additional other cetacean species have also been infected. Additional research evaluating the contribution of co-infections, biotoxin exposure, and contaminants to the UME are pending. Lastly, research is ongoing to better understand the impacts of this large scale outbreak on bottlenose dolphin populations and affected coastal stocks.

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