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Epidemiology of a Phocine Distemper Virus Outbreak Along the North Atlantic Coast of the United States

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Abstract

Due to an increase in pinniped strandings with consistent pathological findings throughout the North Atlantic coast of the United States during the summer and fall of 2006, an unusual mortality event (UME) was declared by the National Oceanic and Atmospheric Administration's (NOAA) National Marine Fisheries Service (NMFS) on 20 October 2006. The goals of this investigation were to describe the magnitude and duration of the peak in mortalities involved in the UME and to evaluate associations with potential causative agents. Seal strandings during the UME were compared to historical strandings in the area to characterize the epidemiologic patterns of the UME. Temporal increases in phocine distemper virus (PDV) prevalence as detected by serology and polymerase chain reaction (PCR) were significantly correlated with increased seal stranding frequency. During July to October 2006, there was a significant spatial and temporal cluster of PDV positive seals centered near Cape Ann, Massachusetts. Our findings provide evidence that PDV infections increased in harbor seals along the North Atlantic coast of the U.S. in 2006, and PDV likely played a role in a UME that involved harbor seals (*Phoca vitulina*), harp seals (*Phoca groenlandica*), hooded seals (*Cystophora cristata*), and gray seals (*Halichoerus grypus*).

Key Words: morbillivirus, rehabilitation, phocine distemper virus, unusual mortality event, UME, seal/pinniped stranding

Introduction

Morbilliviruses have been recognized as a cause of epidemic mortality in pinnipeds over the last 25 y on both sides of the Atlantic but most notably in Europe. Phocine distemper virus (PDV) is a morbillivirus first recognized in marine mammals in Western Europe in the spring of 1988 when, over the next 9 mo, nearly 60% of the North Sea harbor seal (*Phoca vitulina*) population and a few hundred gray seals (*Halichoerus grypus*) died (Heide-Jørgensen et al., 1992). The epidemic quickly spread to the coasts of Sweden, the Netherlands, Norway, Germany, the United Kingdom, and Ireland before ending in early 1989 after an estimated 20,000 harbor seals had died (Dietz et al., 1989; Heide-Jørgensen et al., 1992). A subsequent mass mortality event in 2002, again along the North, Wadden, and Baltic Seas, was estimated to have involved over 30,000 seals (Härkönen et al., 2006). PDV was likely endemic to harp and gray seals in the western North Atlantic waters before the European epidemic in harbor seals (Duignan et al., 2014).

PDV was first recognized in western Atlantic waters in 1992 when an epizootic occurred among harbor seals off the North Atlantic coast of the U.S. (Duignan et al., 1993). One year later, PDV was detected in a harp seal (*Phoca groenlandica*) from the Gulf of St Lawrence, Canada (Daoust et al., 1993). Since then, large mortalities from this virus have not been documented in the U.S.; however, on 20 October 2006, an unusual mortality event (UME) was declared in the northeast U.S. due to

an increase in pinniped strandings with consistent pathological findings. Under the Marine Mammal Protection Act, a UME is declared due to unexpected strandings including a significant die-off of any marine mammal population warranting urgent response (National Oceanic and Atmospheric Administration [NOAA], 2015). The declaration of an UME is important to prompt and fund further investigation that can help understand larger environmental concerns and potential implications for ocean or human health. This UME was declared due to documented clinical presentation of neurologic illness in gray seals, coupled with the high number of mortalities and live-stranded phocidae of multiple species occurring in 2006 throughout the northeast region, extending north to the Canadian border. The declaration of the UME prompted investigations of pinniped stranding events from January 2006 through December 2007 that occurred along the coastline of ten states in the eastern U.S. between southern Virginia and Maine. Subsequent analysis of liver tissue from a harbor seal involved in this UME resulted in the isolation of PDV, suggesting that PDV was a likely cause of at least some of the mortalities reported as part of this UME (Earle et al., 2011). Because PDV can cause mortality among multiple species in a single event, this disease could pose a conservation threat to a naïve population with a high number of susceptible animals.

Herein, we describe the epidemiology of the 2006 UME in pinniped species along the North Atlantic coast (1) to characterize the patterns of pinniped strandings in this region and (2) to describe the pathologic, molecular, and serologic data available for stranded seals sampled during the UME.

Methods

We evaluated retrospective data collected for all harbor, harp, gray, and hooded (*Cystophora cristata*) seals stranding live and dead on the northeast coast of the U.S. from 2002 to 2008 (n = 6,174). Data on species, age class, sex, stranding date, stranding location, rehabilitation center admission, and disposition were collected by members of the National Marine Mammal Stranding Network. Out of the 3,044 seals that stranded live from 2002 to 2008, 2,078 of these seals could be captured and admitted to eight rehabilitation centers from Maine to Virginia. Most of these rehabilitated animals had serum collected, and samples were screened using serology for common pathogens, including PDV, phocine herpes viruses (PhHV) 1 and 2, *Brucella* spp., and *Leptospira* spp. using serology. Beginning in 2006, nasal and ocular swabs, feces, and buffy coat of centrifuged blood were also collected for PDV real-time reverse transcription-polymerase chain reaction (rRT-PCR) testing. A subset of stranded animals in 2006 to 2007 that were found fresh dead or that died during rehabilitation also had a completed necropsy performed with histologic evaluation of all major tissues (n = 69).

Sample Analysis

Swabs and serum from stranded seals were sent to the Oklahoma Animal Disease Diagnostic Laboratory, Center for Veterinary Health Sciences, in Stillwater, Oklahoma, for the pathogen-specific diagnostic assays listed below. For PDV, a serum neutralization test was conducted to measure antibodies against the H and the fusion glycoproteins of the virus (n = 410; Duignan et al., 1994). Paired serum samples demonstrating an increasing antibody titer is the gold standard for assessing recent infection with PDV; however, as repeated samples were often not available, a titer of 1:32 or higher was considered indicative of PDV exposure (Thompson et al., 2002). Swabs and tissues from 254 dead animals were tested by rRT-PCR as described (Saliki et al., 2002; Earle et al., 2011). Seals were considered a *PDV positive case* if they had a serological titer of 1:32 or higher, or if PDV viral RNA was detected via rRT-PCR. A diagnosis of PDV as the cause of death was made by the pathologist if histological lesions included viral inclusions in tissues, meningoencephalitis, bronchointerstitial pneumonia, and/or lymphoid depletion; and seals were either (1) PDV positive by rRT-PCR on one or more tissues or (2) PDV positive by serologic titer of greater than 1:64.

A macroscopic slide agglutination test was performed as described (Colagross-Schouten et al., 2002) on serum samples for *Leptospira bratislava* (n = 286), *Leptospira canicola* (n = 284), *Leptospira grippotyphosa* (n = 284), *Leptospira hardjo* (n = 284), *Leptospira icterohaemorrhagiae* (n = 215), and *Leptospira pomona* (n = 284). Seals with clinical leptospirosis infections generally have titers of 1:1,600 to 1:12,800 (Dunn et al., 2009), but a titer of 1:100 or higher was considered positive (Mackereth et al., 2005) to increase sensitivity. Serum samples (n = 255) were tested for *Brucella abortus* and *Brucella canis* using the standard card agglutination test. PhHV-1 and -2 exposure were evaluated in serum samples (n = 299) using a serum neutralization test against the Atlantic isolate of PhHV-1 from harbor seals, and a titer of 1:8 or higher was considered positive (Goldstein et al., 2004).

Statistical Analysis

Patterns in exposure to PDV, PhHV-1 and -2, *B. abortus*, *L. bratislava*, *L. grippotyphosa*,

L. hardjo, and *L. icterohaemorrhagiae* were evaluated independently among all stranded seals with serologic data. To identify temporal and spatial clustering of pathogen exposure in seals, the spatial, temporal, and space-time *SaTScan* statistics were used with a Bernoulli probability model scanning for clusters with high and low rates of seroprevalence (Salman, 2003; Kulldorff & Information Management Services, 2006). Stranding dates were aggregated into 7- and 30-d aggregates for temporal and spatiotemporal cluster analyses. Serologic results for all pathogens detected during the UME were evaluated for associations with species, sampling year, age classes, sex, live vs dead status at stranding, and disposition (for individuals that stranded live) using the chi square test of independence. When significant spatial, temporal, or space-time clusters were detected, cluster boundaries were used to categorize strandings as within or outside of the specific cluster for multivariate logistic regression analyses using cluster, species, and age (dichotomized as adult and juvenile) to predict the likelihood of being positive for each pathogen.

Bivariate cross-correlation time series analysis was used to evaluate the temporal association of PDV prevalence in stranded animals (as determined by positive serologic assay or positive rRT-PCR divided by the number of animals tested) and stranding frequency from 2004 to 2007 in this geographic region as described above. Because sampling for diagnostic testing was more complete in 2006 and 2007, analysis for the cross-correlation time series was broken up into two time intervals—2004 to 2005 and 2006 to 2007. Time series correlations were evaluated at six consecutive 2-wk time lags to determine whether there was a time lag between increased overall stranding frequency and first detection of *B. abortus*, *L. bratislava*, *L. grippotyphosa*, *L. hardjo*, *L. icterohaemorrhagiae*, PhHV-1, and PDV exposure in stranded seals (x = stranding frequency; y = pathogen prevalence). Temporal associations were not evaluated for *L. grippotyphosa* and *L. icterohaemorrhagiae* in 2004 to 2005 because prevalence was extremely low for these pathogens during this period.

For initially seronegative PDV seals admitted to rehabilitation centers, the association between PDV seroconversion (from negative to positive PDV status) and time in rehabilitation was analyzed. Time spent in rehabilitation was dichotomized using the incubation period of 7 d for PDV (Harder et al., 1990). Multivariate logistic regression was used to determine the likelihood of being released from a rehabilitation center based on species, age class, sex, and PDV serologic status for seals undergoing rehabilitation. *STATA/SE*, Version 9.2 (College Station, TX, USA) was utilized for all statistical analysis, and $p \leq 0.05$ was used to determine significance of statistical tests.

Results

Seal strandings from January 2002 to December 2008 on the northeast coast of the U.S. (n = 6,174) were highly variable in time. While seal strandings were elevated in the months prior to the declaration of a UME in 2006, similar peaks in overall stranding frequency were observed in the spring and fall of 2004 and the spring and summer of 2005 (Figure 1). The highest numbers of strandings in a single calendar year were in 2004 (n = 1,302) due to a major increase in harbor seal strandings compared to the previous year (Table 1). The frequency of harbor seal strandings remained high through 2005 and 2006, after which the number of harbor seal strandings dropped substantially.

As shown in Table 1, species composition among seals stranding along the northeast coast varied significantly by year ($p < 0.001$). Harbor seals were the most commonly stranded species (n = 3,849), with significant increases in 2004 and 2005. Harp seals were the second most common species stranding in this area (n = 1,336) between 2002 and 2008, with the highest number in 2006 (n = 269). Gray seals were the next most common species (n = 766), with the highest number in 2007 (n = 179). Hooded seals (n = 223) stranded infrequently in most years, with the highest number in 2006. The age class of stranded gray, harbor, harp,

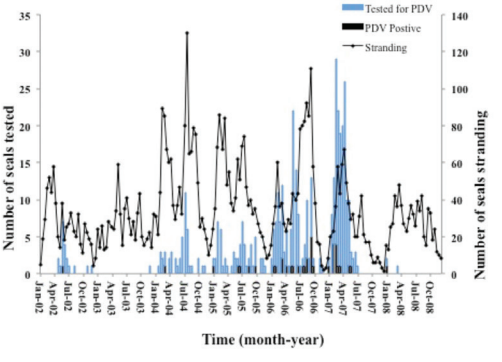

Figure 1. The total number of seals (n = 6,174) that stranded live and dead on the coast of the northeast U.S. from 2002 to 2008 and the number of gray, harbor, harp, and hooded seals tested for phocine distemper virus (PDV) and positive for PDV as determined by serology or real-time reverse transcription-polymerase chain reaction (rRT-PCR) (n = 410)

Table 1. Distribution of species and mean annual strandings among seals that stranded live and dead on the coast of the eastern U.S., along the North Atlantic, from 2002 to 2008 (N = 6,174)

Species	2002	2003	2004	2005	2006	2007	2008	Total
Gray seal	112	138	31	21	147	179	138	766
Harbor seal	299	402	1,066	895	627	240	320	3,849
Harp seal	233	132	179	165	269	214	144	1,336
Hooded seal	22	29	26	47	63	19	17	223
Total	666	701	1,302	1,128	1,106	652	619	6,174

and hooded seals varied significantly by year ($p <$ 0.001). Among seals with known age class (n = 5,481), the majority of seals stranding in 2002 through 2008 were pups and yearlings (76.4%). Approximately half (49.3%) of the stranded seals were live at the time of stranding (see Table 2).

From July to October 2006, the 4 mo prior to the declaration of the UME on 20 October 2006, there were 68.7% more seal strandings than had been observed between July and October in the previous year. Unlike all other years from 2002 to 2008, more seals stranded from July to October in 2006 (n = 619) than stranded during all other months during 2006 (n = 487). Other than this increase in overall number of stranded pinnipeds, there were no significant changes in species or age class composition in the UME months compared to the expected distribution during these months.

However, a spatiotemporal cluster of PDV positive cases occurred from 1 July to 31 October 2006 when stranded seals were significantly more likely to test positive for PDV than stranded seals at other times (p = 0.001). These findings suggest that pinniped strandings during the summer of 2006 corresponded to an outbreak in PDV. The total number of animals stranding during 1 July to 31 October 2006 was 619, with the majority of seals dead at the time of stranding (72.5%). As expected, harbor seals were the dominant species to strand during this time period as 373 harbor seals stranded from July to October 2006, compared to 40 hooded seals, 73 gray seals, and 133 harp seals.

Pathogen-Specific Diagnostic Test Findings

Pathogen-specific diagnostic testing effort on stranded seals increased substantially in summer 2006 and spring 2007 (Figure 1). The majority of samples (391/408, 95.8%) were taken from live seals stranding in 2004 (n = 42), 2005 (n = 54), 2006 (n = 116), and 2007 (n = 154). Results indicated seals were exposed to PDV, *B. abortus*, *L. bratislava*, *L. grippotyphosa*, *L. hardjo*, *L. icterohaemorrhagiae,* and PhHV-1 (Table 3). Seals were not exposed to *L. pomona*, *L. canicola*, and *B. canis*, and seals had an extremely low seroprevalence (0.3%) to PhHV-2. Among the 9.6% (39/408) of stranded seals that tested positive for PDV using serology, harbor seals (28/173, 16.2%) and gray seals (8/90, 8.9%) had the highest seroprevalence, followed by harp seals (3/121, 2.5%), with no exposure detected among 24 hooded seals tested. PDV seroprevalence in harbor seals increased with age from 6.4% of pups (8/125), 18.8% of yearlings (6/32), 75% of subadults (6/8), and 100% of adult harbor seals (7/7) positive for PDV antibodies. Among seals positive for PDV by rRT-PCR (9/254, 3.5%), harbor seals had the highest prevalence (8/97, 8.2%) followed by harp seals (1/61, 1.6%); hooded (n = 8) and gray seals (n = 72) were negative on rRT-PCR.

Positive serology and rRT-PCR cases were combined to identify high-risk temporal and spatiotemporal clusters of PDV positive cases. A spatiotemporal cluster of PDV positive cases occurred from 1 July to 31 October 2006 at 42.816430 N, 70.815370 W, with a diameter of 213.5 km, 5 km southeast of Salisbury, Massachusetts (Figure 2). Within this cluster, risk for PDV was 9.2 times higher than expected as 10 out of 11 stranding harbor seals were positive for PDV, when only one PDV positive case was expected. The purely temporal scan test similarly detected a seven-and-a-half fold higher than expected number of PDV positive cases from 1 July to 31 October 2006 (p = 0.001). Temporal increases in PDV prevalence from 2006 to 2007 were significantly correlated in real time with overall stranding frequency (r^2 = 0.4559, p = 0.003). Increased PDV prevalence was also associated with overall stranding frequency between 2006 and 2007 when bimonthly seal strandings lagged PDV prevalence by 4 wks (r^2 = 0.4269, p = 0.005).

The seroprevalence of PDV in seals was low (5/95, 5.3%) during the 6 mo before the high-risk spatiotemporal cluster. Seroprevalence increased to 28% (7/25) during the high-risk cluster and decreased again 6-mo post-cluster to 9.1% (13/143). This observed pattern was largely due to increased PDV seroprevalence in harbor seals (7/14, 50%) as no hooded seals were seropositive

Table 2. Distribution of species and age class among seals that stranded live or dead on the coast of the eastern U.S., North Atlantic, from 2002 to 2008 (n = 2,579)

Species	Year	Adult	Subadult	Yearling	Pup	Unknown	Total
Gray seal	2002	12	12	23	21	5	73
	2003	14	5	19	51	9	98
	2004	17	10	30	34	5	96
	2005	13	7	20	55	16	111
	2006	12	4	10	42	8	76
	2007	23	11	47	77	10	168
	2008	25	18	43	33	13	132
	Total	116	67	192	313	66	754
Harbor seal	2002	41	18	73	181	22	335
	2003	95	35	63	233	28	454
	2004	210	46	95	417	25	793
	2005	59	15	95	393	44	606
	2006	216	53	58	389	25	741
	2007	46	19	29	132	14	240
	2008	31	15	66	233	22	367
	Total	698	201	479	1,978	180	3,536
Harp seal	2002	14	34	133	0	9	190
	2003	3	15	69	2	7	96
	2004	29	31	256	2	16	334
	2005	6	13	215	2	60	296
	2006	4	2	148	1	13	168
	2007	7	6	164	4	26	207
	2008	3	1	106	0	34	144
	Total	66	102	1,091	11	165	1,435
Hooded seal	2002	0	12	13	2	3	30
	2003	1	3	11	4	0	19
	2004	2	4	28	2	4	40
	2005	0	0	40	0	10	50
	2006	0	0	55	2	13	70
	2007	0	1	17	0	1	19
	2008	0	1	8	0	2	11
	Total	3	21	172	10	33	239

(0/8, 0%), and gray and harp seals were not tested during that time period, likely due to their low stranding frequency. Among PDV cases detected by rRT-PCR, PDV infection was evident in 30% of stranded seals (3/10) in the 6-mo pre-cluster, 14.3% (5/35) during the cluster, and 0.68% (1/146) in the 6-mo post-cluster.

Seroprevalence of *B. abortus*, *L. bratislava*, *L. grippotyphosa*, and *L. hardjo* was also significantly higher from July to October 2006 than at other times (Table 3). While seals were 10 times more likely to be PDV positive during July to October 2006, they were also five times more likely to be *B. abortus* positive. While *B. abortus* and *B. canis* were the only *Brucella* spp., tested herein, there are other *Brucella* spp., such as *B. pinnipedialis*, that could have been present due to the cross-reactivity of *Brucella* spp. However, seals were nearly 50% less likely to be PhHV-1 positive during this time period (Table 3). Temporal and spatial clusters were not detected for the other pathogens, *B. abortus*, *L. bratislava*, *L. grippotyphosa*, *L. hardjo*, *L. icterohaemorrhagiae*, and PhHV-1, and bivariate cross-correlation time series showed no associations with strandings and exposure to these other pathogens.

Table 3. The prevalence of serologic and molecular positive results for 11 pathogens in gray, harbor, harp, and hooded seals shown for two time periods: (1) during the UME period (July to October 2006) and (2) during the non-UME period (2002 to 2008)

	July-October 2006	2002-2008	Overall
Brucella abortus serology*	4/8 = 50%	41/247 = 17%	45/255 = 17.6%
Brucella canis serology	0/8 = 0%	1/247 = 0.4%	1/255 = 0.4%
Leptospirosis spp. serology			
*L. bratislava**	9/14 = 64%	68/272 = 25%	77/286 = 26.9%
*L. grippotyphosa**	3/14 = 21%	14/269 = 5.2%	17/283 = 6.0%
*L. hardjo**	8/12 = 66%	61/272 =25%	69/284 = 24.3%
L. icterohaemorrhagiae	1/4 = 25%	39/211 = 18%	40/215 = 18.6%
L. pomona	0/12 = 0%	0/211 = 0%	0/223 = 0%
L. canicola	0/12 = 0%	0/211 = 0%	0/223 = 0%
Phocine herpes virus (PhHV) 1 serology	3/10 = 30%	147/289 = 51%	150/299 = 50.2%
Phocine herpes virus (PhHV) 2 serology	0/10 = 0%	1/289 = 0.35%	1/299 = 0.3%
Phocine distemper virus (PDV) serology*	7/25 = 28%	26/385 = 7%	33/410 = 8.0%
Phocine distemper virus (PDV) PCR*	5/35 = 14%	4/219 = 1%	9/254 = 3.5%

*Prevalence significantly different between time periods

Figure 2. Map of the northeast U.S. showing the significant spatiotemporal cluster of PDV cases from 1 July to 31 October 2006. The red circles represent the stranding location for positive PDV cases (n = 10), and the blue circle represents a negative case (n = 1). The circle with a star identifies the location of the marine mammal unusual mortality event (MMUME) declared by the National Oceanic Atmospheric Administration on 20 October 2006. The black circle shows the diameter of 213.5-km spatiotemporal cluster of PDV positive cases.

We evaluated the association between PDV serologic status and time in rehabilitation to assess whether PDV was actively circulating and infecting previously uninfected seals at rehabilitation centers. All but one of the PDV positive seals were seropositive to PDV on the first test after admission to a rehabilitation facility. One harp seal was technically seronegative on Day 1 (1:16) and seroconverted (1:48) on Day 20, but this animal could have been recently infected just prior to arriving at the rehabilitation facility. We found seals that had been tested repeatedly using the serologic assay (one harp, four harbor, and two gray seals) had elevated PDV antibody titers (1:32 or higher) for up to 60 d (median = 49 d). The length of stay at a rehabilitation facility was not associated with developing PDV positive status. Furthermore, 26 seals that were seronegative on admission were resampled two or more times and remained seronegative on subsequent tests, suggesting these individuals did not become infected with PDV at rehabilitation facilities. However, repeated sampling of seronegative individuals admitted to rehabilitation facilities was relatively infrequent and this limited our ability to fully assess evidence for PDV transmission at the rehabilitation facilities.

Pathologic Features in Stranded Seals

Pathology reports were available for 69 seals that stranded between January 2006 and December 2007. Only 14 seals with complete necropsy data

also had serologic results for PDV. Cause of death was not determined in nearly 50% (30/69) of seals due to lack of significant findings on pathology. For stranded seals necropsied from July to October 2006, the proportion of seals with PDV infection was 21.9% (7/32). All PDV positive cases were harbor seals (7/24, 29.2%); none of the necropsied hooded seals (0/8, 0%) were PDV positive. The proportionate mortality due to suspected PDV for seals that stranded outside this time period was 8.1% (3/37) among harbor seals (2/23, 8.7%), gray seals (1/4, 25%), harp seals (0/7, 0%), and hooded seals (0/3, 0%) necropsied. Other necropsy findings (n = 17) were nonspecific, but the pathologic features were consistent with morbilliviral infection (bronchopneumonia, bronchointerstitial pneumonia, non-suppurative meningoencephalitis, suppurative meningoencephalitis, and bacterial meningitis). Other causes of death (n =13) included hypernatremia, sepsis, verminous gastroenteritis, enterocolitis, enteritis, leptomeningitis, and cardiac disease.

Factors Influencing Survival at Rehabilitation Centers

Among seals admitted to rehabilitation centers (n = 418), the proportion of seals surviving to be released differed significantly among species, with gray seals (76/98, 77.6%) and harp seals (88/116, 75.9%) having the highest survival followed by harbor seals (92/180, 51.1%) and hooded seals (13/24, 54%; $X^2 = 29.0$, $p < 0.001$). Harbor seals had a very low survival among yearling (10/33, 30.3%) and pup (66/131, 50.4%) age classes, while survival was higher for adults (5/7, 71.4%) and subadults (7/7, 100%; $X^2 = 13.4$, $p = 0.004$). In a multivariate analysis, seals that were PDV PCR positive or seropositive were two times more likely (95% confidence interval [CI] 1.0 to 4.2%) to die than seronegative seals, and harbor and hooded seals were three times more likely (CI 2.0 to 5.0% and 1.2 to 7.1%, respectively) to die during rehabilitation compared to harp and gray seals. When age and species interaction was added to the model alone, harbor seal pups and yearlings were 11.5 times (CI 2.5 to 51.9%) more likely to die compared to hooded, harp, and gray seals.

Discussion

High inter-annual variability in strandings of marine mammals is common, and UMEs can be very difficult to recognize, particularly when multiple species are affected. Epidemiologic data prior to and after stranding events can be used as evidence to further define a biological UME period retrospectively and put the number of strandings in context of expected morbidity and mortality in the area. We found that the number of stranded seals in the northeast region of the U.S. in the summer and fall of 2006 was relatively typical of the overall highly variable stranding pattern that has been observed in this region from 2002 to 2008. However, we provide epidemiological evidence supporting PDV infection as a major cause of the multi-species strandings that peaked in the summer and fall of 2006. We detected a cluster of PDV positive seals stranding between July and October 2006 along the U.S. east coast from Massachusetts extending north to Maine. During this 4-mo time period, PDV seroprevalence increased to nearly 30%, and rRT-PCR confirmed active PDV infection in five seals. We also found that stranding frequency was significantly positively correlated with PDV prevalence in time, providing evidence that PDV played a role in this UME.

Phocine distemper virus is well-recognized for causing large-scale epizootics in pinnipeds from Northern Europe and the UK. Since 1988, PDV exposure has been recognized in stranded harbor and gray seals and in free-ranging harp seals, hooded seals, and ringed seals (*Pusa hispida*) in the Canadian Arctic and western North Atlantic waters (Duignan et al., 1995, 1997). A study confirmed PDV infection in a dead harbor seal from the 2006 UME investigated herein (Earle et al., 2011). Phylogenetic analysis indicated this virus was closely related to the virus found during the 1988 PDV epidemic in the North Sea, suggesting that PDV has been circulating in North American seals for some time or indicating that there was a common source of infection such as in seals in the Arctic. Although there is some speculation as to the source of PDV, it is believed that the virus most likely originated in European harp seals. This virus then may have spread to North American harbor seals due to an unusual migration of harp seals into the North Sea (Duignan et al., 1995).

Evidence of previous exposure and conferred immunity to PDV was generally low among the harbor, gray, and harp seals tested during our study period prior to this epidemic in the northeastern U.S., but we detected a threefold increase in seroprevalence among stranded harbor seals during the 2006 epidemic. As with species-specific differences reported for mortalities in the large-scale European PDV epizootics (Hall et al., 2006), harbor seals in this 2006 epidemic were three times more likely to die compared to gray and harp seals, consistent with other studies that have shown that harbor seals are more susceptible to severe infection (Härkönen et al., 2006). Gray seals are less susceptible to PDV, and PDV epizootics in gray seals are likely prevented because the virus is continuously circulating in the population,

creating a certain level of herd immunity (Duignan et al., 1995).

In 2011, the same population of harbor seals was infected with avian influenza virus (AIV) subtype H8N3. Over a 4-mo period beginning in September 2011, 162 harbor seals less than 6 mo of age were found dead or moribund along the northeast coast of the U.S. (Anthony et al., 2012). In addition to influenza A and B, further pathogens to consider for involvement in this UME in this population of seals are *L. bratislava*, *L. grippotyphosa,* and *L. hardjo*. During the UME period, seroprevalence was significantly higher for these pathogens compared to other periods. However, in light of the fact that temporal and spatial clusters were not detected for leptospirosis, and bivariate cross-correlation time series showed no associations with strandings and exposure to leptospirosis (despite using a low titer of 1:100), it is unlikely that leptospirosis played a role. Lastly, out of the 69 necropsies conducted, only one adult female harbor seal was confirmed serologically as having leptospirosis with a titer of 1:1,600 for *L. hardjo*; and although no Leptospira bacteria were isolated in the tissues, histological lesions supported Leptospira infection. While the cause of the UME was most likely PDV as demonstrated by the epidemiological analysis herein and by previous isolation of PDV by Earle et al. (2011), influenza and other zoonotic pathogens like leptospirosis are important pathogens to consider with any UME involving this population of seals (Earle et al., 2011). Due to the immunosuppressive nature of PDV, animals are more susceptible to secondary infections by other pathogens, and an increased occurrence of co-infections may characterize a PDV-infected individual.

Thousands more seals perished during the European outbreaks in 1988 and 2002 than in both U.S. epizootics documented to date (Heide-Jørgensen et al., 1992; Duignan et al., 1993). In the northeastern U.S. seal population studied herein, seroprevalence was higher in older age classes, with pups having the least serologic evidence of immunity. Higher seroprevalence in adults has been found in other studies (Thompson et al., 1992), and these data indicate some adults exposed to PDV will survive infection. Variation in pathogenicity of morbillivirus strains has been previously reported (Earle et al., 2011). The progression and impact of both epizootics in 1988 and 2002 were fairly similar; however, there were significant differences in mortality between and within the seal populations in the UK. An epidemiological model suggested the mortality differences between the two epizootics was due to the reproductive rate (R_0) of the virus as the R_0 dropped by 27% from 1988 to 2002. The geographic differences in population effects within the epizootics in the UK can be explained by the case mortality but not R_0 as it was similar within populations (Lonergan et al., 2010). This suggests that either more individuals were exposed to PDV or more infected individuals died; however, the underlying reason for this is not known. Similar prospective longitudinal studies are needed in the U.S. to determine whether pathogen strain variation, host susceptibility, or cycles in population-level immunity may also play a role in the timing and severity of PDV outbreaks in the phocid community.

In this epizootic from 1 July to 31 October 2006, biosecurity measures, including isolation of newly admitted seals, were strictly followed at all rehabilitation centers, and no new cases of PDV were detected in seals while in rehabilitation. In future, a clear PDV outbreak protocol, including decision rules for rehabilitation facility isolation, euthanization, and determining viral status, will allow for a highly effective response to infectious disease outbreaks. Similarly, a diagnostic plan with serology, virus identification, and rapid reporting is necessary to learn more about host recovery, immunity, species susceptibility, role of reservoirs, and virulence of the virus. Standardization of sampling frequency (i.e., every 7 d) during rehabilitation would facilitate diagnosis of PDV cases in a timely manner.

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Literature Cited

Anthony, S. J., St. Leger, J. A., Pugliares, K., Ip, H. S., Chan, J. M., Carpenter, Z. W., . . . Lipkin, W. I. (2012). Emergence of fatal avian influenza in New England harbor seals. *mBio*, *3*(4), e00166-12–e00166-12. https://doi.org/10.1128/mBio.00166-12

Colagross-Schouten, A., Mazet, J., Gulland, F. M. D., Miller, M., & Hietala, S. (2002). Diagnosis and seroprevalence of leptospirosis in California sea lions from coastal California. *Journal of Wildlife Diseases*, *38*(1), 7-17. https://doi.org/10.7589/0090-3558-38.1.7

Daoust, P., Haines, D., Thorsen, J., Duignan, P., & Geraci, J. R. (1993). Phocine distemper in a harp seal (*Phoca groenlandica*) from the Gulf of St. Lawrence, Canada. *Journal of Wildlife Diseases*, *29*(1), 114-117. https://doi.org/10.7589/0090-3558-29.1.114

Dietz, R., Heide-Jørgensen, M. P., & Härkönen, T. (1989). Mass deaths of harbor seals (*Phoca vitulina*) in Europe. *Ambio*, *18*, 258-264.

Duignan, P., Duffy, N., Rima, B., & Geraci, J. R. (1997). Comparative antibody response in harbour and grey seals naturally infected by a morbillivirus. *Veterinary Immunology and Immunopathology*, *55*(4), 341-349. https://doi.org/10.1016/S0165-2427(96)05719-4

Duignan, P., Sadove, S., Saliki, J., & Geraci, J. R. (1993). Phocine distemper in harbor seals (*Phoca vitulina*) from Long Island, New York. *Journal of Wildlife Diseases*, *29*(3), 465-469. https://doi.org/10.7589/0090-3558-29.3.465

Duignan, P., Saliki, J., St. Aubin, D., House, J., & Geraci, J. R. (1994). Neutralizing antibodies to phocine distemper virus in Atlantic walruses (*Odobenus rosmarus rosmarus*) from Arctic Canada. *Journal of Wildlife Diseases*, *30*(1), 90-94. https://doi.org/10.7589/0090-3558-30.1.90

Duignan, P., Saliki, J., St. Aubin, D., Early, G., Sadove, S., House, J., . . . Geraci, J. R. (1995). Epizootiology of morbillivirus infection in North American harbor seals (*Phoca vitulina*) and gray seals (*Halichoerus grypus*). *Journal of Wildlife Diseases*, *31*(4), 491-501. https://doi.org/10.7589/0090-3558-31.4.491

Duignan, P. J., Van Bressem, M. F., Baker, J. D., Barbieri, M., Colegrove, K. M., De Guise, S., . . . Wellehan, J. F. (2014). Phocine distemper virus: Current knowledge and future directions. *Viruses*, *6*(12), 5093-5134. https://doi.org/10.3390/v6125093

Dunn, L., Buck, J. J., & Robeck, T. (2009). Bacterial diseases of cetaceans and pinnipeds. In L. A. Dierauf & F. M. D. Gulland (Eds.), *CRC handbook of marine mammal medicine* (pp. 312-314). Boca Raton, FL: CRC Press.

Earle, J., Melia, M., Doherty, N., Nielsen, O., & Cosby, S. (2011). Phocine distemper virus in seals, east coast, United States, 2006. *Emerging Infectious Diseases*, *17*(2), 215-220. https://doi.org/10.3201/eid1702.100190

Goldstein, T., Mazet, J., Gulland, F. M. D., Rowles, T., Harvey, J., Allen, S., . . . Stott, J. L. (2004). The transmission of phocine herpesvirus-1 in rehabilitating and free-ranging Pacific harbor seals (*Phoca vitulina*) in California. *Veterinary Microbiology*, *103*(3-4), 131-141. https://doi.org/10.1016/j.vetmic.2004.06.017

Hall, A., Jepson, P., Goodman, S., & Härkönen, T. (2006). Phocine distemper virus in the North and European Seas – Data and models, nature and nurture. *Biological Conservation*, *131*(2), 221-229. https://doi.org/10.1016/j.biocon.2006.04.008

Harder, T., Willhaus, T., Frey, H. R., & Liess, B. (1990). Morbillivirus infections of seals during the 1988 epidemic in the Bay of Heligoland: III. Transmission studies of cell culture-propagated phocine distemper virus in harbor seals (*Phoca vitulina*) and a grey seal (*Halichoerus grypus*): Clinical, virological and serological results. *Zentralbl Veterinarmed B*, *37*, 641-650. https://doi.org/10.1111/j.1439-0450.1990.tb01109.x

Härkönen, T., Dietz, R., Reijnders, P., Teilmann, J., Harding, K., Hall, A., . . . Thompson, P. (2006). The 1988 and 2002 phocine distemper virus epidemics in European harbour seals. *Diseases of Aquatic Organisms*, *68*, 115-130. https://doi.org/10.3354/dao068115

Heide-Jørgensen, M., Härkönen, T., Dietz, R., & Thompson, P. (1992). Retrospective of the 1988 European seal epizootic. *Diseases of Aquatic Organisms*, *13*, 37-62. https://doi.org/10.3354/dao0130377

Krogsrud, J., Evensen, O., Holt, G., Hoie, S., & Markussen, N. H. (1990). Seal distemper in Norway in 1988 and 1989. *Veterinary Record*, *126*, 460-461.

Kulldorff, M., & Information Management Services. (2006). SaTScan™ *v7.0: Software for the spatial and space-time scan statistics*. Retrieved from www.satscan.org

Lonergan, M., Hall, A., Thompson, H., Thompson, P., Pomeroy, P., & Harwood, J. (2010). Comparison of the 1988 and 2002 phocine distemper epizootics in British harbour seal *Phoca vitulina* populations. *Diseases of Aquatic Organisms*, *88*, 183-188. https://doi.org/10.3354/dao021533

Mackereth, G., Webb, K., O'Keefe, J., Duignan, P., & Kittelberger, R. (2005). Serological survey of preweaned New Zealand fur seals (*Arctocephalus forsteri*) for brucellosis and leptospirosis. *New Zealand Veterinary Journal*, *53*(6), 428-432. https://doi.org/10.1080/00480169.2005.36588

National Oceanic and Atmospheric Administration (NOAA), National Marine Fisheries Service. (2015). *Marine mammal unusual mortality events*. Retrieved from www.nmfs.noaa.gov/pr/health/mmume/events

Saliki, J., Cooper, E., & Gustavson, J. (2002). Emerging morbillivirus infections of marine mammals. *Annals*

of the New York Academy of Sciences, *969*(1), 51-59. https://doi.org/10.1111/j.1749-6632.2002.tb04350.x

Salman, M. D. (Ed.). (2003). *Animal disease surveillance and survey systems: Methods and applications*. Ames: Iowa State Press. 222 pp. https://doi.org/10.1002/9780470344866

Thompson, P., Thompson, H., & Hall, A. (2002). Prevalence of morbillivirus antibodies in Scottish harbour seals. *Veterinary Record*, *151*(20), 609-610. https://doi.org/10.1136/vr.151.20.609

Thompson, P., Cornwell, H., Ross, H., & Miller, D. (1992). Serologic study of phocine distemper in a population of harbor seals in Scotland. *Journal of Wildlife Diseases*, *28*(1), 21-27. https://doi.org/10.7589/0090-3558-28.1.21