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Detection of Wellfleet Bay Virus Antibodies in Sea Birds of the Northeastern USA

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ABSTRACT: Wellfleet Bay virus (WFBV) is a recently described orthomyxovirus isolated from the tissues of Common Eiders (*Somateria mollissima*) collected during recurrent mortality events on Cape Cod, Massachusetts, US. Coastal Massachusetts is the only location where disease or mortality associated with this virus has been detected in wild birds, and a previous seroprevalence study found a significantly higher frequency of viral exposure in eiders from this location than from other areas sampled in North America. This suggests that coastal Massachusetts is an epicenter of WFBV exposure, but the reason for this is unknown. Opportunistic sampling of sympatric species and testing of banked serum was used to investigate potential host range and spatiotemporal patterns of WFBV exposure. Antibodies were detected in Herring Gulls (*Larus argentatus*), Ring-billed Gulls (*Larus delawarensis*), a White-winged Scoter (*Melanitta fusca*), and a Black Scoter (*Melanitta nigra*). These findings demonstrate the likely occurrence of fall/winter transmission, expand our understanding of the host range of the virus, and provide further insight into the epidemiology of WFBV in the northeastern US.

Key words: Black Scoter, Common Eider, Herring Gull, Ring-billed Gull, Wellfleet Bay virus, White-winged Scoter.

Wellfleet Bay virus (WFBV) is a recently described orthomyxovirus isolated from the tissues of Common Eiders (*Somateria mollissima*) collected on Cape Cod, Massachusetts, US (41°54′15″N, –70°2′33″W) during recurrent mortality events (Allison et al. 2015). These mortality events occur regularly in the autumn with less-frequent spring events. Relatively little is known about this virus including its origin, host range, and routes of transmission, but it is suspected to be an arbovirus due to its phylogenetic classification within the genus *Quaranjavirus*. An extensive longitudinal study on the geographic distribution of WFBV in Common Eiders was conducted across the ranges of the Dresser's (*Somateria mollissima dresseri*) and the Northern (*Somateria mollissima borealis*) subspecies (Clements et al. 2015; Ballard et al. 2017). That study demonstrated a significantly higher seroprevalence ($P<0.001$) in Common Eiders from the Massachusetts/Rhode Island area (16.3%; 63/387) compared to the other areas sampled: Iceland (0%; 0/52), Maine (3%; 11/346), Nova Scotia (3%; 6/180), Nunavut (0%; 0/530), and Quebec (1%; 7/501). Massachusetts is also the only location where WFBV-associated mortality has been reported. These findings suggest that coastal Massachusetts may be an epicenter of WFBV exposure, but the reason for this distribution is unknown.

One possible explanation for the unique role of coastal Massachusetts in WFBV epidemiology is its location within the species' range. This area is the southernmost extent of the Common Eider's breeding range and also serves as an important wintering area for birds from nesting colonies in Maine, Quebec, Nova Scotia, and Labrador. Birds at this location may be exposed to sympatric species, arthropod vectors, or environmental factors from which other eider populations are geograph-

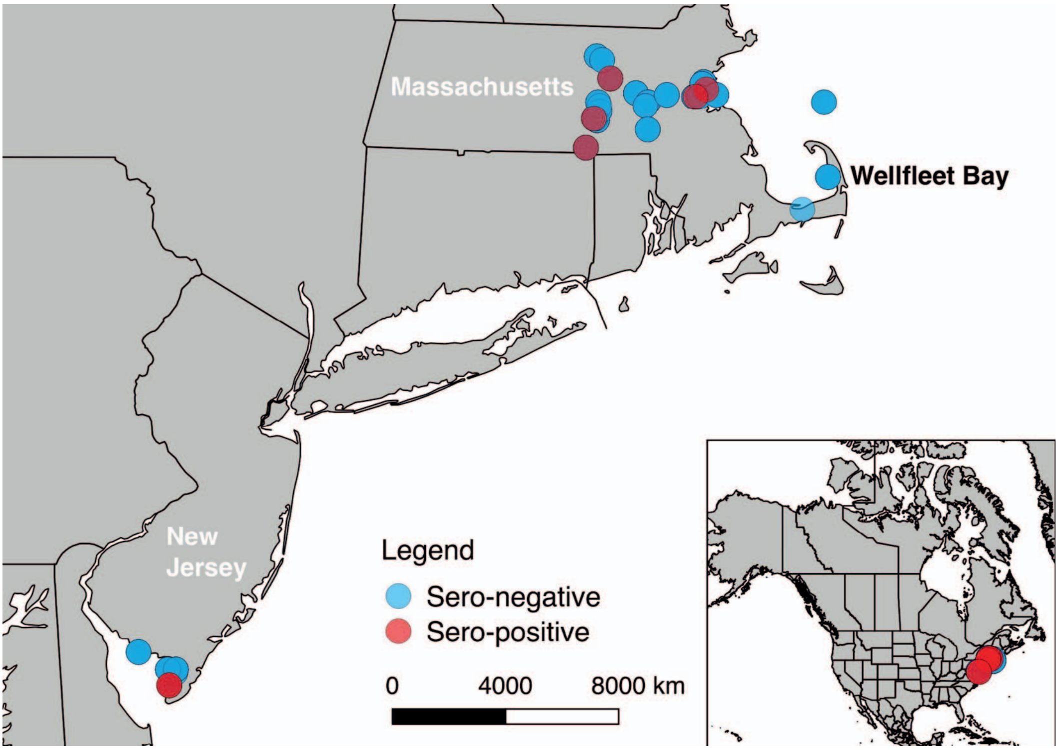

FIGURE 1. Distribution of birds tested for Wellfleet Bay virus in Massachusetts and New Jersey, USA. Light gray and blue dots represent seronegative birds. Dark gray and red dots represent seropositive birds. Wellfleet Bay, Massachusetts is the location of recurrent mortality events in the common eiders (*Somateria mollissima*) associated with this virus.

cally or seasonally isolated. These local factors may include a viral reservoir or a mechanism of transmission that is responsible for the observed geographic or seasonal patterns of exposure. To begin investigating the hypothesis of reservoir species, a survey was conducted to document WFBV exposure in waterbirds sympatric with the Common Eider in and near coastal Massachusetts throughout the year.

Serum samples were collected from multiple sources. Biologists with the US Department of Agriculture's Plant and Animal Health Inspection Service–Wildlife Services collected blood samples from hunter-harvested sea ducks and from birds incidentally captured in floating mist nets during eider surveillance in the autumn in Boston Harbor, Massachusetts (41°53′37″N, −70°4′12″W; Fig. 1). Samples from Cape Cod, Massachusetts also were submitted by a local wildlife rehabilitator. Gulls were sampled in the spring using modified walk-in traps on Calf Island, Boston Harbor, Massachusetts (42°20′23″N, –70°53′19″W; Weaver and Kadlec 1970). Aliquots of banked gull serum were acquired from previous studies conducted at multiple locations in Massachusetts during autumn/winter and in New Jersey during spring (Brown et al. 2010). In both cases, birds were captured using a cannon net or net launcher (Clark et al. 2014).

Blood collection techniques varied according to the institution responsible for sampling. In general, samples from live birds were collected from the jugular, basilic, or median metatarsal veins using a 22-ga needle and 3-mL disposable plastic syringe. Samples from hunter-harvested birds were collected postmortem by intracardiac puncture. All sampling techniques were reviewed and approved by the institutional animal care and use committee, or its equivalent body, for the institution responsible for each study. After collection, blood was allowed to clot before being centrifuged. Serum was removed and stored at –20 C until testing.

Antibody detection was conducted using one of two beta microneutralization assays on Vero-MARU cells (National Veterinary Services Laboratories 1981). Most samples were tested in duplicate at a 1:8 dilution. Positive samples were those with >50% monolayer retention in both replicate wells after 10–11 d incubation, and samples were considered equivocal if one of two wells had >50% monolayer retention. The second assay format was used for larger-volume samples. Those samples were tested in quadruplicate using serial dilutions from 1:4 to 1:256. The titer of each replicate was recorded as the reciprocal of the greatest serum dilution that produced >50% monolayer retention ($TCID_{50}$); positive samples were those with a geometric mean titer $\geq$8. Due to the high concentration ($10^{3.0}$ $TCID_{50}$/25 μL) of challenge virus used in this assay, and the complete monolayer clearance routinely observed in negative samples, equivocal samples (0< geometric mean titer <8) are also reported; these are likely samples containing low concentrations of WFBV antibody, though nonspecific antibody activity cannot be entirely ruled out. Samples reported as negative had no detectable titer.

A total of 480 samples were acquired and, from those, 370 results were generated from 10 species of birds. Samples for which results could not be generated contained excessive hemoglobin or other cytotoxic constituents or were of insufficient volume. Antibodies against WFBV were detected in nine birds from four species (Table 1). Two Herring Gulls (*Larus argentatus*) sampled on the New Jersey coast in spring 2009 were positive and a third Herring Gull sampled in Boston Harbor, Massachusetts in 2014 was equivocal (Fig. 1). Two Ring-billed Gulls (*Larus delawarensis*) collected in 2012, one from Boston (coastal Massachusetts) and the other from Worcester (inland Massachusetts), tested positive. Additionally, one Ring-billed Gull sampled at Clinton (inland Massachusetts) in 2013 tested positive and another had an equivocal result. One hunter-harvested White-winged Scoter (*Melanitta fusca*) sampled from Boston Harbor, Massachusetts in 2013 tested positive and a Black Scoter (*Melanitta americana*) sampled on Cape Cod in 2014 was equivocal. All other samples were negative for WFBV antibodies.

We documented WFBV exposure in four sea bird species, other than the Common Eider, and in birds from the state of New Jersey. Herring Gulls are year-around residents in coastal Massachusetts, although some individuals migrate to other areas for breeding (Clark et al. 2016). The six samples collected from this species in 2014 were from gulls nesting on Calf Island in Boston Harbor, Massachusetts concurrent with Common Eider nesting (Fig. 1). Common Eiders nesting on this island have demonstrated high seroprevalences for WFBV in some years, including in the spring of 2012 (96%; 21/22). Gulls and eiders utilize different habitats on the island, but gulls are frequent predators of eider ducklings. Herring gulls also scavenge carcasses during eider mortality events. It is possible that Herring Gulls in this area, including the one with an equivocal titer, may have been exposed to WFBV through consumption of infected eiders or exposure to the same environmental sources or vectors to which eiders are exposed. The seropositive Herring Gulls detected in New Jersey may indicate that WFBV transmission also occurs in that area or that there is movement of birds between Massachusetts and New Jersey. Herring Gulls and Common Eiders overlap throughout the majority of the eider's North American range, including areas with little or no evidence of WFBV exposure in eiders (Clements et al. 2015; Ballard et al. 2017). Herring Gulls in this survey had a low seroprevalence in areas where the seroprevalence was frequently high in common eiders, making it unlikely that Herring Gulls were a primary reservoir or a source of exposure for eiders.

Ring-billed Gulls are winter-only residents in Massachusetts, nesting across the northern US and southern Canada (Clements et al. 2015). These birds have not been reported to

TABLE 1. Prevalence of antibodies to Wellfleet Bay virus in 10 species of sea birds sampled in New Jersey and Massachusetts, USA, from 2008–14. Prevalence presented as a percentage.

Species	Year	Month	State	n	Prevalence (%)
Herring Gull (*Larus argentatus*)	2008	May	New Jersey	7	0
	2009	May/June	New Jersey	13	15
	2012	May	New Jersey	4	0
	2012	November/December	Massachusetts	13	0
	2013	November	Massachusetts	34	0
	2014	June	Massachusetts	6	16[a]
Total				77	3[a]
Ring-billed Gull (*Larus delawarensis*)	2008	May	New Jersey	3	0
	2012	November/December	Massachusetts	120	1
	2013	January	Massachusetts	129	1[a]
Total				252	1[a]
Great Black-backed Gull (*Larus marinus*)	2008	May	New Jersey	3	0
	2009	May	New Jersey	6	0
	2012	May	New Jersey	1	0
Total				10	0
Laughing Gull (*Larus atricilla*)	2008	May	New Jersey	7	0
	2009	May	New Jersey	4	0
Black Scoter (*Melanitta nigra*)	2014	October	Massachusetts	6	16[a]
White-winged scoter (*Melanitta fusca*)	2013	January	Massachusetts	5	20
	2014	October	Massachusetts	1	0
Total				6	16
Red-breasted Merganser (*Mergus serrator*)	2014	October	Massachusetts	3	0
Surf Scoter (*Melanitta perspicillata*)	2013	January	Massachusetts	1	0
	2014	October	Massachusetts	2	0
Long-tailed Duck (*Clangula hyemalis*)	2013	January	Massachusetts	1	0
Common Murre (*Uria aalge*)	2013	February	Massachusetts	1	0

[a] Count includes one equivocal sample (0< geometric mean titer <8).

scavenge eider carcasses during mortality events and are more common at inland locations where they take advantage of human-derived food sources (Clark et al. 2015). Therefore, the detection of antibodies in this species supports the hypothesis that WFBV transmission may be occurring in the autumn/winter in Massachusetts, although it does not preclude warm season transmission as well. Additionally, the ecology of the Ring-billed Gull, specifically its habitat and feeding preferences, differs from that of other exposed species to such an extent that these findings may indicate the involvement of viral sources or routes of transmission that have not been previously suspected. It appears unlikely that this species is a primary source of WFBV exposure to Common Eiders for the same reasons as those mentioned for the Herring Gull.

White-winged Scoters and Black Scoters are winter residents along the coast of Massachusetts, during which they are not likely to spend time on shore (Johnsgard 2010; Clements et al. 2015). Therefore, detection of antibodies in these species supports the hypothesis that WFBV transmission not only occurs in the autumn/winter but also may be independent of land-based sources of exposure such as terrestrial arthropods. Antibodies against WFBV were detected in both species (Table 1) despite small sample sizes. A notable similarity between these species and the Common Eider is a shared prey base primarily consisting of mollusks. Sampling larger numbers of both scoter species as well as their shared food source may be warranted to better understand their role in WFBV ecology.

The findings of this study provide additional information in the investigation of the host range and epidemiology of WFBV in the northeastern US. Wellfleet Bay virus appears to be capable of infecting a wider range of avian hosts than was previously recognized, although clinical disease has been confirmed only in the Common Eider. There is no strong evidence identifying any of the species examined in our study as WFBV reservoirs; however, additional sampling of these, other sympatric species, and shared prey items may provide valuable information about the mechanisms driving the spatial and temporal distribution of this virus. Species for which Massachusetts represents one of the few areas of overlap with the Common Eider would be of particular interest, including other diving ducks (*Aythya* spp.), egrets and herons (Ardeidae), and other wading birds (Johnsgard 2010; Clements et al. 2015).

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