SHORT COMMUNICATIONS

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Evaluation of Seabirds in Newfoundland and Labrador, Canada, as Hosts of Influenza A Viruses

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ABSTRACT: Influenza A viruses infect a wide range of hosts, including many species of birds. Avian influenza A virus (AIV) infection appears to be most common in Anseriformes (ducks, geese, and swans) and some Charadriiformes (shorebirds and gulls), but many other birds also serve as hosts of AIV. Here, we evaluated the role of seabirds as hosts for AIV. We tested 3,160 swab samples from 13 seabird species between May 2008 and December 2011 in Newfoundland and Labrador, Canada. We also tested 156 serum samples for evidence of previous infection of AIV in Common Murres (*Uria aalge*) and Atlantic Puffins (*Fratercula arctica*). Avian influenza A virus was detected in breeding Common Murres and nonbreeding Thick-billed Murres (*Uria lomvia*), and Common Murres also had high antibody prevalence (44%). From these findings, combined with other studies showing AIV infection in murres, we conclude that murres are important for the ecology of AIV. For other species (Razorbill, *Alca torda*; Leach's Storm-Petrel, *Oceanodroma leucorhoa*; Black-legged Kittiwake, *Rissa tridactyla*; Atlantic Puffin) with good coverage (>100 samples) we did not detect AIV. However, serology indicates infection does occur in Atlantic Puffins, with 22% antibody prevalence found. The possibility of virus spread through dense breeding colonies and the long distance movements of these hosts make a more thorough evaluation of the role for seabirds as hosts of AIV important.

Key words: Common Murre, influenza A virus, guillemot, murre, seabirds, serology, *Uria* sp.

Wild birds are the primary reservoir for influenza A viruses (Webster et al. 1992), and avian influenza A viruses (AIVs) have been isolated from at least 105 wild bird species in 26 families, with the highest prevalence of infection in the Anseriformes (ducks, geese, and swans) and some Charadriiformes (shorebirds and gulls; Olsen et al. 2006). The contributions of most other bird groups to the ecology of AIV are unclear. Seabirds are a behaviorally and ecologically defined group of birds capable of spending much of their lives at sea. Some species stay relatively close to shore and others wander entire oceans, but all are capable of living for extended periods independent of land (Gaston 2004). In this study, we have included the subfamily Sternidae and the kittiwakes (*Rissa* spp.) from the family Laridae (gulls) as seabirds because of their highly pelagic movements (Frederiksen et al. 2012); other members of the family Laridae were not included. The ecology of seabirds makes them logistically challenging to survey for AIV. Many breed on remote islands and then spend the winter months at sea. Further, immature nonbreeding individuals of many species do not approach land for more than a year. Therefore, sampling for AIV in this group

has been limited and little information is available about possible spatial, temporal, and seasonal patterns of infection (Ip et al. 2008).

To investigate the role of seabirds as hosts of AIV, we collected swab samples and serum samples from seabirds along the coast of Newfoundland and Labrador, Canada, between May 2008 and December 2011. Samples were collected from breeding and locally wintering seabirds. Most samples were collected during the summer months, while birds were congregated at breeding colonies (Fig. 1). Either the cloaca (live birds in 2008) or the cloaca and the oropharyngeal cavity (all others) were swabbed. In 2010, moist, freshly deposited fecal samples were collected from ledges used by breeding Common Murres (*Uria aalge*). Fresh carcasses were also opportunistically sampled, and were mostly from murres (*Uria* spp.) harvested during the annual winter hunting season, and from a winter wreck in Conception Bay in 2009 (McFarlane Tranquilla et al. 2010; Fig. 1). Tubes containing samples were kept cool and placed at −80 C within 48 hr of collection. Samples were screened, and viruses isolated following guidelines from Canada's Inter-Agency Wild Bird Influenza Survey using previously published methods (Spackman et al. 2002; Granter et al. 2010). Positive samples had real-time reverse transcriptase PCR (rRT-PCR) threshold cycle values <35 or were successfully cultured. Hemagglutinin and neuraminidase subtypes were determined by sequencing (Wille et al. 2011). In addition to swab samples, blood was collected from 115 adult Common Murres and 41 adult Atlantic Puffins (*Fratercula arctica*) in 2011 using established protocols, and tested for anti-nucleoprotein (NP) antibodies using the AI MultiS-Screen Ab Test (IDEXX, Westbrook, Maine, USA) as recommended by the manufacturer.

We collected 3,160 samples from 13 species in six families (Table 1), and AIVs were detected only in Thick-billed Murres

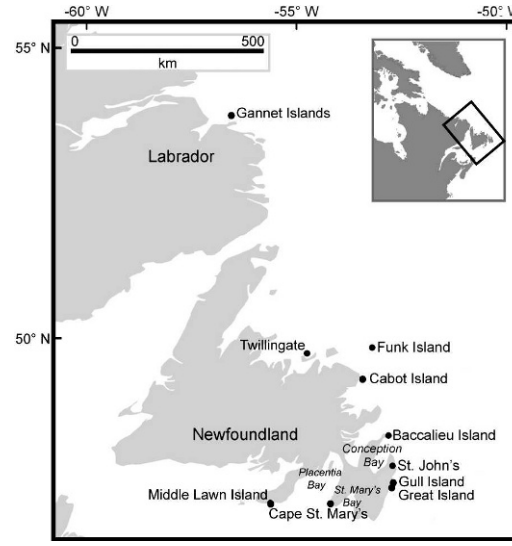

FIGURE 1. Map of Newfoundland and Labrador, Canada, indicating seabird colonies and locations where sample collection occurred. The seabird breeding colonies sampled were Cabot Island (49°10′18″N, 53°21′30″W), Gull Island (47°15′34″N, 52°46′26″W), Great Island (47°10′58.90″N, 52°48′28.55″W), the Gannet Islands (53°56′21″N, 56°40′45″W), Funk Island (49°45′7″N, 53°11′21″W), Baccalieu Island (48°7′0″N, 52°47′39″W), Middle Lawn Island (46°52′9″N, 55°37′3″W) and Cape St. Mary's (46°49′14″N, 54°11′40″W). The most intensively surveyed colonies, particularly for Common and Thick-billed Murres (*Uria aalge* and *Uria lomvia*), were Cabot, Great, and Gull Islands. Hunter-killed murre carcasses were sampled from St. Mary's Bay, Placentia Bay, Conception Bay, and Twillingate; murre carcasses were also sampled from a 2009 wreck event in Conception Bay. The city of St. John's is indicated for reference.

(*Uria lomvia*) and Common Murres within the Alcidae, which were also the sources of the largest number of samples (Table 1). To date, there have been no detections of AIV in the families Stercorariidae, Sulidae, Hydrobatidae, or Diomedeidae, but viruses have been detected in the Alcidae, Laridae, Sternidae, and Procellariidae (Olsen et al. 2006; Ip et al. 2008; Granter et al. 2010; Ramey et al. 2010).

Seventy-one samples from Common Murres were positive, all detected during the summer months at the breeding

TABLE 1. Avian influenza A virus surveillance of seabirds by real-time reverse transcriptase PCR in Newfoundland and Labrador, Canada, May 2008–December 2011.

Family	Species	Samples (positives)	Status		
			Live	Dead	Environmental
Alcidae	Common Murre (*Uria aalge*)	1,317 (71[a])	1,032 (70)	241	44 (1)
	Thick-billed Murre (*Uria lomvia*)	621 (1)	73	548 (1)	
	Atlantic Puffin (*Fratercula arctica*)	365	364	1	
	Dovekie (*Alle alle*)	52	1	51	
	Razorbill (*Alca torda*)	196	188	8	
	Black Guillemot (*Cepphus grylle*)	1	0	1	
Procellariidae	Manx Shearwater (*Puffinus puffinus*)	12	12	0	
	Northern Fulmar (*Fulmarus glacialis*)	4	2	2	
Hydrobatidae	Leach's Storm Petrel (*Oceanodroma leucorhoa*)	377	375	2	
Sulidae	Northern Gannet (*Morus bassanus*)	76	74	2	
Laridae	Black-legged Kittiwake (*Rissa tridactyla*)	109	105	4	
Sternidae	Arctic Tern (*Sterna paradisaea*)	9	9	0	
	Common Tern (*Sterna hirundo*)	21	21	0	
Total		3,160 (72)	2,256 (70)	860 (1)	44 (1)

[a] An additional eight samples were inconclusive, with real-time reverse transcriptase PCR threshold cycle values of 35–40, and no virus was isolated in specific pathogen–free embryonated chicken eggs. Inconclusive samples were collected from live chicks ($n=7$) and environmental samples ($n=1$).

colonies. Most of these were found in 2011 (Table 2) despite similar spatial and temporal sampling schemes across all years. No viruses were detected in Common Murres at our main sampling site on Gull Island in 2008 or 2009 and only one virus was detected in 2010. In contrast, a single sampling occasion on Gull Island in 2011 yielded 60 positives from 68 samples collected. For the 2011 Common Murre AIV samples (Table 2), the prevalence (with 95% confidence intervals) was different for adult birds (33/216, 15.2±4.8%) versus chicks (28/33, 85±12%) on Gull Island, but not Cabot Island (4/101, 4.0±3.8% for adults; 3/40, 8±8% for chicks). No viruses were detected from 211 birds at another nearby colony on Great Island over the course of this study. Overall, 44% of adult Common Murres sampled had anti-NP antibodies, and antibody prevalences were similar across breeding colonies (Table 2). This compares with an overall rRT-PCR–positive rate of 16% for Common Murres at these locations in 2011 (Table 2) and ~5% overall for Common Murres over the 4 yr (Table 1). No antibody-positive birds were positive for ongoing infection by rRT-PCR. Conversely, one individual that was antibody negative was positive by rRT-PCR. One virus was detected in Thick-billed Murres over the 4 yr (Table 1), from a hunter-killed bird in the winter. Most of the samples from Thick-billed Murres were collected during winter (November–April) off the coast of the island of Newfoundland ($n=548$), but some samples were collected from a breeding colony in Labrador in 2009 ($n=73$). Finally, despite a lack of positive swab samples collected from Atlantic Puffins ($n=365$), serology indicated this species is a host for AIV because 22% (9/41) of adult puffins sampled in 2011 were antibody positive. Therefore, more work is needed to begin to characterize the interaction of this species with AIV.

Viruses were recovered from 14 of 32 attempted isolations from matrix-positive samples (isolation rate=~44%), with all but one of these from the 2011 samples. One virus from a Common Murre in 2010 was subtyped as an H4, and 18 viruses

TABLE 2. Avian influenza A virus rRT-PCR and antibody prevalence in Common Murres (*Uria aalge*) from three breeding colonies in Newfoundland, Canada, in 2011.[a]

	rRT-PCR			Serology		
Location	Samples	Positives	%	Samples	Positives	%
Gull Island	249	61	24	71	31	44
Great Island	62	0	0	20	9	45
Cabot Island	141	7	5	24	11	46
Total	452	68	16	115	51	44

[a] rRT-PCR = real-time reverse transcriptase PCR.

from 2011 (representing both Cabot and Gull Islands) were subtyped as H1N2, possibly indicating an outbreak of a single strain in 2011. Previous sequence analyses of viruses from Common and Thick-billed Murres suggest these viruses might be spillover infections from waterfowl because almost all of the genes fall within the avian lineages, dominated by waterfowl viruses (Wallensten et al. 2005; Granter et al. 2010; Ramey et al. 2010). However, ecologically, it is very unlikely that a murre would acquire an infection directly from a duck because they do not share habitat. Additionally, viruses isolated from murres in both North America and Eurasia have been intercontinental reassortants (Wallensten et al. 2005; Ramey et al. 2010). This could result from interactions among birds from the two regions during the nonbreeding season, and AIV has now been detected twice in Thick-billed Murres during the winter. Ecologically, gulls would be more likely to act as intermediates for moving viruses between waterfowl and seabirds, as they often breed on the same islands as seabirds and also use coastal and inland waters that are utilized by waterfowl, but this is not well supported virologically because gulls mostly carry distinct lineages of AIV (Kawaoka et al. 1988; Fouchier et al. 2005; Wille et al. 2011), which have not been identified in seabirds.

It has been suggested that if AIV is introduced to a seabird colony, transmission should be high because of high host densities and immunologically naïve chicks (Clancy et al. 2006). Our findings support this suggestion, given the high prevalence of infection and the dominance of a single subtype found 1 yr amongst nesting Common Murres. Common and Thick-billed Murres are among the most densely nesting seabirds at up to 20 pairs/m^2 and 37 pairs/m^2, respectively (Cramp et al. 1985), and are in frequent physical contact with their neighbors. As well, many seabird colonies have multiple species in close proximity, such that movement of AIV between species seems likely, hence the chance for reassortment could be enhanced. Because of the highly pelagic movements of seabirds, they could also be important in the spread of AIV across the globe. Marine birds have also been implicated in the spread of influenza viruses to marine mammals (Webster et al. 1992; Nielson et al. 2001). To date, AIVs have been detected in very few seabird species and at very low prevalence, despite over 10,000 samples having been collected since the 1970s (Olsen et al. 2006; Ip et al. 2008; Granter et al. 2010; Ramey et al. 2010). However, serology data combined with repeated and widespread detection of viruses in some species, such as murres, indicate that seabirds are infected with AIV and, therefore, likely play a role in the global dynamics of the virus.

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