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1 **Short Communications**

2

3 **Evaluation of seabirds in Newfoundland and Labrador, Canada, as hosts of influenza A**

4 **viruses**

5

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32

33 **Abstract**

34 Influenza A viruses infect a wide range of hosts, including many species of birds. Avian  
35 influenza A virus (AIV) infection appears to be most common in Anseriformes (ducks, geese,  
36 and swans) and some Charadriiformes (shorebirds and gulls), but many other birds also serve  
37 as hosts of AIV. Here, we evaluated the role of seabirds as hosts for AIV. In this study we  
38 tested 3158 swab samples from 13 seabird species between May 2008 and December 2011 in  
39 Newfoundland and Labrador, Canada. We also tested 156 serum samples for evidence of  
40 previous infection of AIV in Common Murres (*Uria aalga*) and Atlantic Puffins (*Fratercula*  
41 *arctica*). AIV was detected in breeding Common Murres and non-breeding Thick-billed  
42 Murres (*U. lomvia*), and Common Murres also had high seroprevalence (44%). From these  
43 findings, combined with other studies showing AIV infection in murres, we conclude that  
44 murres are important for the epidemiology of AIV. For other species (Razorbill, *Alca torda*;  
45 Leach's Storm-Petrel, *Oceanodroma leucorhoa*; Black-legged Kittiwake, *Rissa tridactyla*;  
46 Atlantic Puffin) with good coverage (>100 samples) we did not detect AIV. However,  
47 serology indicates infection does occur in Atlantic Puffins, with 22% seroprevalence found.  
48 The possibility of virus spread through dense breeding colonies and the long distance  
49 movements of these hosts makes a more thorough evaluation of the role for seabirds as hosts  
50 of AIV important.

51

52 **Keywords:** Common Murre, influenza A virus, guillemot, murre, seabirds, serology, *Uria* sp.

53

54 Wild birds are the primary reservoir for influenza A viruses (Webster *et al.* 1992), and  
55 avian influenza viruses (AIV) have been isolated from at least 105 wild bird species across 26  
56 different families, with the highest prevalence of infection in the Anseriformes (ducks, geese,

57 and swans) and some Charadriiformes (shorebirds and gulls) (Olsen *et al.* 2006). The  
58 contributions of most other bird groups to the epidemiology of AIV are unclear.

59 Seabirds are a behaviorally and ecologically defined group of birds capable of  
60 spending much of their lives at sea. Some species stay relatively close to shore and others  
61 wander entire oceans, but all are capable of living for extended periods independent of land  
62 (Gaston 2004). In this study, we have included the subfamily Sternidae and the kittiwakes  
63 (*Rissa* spp.) from the family Laridae (gulls) as seabirds because of their highly pelagic  
64 movements (Frederiksen *et al.* 2012); other members of the family Laridae were not  
65 included. The ecology of seabirds makes them logistically challenging to comprehensively  
66 survey for AIV. Many breed on remote islands and then spend the winter months at sea.  
67 Further, immature non-breeding individuals of many species do not approach land for more  
68 than a year. Therefore, sampling for AIV in this group has been limited and little information  
69 is available about possible spatial, temporal, and seasonal patterns of infection (Ip *et al.*  
70 2008).

71 To investigate the role of seabirds as hosts of AIV we collected swab samples and  
72 serum samples from seabirds along the coast of Newfoundland and Labrador, Canada,  
73 between May 2008 and December 2011. Samples were collected from breeding and locally  
74 wintering seabirds. Most samples were collected during the summer months, while birds  
75 were congregated at breeding colonies (Figure 1). The cloaca and the oropharyngeal cavity  
76 (excluding live birds sampled in 2008) were swabbed. In 2010, moist, freshly deposited fecal  
77 samples were collected from ledges used by breeding Common Murres (*Uria aalge*). Fresh  
78 carcasses were also opportunistically sampled, and were mostly from murres (*Uria* spp.)  
79 harvested during the annual winter hunting season, and from a winter wreck in Conception  
80 Bay in 2009 (McFarlane Tranquilla *et al.* 2010) (Figure 1). Tubes containing samples were  
81 kept cool and placed at -80°C within 48 hours of collection. Samples were screened, and

82 viruses isolated as per guidelines from Canada's Inter-Agency Wild Bird Influenza Survey  
83 using previously published methods (Spackman *et al.* 2002, Granter *et al.* 2010). Positive  
84 samples had rRT-PCR Ct values <35 and/or were successfully cultured. Hemagglutinin (HA)  
85 and neuraminidase (NA) subtypes were determined by sequencing (Wille *et al.* 2011). In  
86 addition to swab samples, blood was collected from 115 adult Common Murres and 41 adult  
87 Atlantic Puffins (*Fratercula arctica*) in 2011 using established protocols, and tested for anti-  
88 nucleoprotein (NP) antibodies using the AI MultiS-Screen Ab Test (IDEXX, Westbrook,  
89 Maine) as recommended by the manufacturer.

90 A total of 3158 samples were collected from 13 species across 6 families (Table 1),  
91 and AIV were only detected in Thick-billed Murres (*Uria lomvia*) and Common Murres  
92 within the Alcidae, which were also the sources of the largest number of samples (Table 1).  
93 To date, there have been no detections of AIV in the families Stercorariidae, Sulidae,  
94 Hydrobatidae, or Diomedidae, but viruses have been detected in the Alcidae, Laridae,  
95 Sternidae and Procellariidae (Olsen *et al.* 2006, Ip *et al.* 2008, Granter *et al.* 2010, Ramey *et*  
96 *al.* 2010).

97 Seventy-one samples from Common Murres were positive, all detected during the  
98 summer months at the breeding colonies. Most of these were found in 2011 (Table 2) despite  
99 similar spatial and temporal sampling schemes across all years. No viruses were detected in  
100 Common Murres at our main sampling site on Gull Island in 2008 or 2009, and only 1 virus  
101 was detected in 2010. In contrast, a single sampling occasion on Gull Island in 2011 yielded  
102 60 positives from the 68 samples collected. For the 2011 Common Murre AIV samples  
103 (Table 2), the prevalence was different for adult birds versus chicks at both Gull (33/216  
104 adult, 28/33 chick) and Cabot Islands (4/101 adult, 3/40 chick). No viruses were detected  
105 from 211 birds at another nearby colony on Great Island over the course of this study.  
106 Overall, 44% of adult Common Murres sampled had anti-NP antibodies, and seroprevalence

107 was similar across breeding colonies (Table 2). This compares with an overall rRT-PCR  
108 positive rate of 16% for Common Murres at these locations in 2011 (Table 2) and ~5%  
109 overall for Common Murres over the 4 years (Table 1). No seropositive birds were positive  
110 for ongoing infection by rRT-PCR. Conversely, one individual that was seronegative was  
111 positive by rRT-PCR. One virus was detected in Thick-billed Murres over the 4 years (Table  
112 1), from a hunter-killed bird in the winter. Most of the samples from Thick-billed Murres  
113 were collected during the winter months (November-April) off the coast of the island of  
114 Newfoundland (n=548), but some samples were collected from a breeding colony in  
115 Labrador in 2009 (n=73). Finally, despite a lack of positive swab samples collected from  
116 Atlantic Puffins (n=365), serology indicated this species is a host for AIV because 22%  
117 (9/41) of adult puffins sampled in 2011 were seropositive. Therefore, more work is needed to  
118 begin to characterize the interaction of this species with AIV.

119 Viruses were recovered from 14 of 32 attempted isolations (isolation rate = ~44%),  
120 with all but one of these from the 2011 samples. One virus from a Common Murre in 2010  
121 was subtyped as an H4, and 18 viruses from 2011 (representing both Cabot and Gull Islands)  
122 were subtyped as H1N2, possibly indicating an outbreak of a single strain in 2011. Previous  
123 sequence analyses of viruses from Common and Thick-billed Murres suggest these viruses  
124 might be spillover infections from waterfowl because almost all of the genes fall within the  
125 avian lineages, dominated by waterfowl viruses (Wallensten *et al.* 2005, Granter *et al.* 2010,  
126 Ramey *et al.* 2010). However, ecologically, it is very unlikely that a murre would acquire an  
127 infection directly from a duck because they do not share habitat. Additionally, viruses  
128 isolated from murres in both America and Eurasia have been intercontinental reassortants  
129 (Wallensten *et al.* 2005, Ramey *et al.* 2010). This could result from interactions among birds  
130 from the two regions during the non-breeding season, and AIV has now been detected twice  
131 in Thick-billed Murres during the winter. Ecologically, gulls would be more likely to act as

132 intermediates for moving viruses between waterfowl and seabirds as they often breed on the  
133 same islands as seabirds and also use coastal and inland waters that are utilized by dabbling  
134 ducks, but this is not well supported virologically because gulls mostly carry specialized  
135 lineages of AIV (Kawaoka *et al.* 1988, Fouchier *et al.* 2005, Wille *et al.* 2011), which have  
136 not yet been identified in seabirds.

137         It has been suggested that if AIV is introduced to a seabird colony, transmission  
138 should be high due to high host densities and immunologically naïve chicks (Clancy *et al.*  
139 2006). Our findings support this suggestion, given the high prevalence of infection, and  
140 dominance of a single subtype found one year amongst nesting Common Murres. Common  
141 and Thick-billed Murres are amongst the most densely nesting seabirds at up to 20 pairs/m<sup>2</sup>  
142 and 37 pairs/m<sup>2</sup>, respectively (Cramp *et al.* 1985), and are in frequent physical contact with  
143 their neighbours. As well, many seabird colonies have multiple species in close proximity,  
144 such that movement of AIV between species seems likely, hence the chance for reassortment  
145 could be enhanced. Due to the highly pelagic movements of seabirds, they could also be  
146 important in the spread of AIV across the globe. Marine birds have also been implicated in  
147 the spread of influenza viruses to marine mammals (Webster *et al.* 1992, Nielson *et al.* 2001).  
148 To date, AIV have been detected in very few seabird species and at very low prevalence,  
149 despite over 10,000 samples having been collected since the 1970's (Olsen *et al.* 2006, Ip *et*  
150 *al.* 2008, Granter *et al.* 2010, Ramey *et al.* 2010). However, serology data combined with  
151 repeated and widespread detection of viruses in some species, such as murres, indicate that  
152 seabirds are infected with AIV, and therefore likely play a role in the global dynamics of the  
153 virus.

154



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176

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- 233
- 234

235 Table 1. AIV surveillance of seabirds by rRT-PCR in Newfoundland and Labrador between  
 236 May 2008 and December 2011.

Family	Species	Samples (positives)	Status		
			Live	Dead	Environmental
Alcidae	Common Murre ( <i>Uria aalge</i> )	1315 (71 <sup>a</sup> )	1032 (70)	241	44 (1)
	Thick-billed Murre ( <i>U. lomvia</i> )	621 (1)	73	548 (1)	
	Atlantic Puffin ( <i>Fratercula arctica</i> )	365	364	1	
	Dovekie ( <i>Alle alle</i> )	52	1	51	
	Razorbill ( <i>Alca torda</i> )	196	188	8	
	Black Guillemot ( <i>Cephus grylle</i> )	1	0	1	
	Procelariidae	Manx Shearwater ( <i>Puffinus puffinus</i> )	12	12	0
Northern Fulmar ( <i>Fulmarus glacialis</i> )		4	2	2	
Leach's Storm Petrel		377	375	2	
( <i>Oceanodroma leucorhoa</i> )					
Sulidae	Northern Gannet ( <i>Morus bassanus</i> )	76	74	2	
Laridae	Black-legged Kittiwake ( <i>Rissa tridactyla</i> )	109	105	4	
Sternidae	Arctic Tern ( <i>Sterna paradisaea</i> )	9	9	0	
	Common Tern ( <i>S. hirundo</i> )	21	21	0	
Total		3158			

237

238 <sup>a</sup>An additional 8 samples were inconclusive, with rRT-PCR Ct values of 35-40 and no virus  
 239 was isolated in SPF embryonated chicken eggs. Inconclusive samples were collected from  
 240 live chicks (n=7) and environmental samples (n=1).

241

242 Table 2. AIV rRT-PCR and seroprevalence in Common Murres from three breeding colonies  
 243 in Newfoundland in 2011.

Location	rRT-PCR			Serology		
	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

244

245

246 **Figure legend**

247 Figure 1. Map of Newfoundland and Labrador, Canada, indicating the seabird colonies and  
248 locations where sample collection occurred in this study. The seabird breeding colonies  
249 sampled were Cabot Island (49°10'18"N, 53°21'30"W), Gull Island (47°15'34"N,  
250 52°46'26"W), Great Island (47°10'58.90"N, 52°48'28.55"W), the Gannet Islands  
251 (53°56'21"N, 56°40'45"W), Funk Island (49°45'7"N, 53°11'21"W), Baccalieu Island  
252 (48°7'0"N, 52°47'39"W), Middle Lawn Island (46°52'9"N, 55°37'3"W) and Cape St. Mary's  
253 (46°49'14"N, 54°11'40"W). The most intensively surveyed colonies, particularly for  
254 Common and Thick-billed Murres, were Cabot, Great, and Gull Islands. Hunter-killed murre  
255 carcasses were sampled from St. Mary's Bay, Placentia Bay, Conception Bay and  
256 Twillingate; and murre carcasses were also sampled from a 2009 wreck event in Conception  
257 Bay. The city of St. John's is indicated for reference. Major lines of latitude and longitude are  
258 denoted on map frame.

