



Avian influenza virus ecology in Iceland shorebirds: Intercontinental reassortment and movement



Jeffrey S. Hall^{a,*}, Gunnar Thor Hallgrimsson^d, Kamol Suwannanarn^c, Srinand Sreevatsen^c, Hon S. Ip^a, Ellen Magnusdottir^b, Joshua L. TeSlaa^a, Sean W. Nashold^a, Robert J. Dusek^a

^aU.S. Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711, United States

^bSouthwest Iceland Nature Research Institute, Garðvegur 1, 245 Sandgerði, Iceland

^cVeterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, United States

^dUniversity of Iceland, Reykjavik, Iceland

ARTICLE INFO

Article history:

Received 4 June 2014

Received in revised form 4 August 2014

Accepted 11 September 2014

Available online 18 September 2014

Keywords:

Influenza

Reassortment

Shorebirds

Iceland

Intercontinental

Movement

ABSTRACT

Shorebirds are a primary reservoir of avian influenza viruses (AIV). We conducted surveillance studies in Iceland shorebird populations for 3 years, documenting high serological evidence of AIV exposure in shorebirds, primarily in Ruddy Turnstones (*Arenaria interpres*; seroprevalence = 75%). However, little evidence of virus infection was found in these shorebird populations and only two turnstone AIVs (H2N7; H5N1) were able to be phylogenetically examined. These analyses showed that viruses from Iceland shorebirds were primarily derived from Eurasian lineage viruses, yet the H2 hemagglutinin gene segment was from a North American lineage previously detected in a gull from Iceland the previous year. The H5N1 virus was determined to be low pathogenic, however the PB2 gene was closely related to the PB2 from highly pathogenic H5N1 isolates from China. Multiple lines of evidence suggest that the turnstones were infected with at least one of these AIV while in Iceland and confirm Iceland as an important location where AIV from different continents interact and reassort, creating new virus genomes. Mounting data warrant continued surveillance for AIV in wild birds in the North Atlantic, including Canada, Greenland, and the northeast USA to determine the risks of new AI viruses and their intercontinental movement in this region.

Published by Elsevier B.V.

1. Introduction

Shorebirds (Families *Charadriidae*, *Scolopacidae*) are a primary reservoir of avian influenza virus (Webster et al., 1992). However, the overwhelming documentation of avian influenza virus (AIV) in shorebirds comes from surveillance studies conducted at a single location, Delaware Bay on the Atlantic coast of the US. Spring migrant shorebirds congregate at Delaware Bay in large numbers to feed on horseshoe crab (*Limulus polyphemus*) eggs prior to continuing their migration to breeding areas in the Arctic (Krauss et al., 2010). Even at Delaware Bay, the majority of AIV detection occurs primarily in one species, Ruddy Turnstones (*Arenaria interpres*) (Kawaoka et al., 1988; Stallknecht et al., 2012). Over the several weeks that the birds are at Delaware Bay, the AIV infection rate increases as does the proportion of birds having antibodies to AIV (Hanson et al., 2008; Maxted et al., 2012). Very few AIV infections have been documented in shorebird populations at

locations or times other than DE Bay in spring, despite significant surveillance efforts (Olsen et al., 2006; Munster et al., 2007; Ip et al., 2008; Winker et al., 2008; Gaidet et al., 2012).

However, recent virological and serological surveys of shorebirds at other locations showed that populations of shorebirds have high prevalence of antibodies to AIV despite no detection of AIV infections (Pearce et al., 2012; Gaidet et al., 2012; Johnson et al., 2014). These findings indicate significant virus exposure histories at times or locations other than where they were sampled. The reasons for the uniqueness of Delaware Bay and the role of shorebirds in the ecology of avian influenza elsewhere in the world are currently unclear although many shorebird stopover locations and non-breeding areas around the world have not been examined for AIV in these hosts.

Iceland represents another important stopover location for thousands of migrant birds in the East Atlantic flyway as they travel between their breeding grounds in Greenland and northeastern Canada and their wintering areas in Europe and Africa (Branson et al., 1978). Recent evidence confirms that the North Atlantic region in general (Wille et al., 2011; Hall et al., 2013b), and this

* Corresponding author. Tel.: +1 608 270 2458.

E-mail address: jshall@usgs.gov (J.S. Hall).

migration route particularly, are important in intercontinental virus movement and the generation of new genetic combinations, particularly in gulls (Family *Laridae*) that are a closely related taxa to shorebirds (Dusek et al., 2014). Therefore, North Atlantic and Icelandic wild bird populations, including migrant shorebirds, need to be examined for AIV infection and exposure histories to determine their roles and the potential risks of virus movement and virus introduction into new geographic regions. This study presents the findings from 3 years of AI virological and serological surveillance in migratory Icelandic shorebirds, as well as the phylogeography of AIV recovered from these birds.

2. Results

2.1. Detection of avian influenza RNA in Icelandic shorebirds

Table 1 summarizes RT-PCR analysis of shorebird cloacal swabs and showed a total of 2 samples were positive for AIV RNA out of 673 samples tested (0.3%). Both of the positive samples came from one shorebird species, single swab samples from Ruddy Turnstones in 2012 and 2013.

2.2. Detection of avian influenza antibodies in Icelandic shorebirds

The results of ELISA analysis of shorebird sera reporting the seroprevalence in these populations are shown in Table 2. Overall, 47% of the shorebird sera tested contained antibodies to AIV, however the majority of seropositive birds were Ruddy Turnstones. In 2012, 85% of the Ruddy Turnstones tested positive and in 2013, 66% were positive. In contrast, the remaining shorebird samples over both years had only 4 out of 84 samples that tested positive (4.8%). These data indicate that, as at Delaware Bay in the North American Atlantic Flyway, Ruddy Turnstones are also the predominant species involved in shorebird AIV transmission cycles in the East Atlantic flyway.

Table 1

RT-PCR detection of avian influenza virus RNA in cloacal swabs from Icelandic shorebirds. Swab samples with Ct values <35 were considered positive for viral RNA.

Year	Species	No. sampled	No. positive (%)
2010	Common Redshank (<i>Tringa totanus</i>)	1	0
	Common Ringed Plover (<i>Charadrius hiaticula</i>)	4	0
	Common Snipe (<i>Gallinago gallinago</i>)	1	0
	Dunlin (<i>Calidris alpina</i>)	2	0
	Eurasian Oystercatcher (<i>Haematopus ostralegus</i>)	16	0
	Purple Sandpiper (<i>Calidris maritima</i>)	2	0
	Red Knot (<i>Calidris canuta</i>)	1	0
	Ruddy Turnstone (<i>Arenaria interpres</i>)	64	0
	Sanderling (<i>Calidris alba</i>)	129	0
		220	0
2012	Common Ringed Plover	10	0
	Dunlin	3	0
	Ruddy Turnstone	68	1 (1.5)
	Sanderling	108	0
		189	1 (0.5)
2013	Common Ringed Plover	13	0
	Dunlin	12	0
	Purple Sandpiper	6	0
	Red Knot	21	0
	Ruddy Turnstone	88	1 (1.1)
	Sanderling	124	0
		264	1 (0.4)
Total		673	2 (0.3)

Table 2

Avian influenza seroprevalence in spring Icelandic shorebirds.

Year	Species	No. sampled	No. positive (%)
2012	Ruddy Turnstone (<i>Arenaria interpres</i>)	60	51 (85)
	Sanderling (<i>Calidris alba</i>)	48	1 (2)
	Common Ringed Plover (<i>Charadrius hiaticula</i>)	10	0
	Dunlin (<i>Calidris alpina</i>)	3	0
		121	52 (43)
2013	Ruddy Turnstone	70	46 (66)
	Sanderling	1	0
	Purple Sandpiper (<i>Calidris maritima</i>)	1	0
	Red Knot (<i>Calidris canutus</i>)	21	3 (14)
		93	49 (53)
Total		214	101 (47)

2.3. Isolation of avian influenza virus from Icelandic shorebirds

Virus isolation was attempted on all cloacal swabs obtained from Icelandic shorebirds. One viable virus isolate was obtained from the RT-PCR positive ruddy turnstone in 2012 (A/Ruddy Turnstone/Iceland/1946/2012).

2.4. Sequence analysis of Iceland shorebird AIV

The swab sample from the 2013 RT-PCR positive Ruddy Turnstone had a relatively low RT-PCR Ct value (29) but no virus was obtained from egg culture. Therefore we directly sequenced the viral RNA from that swab (A/Ruddy Turnstone/Iceland/2899/2013). The A/Ruddy Turnstone/Iceland/1946/2012 virus was also sequenced as described in the methods. From these sequence data we determined that the 2012 virus was an H2N7 subtype and the 2013 Ruddy Turnstone virus an H5N1 subtype containing a cleavage site in the hemagglutinin (HA) gene characteristic of a low pathogenic influenza virus (Klenk et al., 2008).

Phylogenetic analysis of the hemagglutinin genes showed that the 2013 H5 gene was derived from a Eurasian waterfowl lineage (Fig. 1a) while the 2012 H2 gene belonged to a North American waterfowl lineage previously identified in Icelandic gull populations sampled in 2011 (Dusek et al., 2014) (Fig. 1b). This result indicates that the H2 North American gene had recently become established in Iceland, and was circulating for at least 2 years in gull and shorebird populations there, albeit in association with different virus neuraminidase subtypes. The neuraminidase (NA) segments were from Eurasian lineages in both viruses (Fig. 2a and b). Comparison of the two Ruddy Turnstone virus sequences showed that the NS gene was >99% identical with only 4 nucleotides different between the two viruses. The other internal protein coding gene segments were less similar with the next most closely related sequences being the matrix genes that were 97% similar. The NP genes were 96% similar, PB1 was 96%, PA-95%, and the PB2 genes were 93% similar.

Analysis of the other 7 gene segments of the two shorebird viruses showed that they all were derived from Eurasian waterfowl lineages (data not shown). Interestingly, BLAST queries of GenBank showed the most similar sequence to the A/Ruddy Turnstone/Iceland /2899/2013 (H5N1) PB2 gene was a H9N2 isolate from France (A/mallard/France/090360/2009(H9N2) (Dundon et al., 2012). However, the next 9 most similar sequences were from highly pathogenic avian influenza H5N1 viruses (clade 2.3.4) isolated from Chinese live poultry markets between 2006 and 2008 (Chen et al., 2009). The PB2 phylogenetic relationships of these viruses are shown in Fig. 3. Additionally, this figure shows that the PB2 gene of the 2012 turnstone virus was most similar to the

Download English Version:

<https://daneshyari.com/en/article/5909400>

Download Persian Version:

<https://daneshyari.com/article/5909400>

[Daneshyari.com](https://daneshyari.com)