



The Genome Sequence of an H6N5 Influenza A Virus Strain Isolated from a Northern Pintail (*Anas acuta*) Sampled in Alaska, USA, Shares High Identity with That of a South Korean Wild Bird Strain

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ABSTRACT We report the genome sequence of an H6N5 influenza A virus isolated from a northern pintail sampled in Alaska in 2017. All segment sequences shared >99% nucleotide identity with those of a wild bird strain from South Korea. This finding supports viral dispersal between East Asia and North America by wild birds.

There is a growing body of evidence that wild migratory aquatic birds disperse influenza A viruses (IAVs; family *Orthomyxoviridae*, genus *Alphainfluenzavirus*) between East Asia and North America via the Bering Strait (1–5). Here, we report the genome sequence of A/northern pintail/Alaska/UGAI17-4733/2017(H6N5), here pintail/Alaska/UGAI-4733, which provides additional support for this premise.

As part of annual IAV research and surveillance, combined cloacal and oropharyngeal samples from hunter-harvested ducks were collected at Izembek National Wildlife Refuge (55.3°N, 162.8°W), Alaska in September and October of 2017. Samples were screened via real-time reverse transcriptase PCR (rRT-PCR) for IAV, and viruses were isolated from eggs using previously described methods (6, 7). From a northern pintail (*Anas acuta*) sampled on 20 September 2017, the rRT-PCR screen resulted in a threshold cycle (C_T) value of 24.46, and an H6N5 virus was isolated. RNA was extracted using a Qiagen viral RNA minikit and amplified by multiplex RT-PCR using published primers and methods (8). Following library preparation (Nextera XT DNA library preparation kit; Illumina, Inc.), next-generation sequencing (NGS) of paired-end reads was performed using a V3 600-cycle kit on a MiSeq instrument (Illumina, Inc.). From this library, 626,806 reads were generated. The genome was assembled using Geneious 11.0.4 (Biomatters, Inc.) by mapping paired reads to reference sequences from GenBank (9). The best-fitting reference sequence for each segment is reported in Table 1. NGS covered 100% of all coding regions of the genome (Table 1). Results from a nucleotide BLAST analysis conducted on 29 April 2020 for all gene segment sequences of pintail/Alaska/UGAI-4733 indicated that this isolate shared high genomic similarity with A/Aix galericulata/South Korea/K17-1638-5/2017(H6N5), here mandarin duck/South Korea/K17-1638-5. To determine the extent of genomic similarity, we used Geneious 11.04 to construct nucleotide and protein sequence alignments using the two genomes. The coding regions of gene segment sequences for northern pintail/Alaska/UGAI-4733 and mandarin duck/South Korea/K17-1638-5 shared nucleotide identities of 99.4 to 100% and predicted amino acid similarities of 99.2 to 100% (Table 1).

Virus strain mandarin duck/South Korea/K17-1638-5 was reported to have been isolated from wild bird feces collected at a wetland in northwestern South Korea on 7 December 2017 (5). All eight gene segments of mandarin duck/South Korea/K17-1638-5 were previously reported to be of a North American lineage based on BLAST results and

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TABLE 1 Genome sequence information for A/northern pintail/Alaska/UGAI17-4733/2017(H6N5)^a

Gene segment	GenBank accession no.	GenBank accession no. for closest reference sequence	No. of reads aligning to reference sequence	Coverage (nt; % of CDS) ^b	GC content (%)	Shared nt identity with A/Aix galericulata/South Korea/K17-1638-5/2017 (%)	Protein	Protein size (aa) ^c	Shared aa identity with A/Aix galericulata/South Korea/K17-1638-5/2017(H6N5) (%)
PB2	MT420712	CY056275	808	2,280; 100	44.65	99.76	PB2	760	99.74
PB1	MT420713	CY196039	3,284	2,274; 100	43.40	99.96	PB1	758	99.87
PA	MT420714	CY016129	1,068	2,151; 100	44.35	99.42	PA	717	99.45
							PA-X	253	99.60
HA	MT420715	CY041402	15,072	1,701; 100	41.56	99.53	HA	567	99.29
NP	MT420716	KY551144	15,882	1,497; 100	48.23	100	NP	499	100
NA	MT420717	CY012842	58,862	1,422; 100	42.83	99.86	NA	474	99.79
MA	MT420718	GQ257412	122,320	982; 100	49.08	99.53	M1	253	100
							M2	98	100
NS	MT420719	CY003940	216,222	838; 100	45.23	99.76	NS	231	99.57
							NEP	122	99.18

^a All alignments and calculations were made in Geneious 11.04 (Biomatters, Inc.).

^b nt, nucleotides; CDS, coding DNA sequences.

^c aa, amino acids.

phylogenetic analysis (5). Isolate pintail/Alaska/UGAI-4733 was collected 78 days before the South Korean sample. Given the timing of both sample collection efforts and typical migratory bird movements within and between the East Asian-Australasian and Pacific Americas flyways, it is possible that a viral ancestor to mandarin duck/South Korea/K17-1638-5 was dispersed from Alaska to East Asia during the summer or autumn of 2017. There is evidence that numerous species, including northern pintails, have intercontinental migratory tendencies that may have facilitated such a dispersal event (10–12).

The genomic similarity of pintail/Alaska/UGAI-4733 and mandarin duck/South Korea/K17-1638-5 is the second instance of nearly identical viruses identified at the Izembek National Wildlife Refuge in Alaska and wetlands in western South Korea (1). Furthermore, evidence suggests that viral dispersal across the Bering Strait is bidirectional (2–5). Therefore, the detection of IAVs in wild birds in western South Korea that represent potential economic or public health threats may serve as an early warning for potential introduction into North America via Alaska and vice versa.

Data availability. The coding-complete genome sequence of strain A/northern pintail/Alaska/UGAI17-4733/2017(H6N5) has been deposited in GenBank under the accession numbers MT420712 to MT420719. The raw sequencing reads were deposited in the NCBI Sequence Read Archive (SRA) under the accession number SRR11908080 and BioProject accession number PRJNA636765.

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REFERENCES

- Ramey AM, Reeves AB, Sonsthagen SA, TeSlaa JL, Nashold S, Donnelly T, Casler B, Hall JS. 2015. Dispersal of H9N2 influenza A viruses between East Asia and North America by wild birds. *Virology* 482:79–83. <https://doi.org/10.1016/j.virol.2015.03.028>.
- Lee DH, Torchetti MK, Winker K, Ip HS, Song CS, Swayne DE. 2015. Intercontinental spread of Asian-origin H5N8 to North America through Beringia by migratory birds. *J Virol* 89:6521–6524. <https://doi.org/10.1128/JVI.00728-15>.
- Ramey AM, Reeves AB, TeSlaa JL, Nashold S, Donnelly T, Bahl J, Hall JS. 2016. Evidence for common ancestry among viruses isolated from wild birds in Beringia and highly pathogenic intercontinental reassortant H5N1 and H5N2 influenza A viruses. *Infect Genet Evol* 40:176–185. <https://doi.org/10.1016/j.meegid.2016.02.035>.
- Ramey AM, Reeves AB, Donnelly T, Poulson RL, Stallknecht DE. 2018. Introduction of Eurasian-origin influenza A(H8N4) virus into North America by migratory birds. *Emerg Infect Dis* 24:1950–1953. <https://doi.org/10.3201/eid2410.180447>.
- Jeong S, Lee DH, Kim YJ, Lee SH, Cho AY, Noh JY, Tseren-Ochir EO, Jeong

- JH, Song CS. 2019. Introduction of avian influenza A(H6N5) virus into Asia from North America by wild birds. *Emerg Infect Dis* 25:2138–2140. <https://doi.org/10.3201/eid2511.190604>.
6. Spackman E, Senne DA, Myers TJ, Bulaga LL, Garber LP, Perdue ML, Lohman K, Daum LT, Suarez DL. 2002. Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. *J Clin Microbiol* 40:3256–3260. <https://doi.org/10.1128/jcm.40.9.3256-3260.2002>.
 7. Stallknecht DE, Shane SM, Zwank PJ, Senne DA, Kearney MT. 1990. Avian influenza viruses from migratory and resident ducks of coastal Louisiana. *Avian Dis* 34:398–405. <https://doi.org/10.2307/1591427>.
 8. Reeves AB, Hall JS, Poulson RL, Donnelly T, Stallknecht DE, Ramey AM. 2018. Influenza A virus recovery, diversity, and intercontinental exchange: a multi-year assessment of wild bird sampling at Izembek National Wildlife Refuge, Alaska. *PLoS One* 13:e0195327. <https://doi.org/10.1371/journal.pone.0195327>.
 9. Bao YM, Bolotov P, Dernovoy D, Kiryutin B, Zaslavsky L, Tatusova T, Ostell J, Lipman D. 2008. The Influenza Virus Resource at the National Center for Biotechnology Information. *J Virol* 82:596–601. <https://doi.org/10.1128/JVI.02005-07>.
 10. Winker K, Gibson DD. 2010. The Asia-to-America influx of avian influenza wild bird hosts is large. *Avian Dis* 54:477–482. <https://doi.org/10.1637/8741-032509-Reg.1>.
 11. Hupp JW, Yamaguchi N, Flint PL, Pearce JM, Tokita K, Shimada T, Ramey AM, Kharitonov S, Higuchi H. 2011. Variation in spring migration routes and breeding distribution of northern pintails *Anas acuta* that winter in Japan. *J Avian Biol* 42:289–300. <https://doi.org/10.1111/j.1600-048X.2011.05320.x>.
 12. Ramey AM, Uher-Koch BD, Reeves AB, Schmutz JA, Poulson RL, Stallknecht DE. 2019. Emperor geese (*Anser canagicus*) are exposed to a diversity of influenza A viruses, are infected during the non-breeding period and contribute to intercontinental viral dispersal. *Transbound Emerg Dis* 66:1958–1970. <https://doi.org/10.1111/tbed.13226>.