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Introduction of Eurasian-Origin Influenza A(H8N4) Virus into North America by Migratory Birds

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We identified a Eurasian-origin influenza A(H8N4) virus in North America by sampling wild birds in western Alaska, USA. Evidence for repeated introductions of influenza A viruses into North America by migratory birds suggests that intercontinental dispersal might not be exceedingly rare and that our understanding of viral establishment is incomplete.

Research of and surveillance for influenza A viruses in wild birds inhabiting western Alaska have consistently provided support for the exchange of viruses between East Asia and North America via Beringia (*1*,*2*). Sampling of wild birds inhabiting Izembek National Wildlife Refuge (NWR) and surrounding areas in Alaska (≈55°N, 163°W) conducted during 2011–2015 has been used in recent research to identify the dispersal of influenza A(H9N2) viruses among China, South Korea, and Alaska (*3*); provide inference about the evolutionary pathways of economically important foreign-origin poultry pathogens introduced into North America (*4*); and identify sampling efficiencies for optimizing the detection of evidence for intercontinental virus exchange (*5*).

During September–October 2016, we collected 541 combined oral-pharyngeal and cloacal swab samples from hunter-harvested waterfowl (*Anseriformes* spp.) and 401 environmental fecal samples from monospecific flocks of either emperor geese (*Chen canagica*) or glaucous-winged gulls (*Larus glaucescens*) within and around Izembek NWR. Samples were deposited into viral transport media, placed in dry shippers charged with liquid nitrogen within 24 h, shipped, and stored frozen at –80°C before laboratory analysis. We screened samples for the influenza A virus matrix gene and subjected them to virus isolation; resultant isolates were genomically sequenced in accordance with previously reported methods (*5*). A total of 116 samples tested positive for the matrix gene, and 38 isolates were recovered of the following combined subtypes: H1N2, H3N2, H3N2/N6 (mixed infection), H3N8, H4N6, H5N2, H6N2, H7N3,

H8N4, and H12N2. We selected the single H8N4 isolate, A/northern pintail/Alaska/UGAI16-3997/2016(H8N4) (GenBank accession nos. MG976689–96), for genomic characterization as part of this investigation.

We queried sequence information for the complete coding region of each gene segment of A/northern pintail/Alaska/UGAI16-3997/2016(H8N4) against the GenBank database to identify strains sharing ≥99% nt identity. We then reconstructed maximum-likelihood phylogenetic trees for each gene segment in MEGA 7.0.21 (https://www.megasoftware.net/) by incorporating sequence information for representative reference sequences from avian-origin influenza A virus isolates from Eurasia and North America using the general time–reversible plus invariant sites (G+I) model with 1,000 bootstrap replications.

Gene segments for A/northern pintail/Alaska/UGAI16-3997/2016(H8N4), isolated from a sample collected from a hunter-harvested duck on September 6, 2016, shared ≥99% nt identity to those of ≥1 isolates recovered from wild and domestic birds sampled in East Asia during 2006–2016 (online Technical Appendix Table, https://wwwnc.cdc.gov/EID/article/24/10/18-0447-Techap1.pdf). This isolate also shared ≥99% nt identity with 1–4 isolates recovered from wild bird samples collected at Izembek NWR during 2012–2015 at the polymerase acidic and polymerase basic 2 gene segments (online Technical Appendix Table). A/northern pintail/Alaska/UGAI16–3997/2016(H8N4) did not, however, share ≥99% nt identity at all 8 gene segments with any other influenza A virus isolate for which genomic information was available, indicating that this H8N4 isolate might

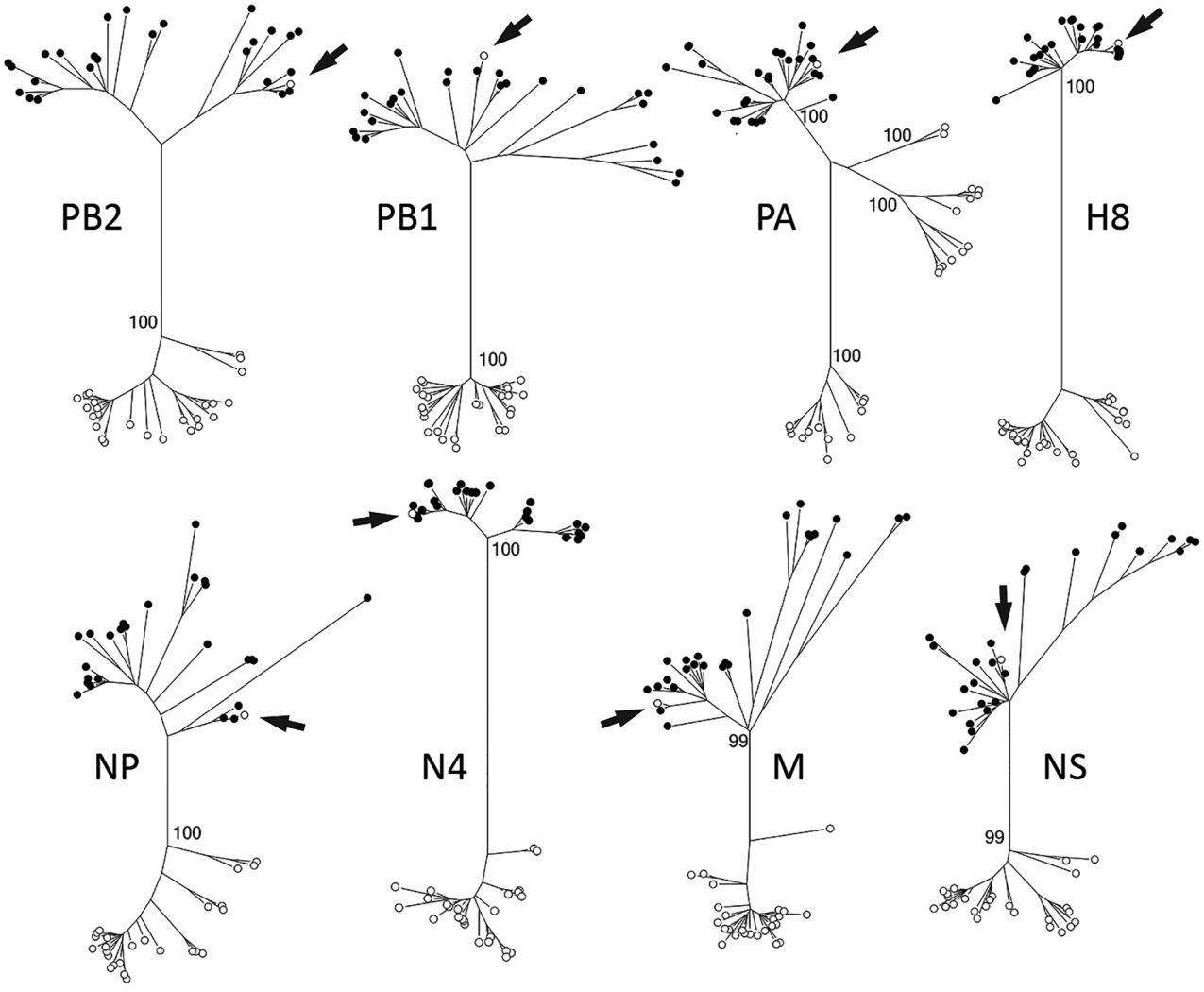

Figure. Maximum-likelihood phylogenetic trees showing inferred relationships among nucleotide sequences for the complete coding regions of gene segments for influenza A virus strain A/northern pintail/Alaska/UGAI16–3997/2016(H8N4) (white circle indicated with an arrow) and reference sequences from viruses isolated from birds in Eurasia (black circles) and North America (white circles). Bootstrap support values for continentally affiliated clades are shown. Phylogenetic trees with complete strain names as tip labels are provided in the online Technical Appendix Figure (https://wwwnc.cdc.gov/EID/article/24/10/18-0447-Techap1.pdf). H, hemagglutinin; M, matrix; N, neuraminidase; NP, nucleoprotein; NS, nonstructural; PA, polymerase acidic; PB, polymerase basic.

represent a previously unidentified or unreported genome constellation (online Technical Appendix Table).

Phylogenetic analyses strongly supported structuring of tree topologies into major clades by continental affiliation of reference sequences (bootstrap values ≥99; online Technical Appendix Figure). Sequence information for all 8 gene segments of A/northern pintail/Alaska/UGAI16-3997/2016(H8N4) clustered within clades composed of reference sequences for influenza A viruses originating from samples collected in Eurasia (Figure; online Technical Appendix Figure). Therefore, phylogenetic analyses provided support for Eurasian ancestry of this genomic constellation. We inferred our results to provide evidence for the introduction of this foreign-origin H8N4 virus into North America by migratory birds given previous support for intercontinental viral dispersal derived through genetic characterization of avian influenza A viruses originating from western Alaska (*1*–*3*,*5*), the intercontinental migratory tendencies of northern pintails (*6*,*7*) and other species inhabiting Izembek NWR at the time of sampling (*8*), the paucity of domestic poultry in this region, and the proximity of Izembek NWR to East Asia.

During 2010–2016, research and surveillance for influenza A viruses in wild birds inhabiting North America have provided evidence for the intercontinental dispersal of the following 4 viral genome constellations between Eurasia and North America: H16N3 (*9*), H9N2 (*3*), highly pathogenic clade 2.3.4.4 H5N8 (*10*), and H8N4 (this study). Four reports of independent purported intercontinental dispersal events for influenza A viruses via migratory birds during 7 years of sampling do not disprove the paradigm of restricted viral dispersal between Eurasia and North America. However, repeated detections of these viruses crossing the Bering Strait (*3*,*10*; this study) suggest that viral dispersal between East Asia and North America might not be exceedingly rare. Thus, a lack of selective advantage for comparatively rare foreign-origin influenza A viruses, purifying selection for endemic viruses, or both might be important mechanisms regulating the establishment of these viruses within the wild bird reservoir. Therefore, additional research directed toward understanding selection pressures regulating the establishment of these viruses might provide useful inference for informing surveillance and response activities for economically costly or potentially pandemic foreign-origin viruses in wild birds inhabiting North America.

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New Reassortant Clade 2.3.4.4b Avian Influenza A(H5N6) Virus in Wild Birds, South Korea, 2017–2018

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We isolated new reassortant avian influenza A(H5N6) viruses from feces of wild waterfowl in South Korea during 2017–18. Phylogenetic analysis suggested that reassortment occurred between clade 2.3.4.4b H5N8 and Eurasian low pathogenicity avian influenza viruses circulating in wild birds. Dissemination to South Korea during the 2017 fall migratory season followed.

Clade 2.3.4.4 H5 highly pathogenic avian influenza viruses (HPAIVs) have evolved by reassortment with different neuraminidase (NA) and internal genes of prevailing low pathogenicity avian influenza viruses (LPAIVs) and other HPAIVs to generate new genotypes and further evolved into genetic subgroups A–D since 2014 (*1*). Among these, subgroups A and B viruses were disseminated over vast geographic regions by migratory wild birds (*2*,*3*). Subgroup B influenza A(H5N8) viruses were detected in Qinghai Lake, China, and Uvs-Nuur Lake, Russia, during May–June 2016 (Qinghai/Uvs-like), followed by the identification of reassortant viruses in multiple Eurasian countries (*4*–*6*). Recently, subgroup B H5N6 viruses were isolated from birds in Greece during February 2017 and England, Germany, the Netherlands, Japan, and Taiwan during winter 2017–18 (*7*,*8*).

[1]These authors contributed equally to this article.

During December 2017–January 2018 in South Korea, we isolated 6 H5N6 HPAIVs from 231 fecal samples of wild birds collected from the banks of the Cheongmi-cheon River (37°06′56.9″N, 127°25′18.3″E) and 34 from 222 fecal samples collected from the banks of the Gokgyo-cheon River (36°45′12.3′N, 127°07′12.7′E) (online Technical Appendix 1, https://wwwnc.cdc.gov/EID/article/24/10/18-0461-Techapp1.pdf). These wild bird habitats are wintering sites of migratory waterfowl, including mallard (*Anas platyrhynchos*), spot-billed duck (*Anas poecilorhyncha*), Mandarin duck (*Aix galericulata*), and common teal (*Anas crecca*). The Gokgyo-cheon River is a major habitat site for Mandarin ducks, and numerous HPAIVs were detected in fecal samples from Mandarin ducks during 2011, 2015, and 2016 (*9*). We identified avian influenza virus–positive fecal samples from 38 Mandarin ducks and 2 mallards, based on DNA barcoding technique (*10*). We performed full-length genome sequencing and comparative phylogenetic analysis on 19 of the 40 isolates (online Technical Appendix 1; online Technical Appendix 2, https://wwwnc.cdc.gov/EID/article/24/10/18-0461-Techapp2.xlsx).

All H5N6 isolates shared high nucleotide sequence identities in all 8 gene segments (99.58%–100%) and were identified as HPAIVs based on the presence of multiple basic amino acids at the HA proteolytic cleavage site (PLREKRRKR/G). Searches of the GISAID (https://www.gisaid.org) and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) databases indicated that all 8 genomes had the highest nucleotide identity with A/Great_Black-backed_Gull/Netherlands/1/2017 (Netherlands/1) clade 2.3.4.4 subgroup B H5N6 strain from December 2017 (99.17%–99.79%), rather than subgroup B H5N6 viruses from Japan and Taiwan collected during December 2017 (97.18%–99.27%).

In phylogenetic analysis, we identified 2 genotypes of subgroup B H5N6 viruses (online Technical Appendix 1 Figures 1, 2): genotypes B.N6.1 and B.N6.2. The genotype B.N6.1 viruses were identified from South Korea, Japan, Taiwan, Greece, and the Netherlands (Netherlands/1 strain), and the genotype B.N6.2 viruses were detected from England, Germany, and the Netherlands. For genotype B.N6.1, all genes except NA clustered with H5N8 HPAIV of previously reported genotypes, H5N8-NL cluster I in the Netherlands (*6*), Ger-11-16 in Germany (*5*), and Duck/Poland/82a/16-like in Italy (*4*). The NA gene clustered with LPAIVs circulating in wild birds in Eurasia and separated into 2 clusters, suggesting the potential for >2 independent reassortment events between H5N8 virus and unidentified wild bird origin N6 segments. Consistent clustering of South Korea isolates with the Netherlands/1 strain in maximum-likelihood (ML) phylogenies for each gene supported by high ML bootstrap values (86–100) suggests their close relationship. The genotype B.N6.2 viruses