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Intercontinental Spread of Asian-Origin H5N8 to North America through Beringia by Migratory Birds

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Phylogenetic network analysis and understanding of waterfowl migration patterns suggest that the Eurasian H5N8 clade 2.3.4.4 avian influenza virus emerged in late 2013 in China, spread in early 2014 to South Korea and Japan, and reached Siberia and Beringia by summer 2014 via migratory birds. Three genetically distinct subgroups emerged and subsequently spread along different flyways during fall 2014 into Europe, North America, and East Asia, respectively. All three subgroups reappeared in Japan, a wintering site for waterfowl from Eurasia and parts of North America.

Since 1996, the Asian-origin H5N1 A/goose/Guangdong/1/1996 (Gs/GD) lineage of highly pathogenic avian influenza viruses (HPAIV) has caused outbreaks in poultry, infections in wild birds, and clinical, often fatal, cases in humans in Eurasia and North Africa (1). Historically, geographic barriers appeared to limit the spread of low-pathogenicity avian influenza viruses (LPAIV) through migratory aquatic birds between the Old and New Worlds, allowing distinct lineages of Eurasian and North American viruses to evolve, but such barriers were not complete, as occasional spillovers of gene segments occurred (2). Migratory birds have previously been implicated in the spread of H5N1 HPAIV in Eurasian (3), but geographic barriers, such as the Bering Strait and the Chukchi Sea, prevented the spread of the Gs/GD virus lineage to North America (4, 5).

In early 2014, outbreaks of novel reassortant H5N8 HPAIV of the Gs/GD lineage H5 clade 2.3.4.4 were reported in South Korea (6), and aquatic migratory birds were strongly suspected of playing a key role in the introduction of this strain from China and in its subsequent spread during the outbreak (7). In late autumn 2014, H5N8 clade 2.3.4.4 was reported in Europe and East Asia. Concurrently, this virus lineage was detected in Washington in captive falcons, wild birds, and backyard poultry (8). Verhagen et al. hypothesized that the timing and direction of intercontinental spread coincided with autumn bird migration out of Russia; the H5N8 virus was identified in a long-distance migratory bird in Russia in September 2014 and subsequently in Japan and Europe, and on the west coast of North America (9). Similarly, wide geographic dissemination of the Gs/GD lineage virus was seen between 2005 and 2006, as clade 2.2 viruses spread from Qinghai Lake, China, to Siberia and then to various countries of Asia, Europe, and Africa (2, 10). In addition, another novel reassortant HPAIV of H5 clade 2.3.4.4 H5N2 (Eurasian [EA] polymerase basic 2 [PB2], polymerase acidic [PA], hemagglutinin [HA], matrix [M], and nonstructural [NS] protein-encoding gene segments) with North American [AM] lineage neuraminidase (NA), polymerase basic 1 (PB1), and nucleoprotein (NP) gene segments was identified as the cause of outbreaks in poultry farms in British Columbia and subsequently detected in wild waterfowl in Oregon near the Washington State border (8, 11). Both the H5N8 and H5N2 reassortants have been detected in wild waterfowl, as well as backyard and commercial poultry along the Pacific flyway (8, 12).

In this study, a molecular epidemiological approach based on genome sequences and outbreak information from Asia, Europe, and North America during 2014 was used to describe the pattern of global spread of this Asian-origin H5N8 reassortant clade 2.3.4.4 HPAIV.

The nucleotide sequences used for phylogenetic analysis in this study (for the accession numbers, see Table S1 in the supplemental material) were previously deposited in GISAID (http://platform.gisaid.org/epi3/frontend#1b81fe) and GenBank (http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html) (see Table S2 in the supplemental material). Neighbor-joining (NJ) phylogenetic trees were constructed for each of the eight gene segments. The median-joining phylogenetic network and time-scaled phylogenetic tree of the HA gene were constructed by using nucleotide sequences genetically close to H5 viruses identified in 2014 based on the result of NJ phylogenetic analyses. For details of the methods used, see the supplemental material.

The South Korean H5N8 outbreak in January 2014 was the first reported case of H5N8 clade 2.3.4.4 outside China. During this outbreak, two distinct H5N8 virus groups were identified, A (Buan2-like) and B (Gochang1-like) (see Fig. S1 to S9 in the supplemental material) (6). Group A viruses predominated and were identified in poultry farm outbreaks and subsequently along bird migration path-

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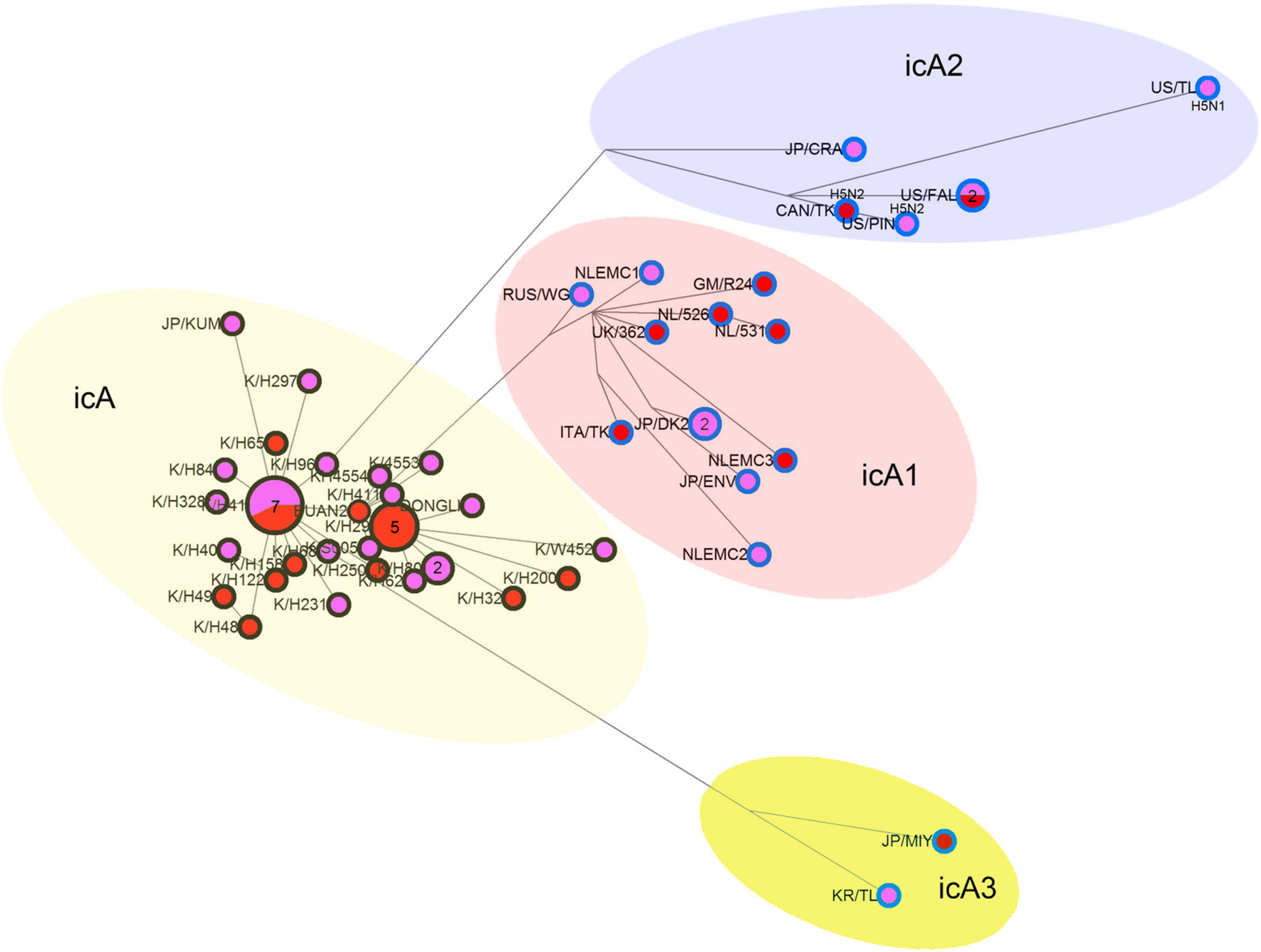

FIG 1 Median-joining phylogenetic network of HPAI H5 clade 2.3.4.4 viruses. The median-joining network was constructed from the HA-encoding gene. This network includes all of the most parsimonious trees linking the sequences. Each unique sequence is represented by a circle whose size reflects the frequency of the sequence in the data set. Branch length is proportional to the number of mutations. Isolates are colored according to the origin and season of the sample as follows: red inner circle, poultry farm isolates; purple inner circle, wild-bird isolates; black outer circle, early 2014 isolates; blue outer circle, late 2014 isolates.

ways (7). This group now comprises Chinese, Russian, South Korean, Japanese, European, and North American H5N8 2.3.4.4 viruses representing intercontinental group A (icA). The icA H5 viruses further evolved into three distinct subgroups, icA1, icA2, and icA3, by late 2014 (Fig. 1; see Fig. S2 in the supplemental material). The icA1 subgroup is composed of H5N8 viruses from Europe and Russia from late 2014 and three viruses detected in Japan in December 2014. The icA2 subgroup is composed of H5N8, as well as H5 clade 2.3.4.4 North American HPAIV reassortants (H5N2 and H5N1) detected in North America starting in late 2014 (12). A Japanese virus, A/crane/Kagoshima/KU1/2014 (H5N8), detected in November 2014 is also within the icA2 subgroup. The icA3 subgroup is composed of H5N8 viruses isolated in Japan in December 2014 and Korea in January 2015 (Fig. 1).

An important route of the East Asian flyway goes from China to Japan (China-South Korea-Russian Far East-Japan), and the timing of H5N1 outbreaks and virus movements has been closely associated within this flyway (13). In addition, satellite tracking of the movement patterns of wild birds in East Asia showed that wild ducks followed the East Asian flyway along the coast to breeding areas in eastern Russia (14). The phylogenetic analyses of early and late 2014 icA H5N8 (Fig. 1 and 2) and aquatic bird migration patterns suggest that viruses likely moved to parts of Siberia and Beringia via migratory birds in spring 2014, evolved into subgroups during the breeding season, and subsequently spread along different flyways during autumn migrations into Europe, North America, and East Asia (Fig. 2). For example, the earliest icA1 virus was isolated in September 2014 in Russia [A/wigeon/Sakha/1/2014(H5N8)] prior to the November outbreaks in Europe (Fig. 2). Interestingly, viruses from all genetic subgroups were subsequently detected in Japan in late 2014, where some waterfowl from Eurasia and North America winter.

The relatively high incidence of icA isolates from migratory waterfowl suggests that they are important hosts in both the maintenance and spread of these viruses. Areas such as the Beringian Crucible (Alaska and the Russian Far East), where the New World and Old World migration systems use the same breeding grounds,

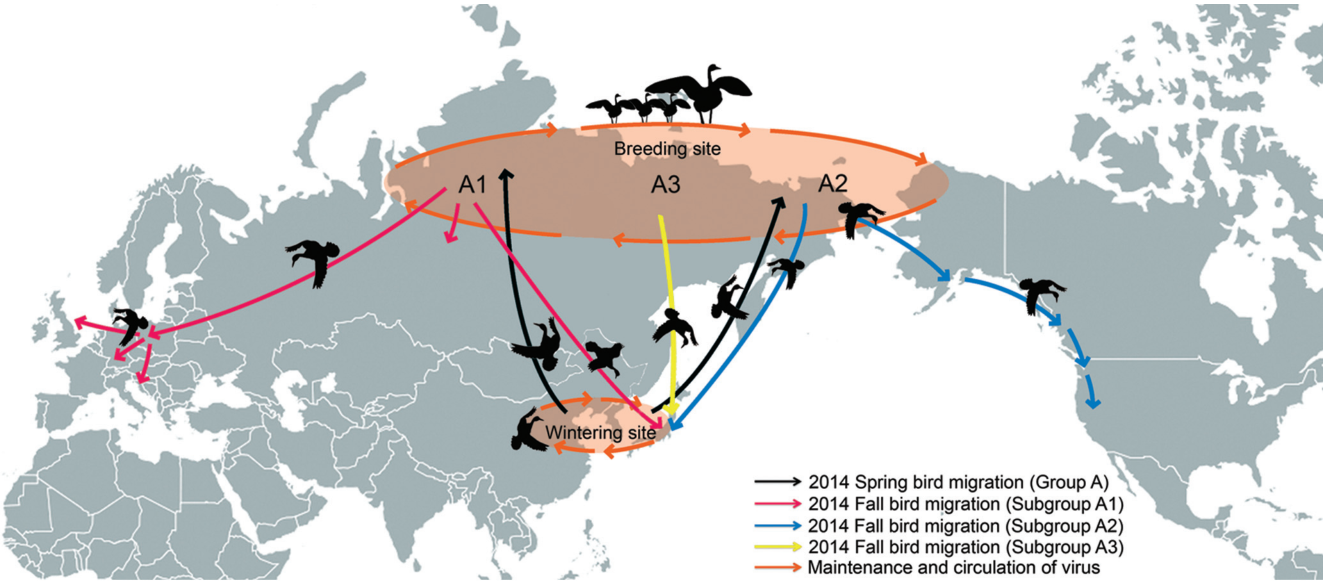

FIG 2 Geographic map showing the movement of H5N8 HPAIV in Asia, Europe, and North America in relation to regional waterfowl migration routes. The map, by Dmthoth, is from Wikipedia Commons (http://commons.wikimedia.org/wiki/File:Blank_Map_Pacific_World.svg).

provide ample opportunity for virus reassortment because of the large-scale intercontinental associations of waterfowl; it is estimated that 1.5 to 2.9 million aquatic migratory birds move from Asia to Alaska annually (4, 5). While influenza virus surveillance in Alaskan waterfowl species found predominantly LPAIV (15), frequent reassortment was noted in one study of northern pintails, with nearly half (44.7%) of the LPAIV tested having at least one gene segment demonstrating closer relatedness to Eurasian than to North American LPAIV genes (16). In contrast, relatively few aquatic birds move from Asia to North America south of Alaska and relatively few from Alaska are associated with the Atlantic flyway, these being birds that winter in the eastern United States and come northwest in spring (17). Although the North Atlantic area is a potential route for migratory birds to transport avian influenza viruses between North America and Europe (18), the possibility of viral spread through the Atlantic rim was excluded on the basis of the timing of the detections and the very low level of viral gene flow between Pacific and Atlantic flyways (19) and because the icA H5 viruses identified in North America were genetically closer to East Asian viruses than to European viruses. Additionally, ongoing routine surveillance has detected no icA H5 virus in poultry or wild aquatic bird in the Atlantic flyway. Detection of icA H5N8 HPAIV from apparently healthy wild waterfowl from multiple countries and high viral shedding in the absence of illness in experimentally icA H5N8-infected waterfowl (20) support the theory that the global spread of icA H5N8 HPAIV has been driven by migratory birds. While the closest relatives of the icA H5N2 viruses appear to be South Korean/Japanese H5N8 viruses, introduction of icA H5N8 via illegal importation of poultry or poultry products into either Europe or North America seems unlikely, as Koreans and Japanese have a low cultural preference for live-poultry market systems, which in mainland Asia are the source of illegally moved poultry and poultry products, plus the added difficulty of smuggling live birds or uncooked product across the Pacific Ocean with current transportation security measures. Examination of the timing and genetic footprints of these viruses through phylogenetic analysis and waterfowl migratory patterns supports the hypothesis that icA H5N8 clade 2.3.4.4 was introduced into North America through the Beringian Crucible via intercontinental associations of waterfowl (5, 6). In addition, reassortment of icA2 H5N8 with North American LPAIV has already occurred within the Pacific flyway, generating reassortant icA2 H5N2 and icA H5N1 HPAIV. icA2 H5N8 has been detected as far south as California and Utah; the icA2 H5N2 reassortant has been detected in British Columbia, the northwestern United States, and more recently in the midwestern United States (Mississippi flyway); and the icA2 H5N1 reassortant has been detected in a wild bird in Washington and a backyard flock in British Columbia (8). Enhanced active surveillance is required to monitor the spread and potential for onward reassortment of icA 2.3.4.4; such efforts could further the epidemiological understanding of HPAIV and the design of improved prevention strategies.

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REFERENCES

1. **Machalaba CC, Elwood SE, Forcella S, Smith KM, Hamilton K, Jebara KB, Swayne DE, Webby RJ, Mumford E, Mazet JA, Gaidet N, Daszak P, Karesh WB.** 2015. Global avian influenza surveillance in wild birds: a strategy to capture viral diversity. Emerg Infect Dis **21:**e141415. http://dx.doi.org/10.3201/eid2104.141415.
2. **Olsen B, Munster VJ, Wallensten A, Waldenstrom J, Osterhaus AD, Fouchier RA.** 2006. Global patterns of influenza a virus in wild birds. Science **312:**384–388. http://dx.doi.org/10.1126/science.1122438.
3. **Liu J, Xiao H, Lei F, Zhu Q, Qin K, Zhang XW, Zhang XL, Zhao D, Wang G, Feng Y, Ma J, Liu W, Wang J, Gao GF.** 2005. Highly patho-

genic H5N1 influenza virus infection in migratory birds. Science **309:** 1206. http://dx.doi.org/10.1126/science.1115273.

4. **Winker K, McCracken KG, Gibson DD, Pruett CL, Meier R, Huettmann F, Wege M, Kulikova IV, Zhuravlev YN, Perdue ML, Spackman E, Suarez DL, Swayne DE.** 2007. Movements of birds and avian influenza from Asia into Alaska. Emerg Infect Dis **13:**547–552. http://dx.doi.org/10.3201/eid1304.061072.
5. **Winker K, Gibson DD.** 2010. The Asia-to-America influx of avian influenza wild bird hosts is large. Avian Dis **54:**477–482. http://dx.doi.org/10.1637/8741-032509-Reg.1.
6. **Lee YJ, Kang HM, Lee EK, Song BM, Jeong J, Kwon YK, Kim HR, Lee KJ, Hong MS, Jang I, Choi KS, Kim JY, Lee HJ, Kang MS, Jeong OM, Baek JH, Joo YS, Park YH, Lee HS.** 2014. Novel reassortant influenza A(H5N8) viruses, South Korea, 2014. Emerg Infect Dis **20:**1087–1089. http://dx.doi.org/10.3201/eid2006.140233.
7. **Jeong J, Kang HM, Lee EK, Song BM, Kwon YK, Kim HR, Choi KS, Kim JY, Lee HJ, Moon OK, Jeong W, Choi J, Baek JH, Joo YS, Park YH, Lee HS, Lee YJ.** 2014. Highly pathogenic avian influenza virus (H5N8) in domestic poultry and its relationship with migratory birds in South Korea during 2014. Vet Microbiol **173:**249–257. http://dx.doi.org/10.1016/j.vetmic.2014.08.002.
8. **Ip HS, Torchetti MK, Crespo R, Kohrs P, DeBruyn P, Mansfield KG, Baszler T, Badcoe L, Bodenstein B, Shearn-Bochsler V, Killian ML, Pedersen JC, Hines N, Gidlewski T, DeLiberto T, Sleeman JM.** 2015. Novel Eurasian highly pathogenic influenza A H5 viruses in wild birds, Washington, USA, 2014. Emerg Infect Dis **21:**May 2015. http://dx.doi.org/10.3201/eid2105.142020.
9. **Verhagen JH, Herfst S, Fouchier RA.** 2015. Infectious disease. How a virus travels the world. Science **347:**616–617. http://dx.doi.org/10.1126/science.aaa6724.
10. **Salzberg SL, Kingsford C, Cattoli G, Spiro DJ, Janies DA, Aly MM, Brown IH, Couacy-Hymann E, De Mia GM, Dung do, Guercio HA, Joannis T, Maken Ali AS, Osmani A, Padalino I, Saad MD, Savic V, Sengamalay NA, Yingst S, Zaborsky J, Zorman-Rojs O, Ghedin E, Capua I.** 2007. Genome analysis linking recent European and African influenza (H5N1) viruses. Emerg Infect Dis **13:**713–718. http://dx.doi.org/10.3201/eid1305.070013.
11. **Pasick J, Berhane Y, Joseph T, Bowes V, Hisanaga T, Handel K, Alexandersen S.** 2015. Reassortant highly pathogenic influenza A H5N2 virus containing gene segments related to Eurasian H5N8 in British Columbia, Canada, 2014. Sci Rep **5:**9484. http://dx.doi.org/10.1038/srep09484.
12. **World Organization for Animal Health.** 2014. Summary of immediate notifications and follow-ups—2014: highly path. avian influenza. http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Immsummary. Accessed 13 March 2015.
13. **Tian H, Zhou S, Dong L, Van Boeckel TP, Cui Y, Wu Y, Cazelles B, Huang S, Yang R, Grenfell BT, Xu B.** 2015. Avian influenza H5N1 viral and bird migration networks in Asia. Proc Natl Acad Sci U S A **112:**172–177. http://dx.doi.org/10.1073/pnas.1405216112.
14. **Takekawa JY, Newman SH, Xiao X, Prosser DJ, Spragens KA, Palm EC, Yan B, Li T, Lei F, Zhao D, Douglas DC, Muzaffar SB, Ji W.** 2010. Migration of waterfowl in the East Asian flyway and spatial relationship to HPAI H5N1 outbreaks. Avian Dis **54:**466–476. http://dx.doi.org/10.1637/8914-043009-Reg.1.
15. **Ip HS, Flint PL, Franson JC, Dusek RJ, Derksen DV, Gill RE, Jr, Ely CR, Pearce JM, Lanctot RB, Matsuoka SM, Irons DB, Fischer JB, Oates RM, Petersen MR, Fondell TF, Rocque DA, Pedersen JC, Rothe TC.** 2008. Prevalence of influenza A viruses in wild migratory birds in Alaska: patterns of variation in detection at a crossroads of intercontinental flyways. Virol J **5:**71. http://dx.doi.org/10.1186/1743-422X-5-71.
16. **Koehler AV, Pearce JM, Flint PL, Franson JC, Ip HS.** 2008. Genetic evidence of intercontinental movement of avian influenza in a migratory bird: the northern pintail (Anas acuta). Mol Ecol **17:**4754–4762. http://dx.doi.org/10.1111/j.1365-294X.2008.03953.x.
17. **Gabrielson IN, Lincoln FC.** 1959. The birds of Alaska. Stackpole Co., Harrisburg, PA.
18. **Dusek RJ, Hallgrimsson GT, Ip HS, Jonsson JE, Sreevatsan S, Nashold SW, TeSlaa JL, Enomoto S, Halpin RA, Lin X, Fedorova N, Stockwell TB, Dugan VG, Wentworth DE, Hall JS.** 2014. North Atlantic migratory bird flyways provide routes for intercontinental movement of avian influenza viruses. PLoS One **9:**e92075. http://dx.doi.org/10.1371/journal.pone.0092075.
19. **Lam TT, Ip HS, Ghedin E, Wentworth DE, Halpin RA, Stockwell TB, Spiro DJ, Dusek RJ, Bortner JB, Hoskins J, Bales BD, Yparraguirre DR, Holmes EC.** 2012. Migratory flyway and geographical distance are barriers to the gene flow of influenza virus among North American birds. Ecol Lett **15:**24–33. http://dx.doi.org/10.1111/j.1461-0248.2011.01703.x.
20. **Kang HM, Lee EK, Song BM, Jeong J, Choi JG, Jeong JJ, Moon OK, Yoon H, Cho YM, Kang YM, Lee HS, Lee YJ.** 2015. Novel reassortant influenza A(H5N8) viruses among inoculated domestic and wild ducks, South Korea, 2014. Emerg Infect Dis **21:**298–304. http://dx.doi.org/10.3201/eid2102.141268.