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Reoccurrence of Avian Influenza A(H5N2) Virus Clade 2.3.4.4 in Wild Birds, Alaska, USA, 2016

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DOI: <http://dx.doi.org/10.3201/eid2302.161616>

We report reoccurrence of highly pathogenic avian influenza A(H5N2) virus clade 2.3.4.4 in a wild mallard in Alaska, USA, in August 2016. Identification of this virus in a migratory species confirms low-frequency persistence in North America and the potential for re-dissemination of the virus during the 2016 fall migration.

Historically, apparently effective geographic barriers (Bering and Chukchi Seas of the North Pacific Ocean) appeared to limit dissemination of Asian-origin, highly pathogenic avian influenza virus (HPAIV), such as influenza A(H5N1) virus A/goose/Guangdong/1/1996 (Gs/GD), between the Old and New Worlds (1). However, such barriers are incomplete; occasional spillovers of virus genes move from 1 gene pool to another (2). Asian-origin HPAIV H5N8 was identified in North America at the end of 2014 (3).

Novel HPAIVs H5N1, H5N2, and H5N8 emerged in late 2014 by reassortment with North American low pathogenicity avian influenza viruses (4). A novel reassortant H5N2 virus originating from Asian-origin H5N8 virus clade 2.3.4.4 and containing Eurasian polymerase basic 2, polymerase acidic, hemagglutinin, matrix, and nonstructural protein genes and North American lineage neuraminidase (NA), polymerase basic 1 (PB1), and nucleoprotein genes was identified on poultry farms in British Columbia, Canada, and in wild waterfowl in the northwestern United States. This virus subsequently predominated during influenza outbreaks in the United States in 2015.

During the boreal summer, birds from 6 continents (North America, South America, Asia, Africa, Australia, and Antarctica) fly to Alaska, USA, to breed. Thus, Alaska is a potentially major location for intercontinental virus transmission (1,2). Recent data provide direct evidence for viral dispersal through Beringia (5,6). Genetic evidence and waterfowl migratory patterns support the hypothesis that H5 virus clade 2.3.4.4 was introduced into North America through the Beringian Crucible by intercontinental associations with waterfowl (3). In addition, low pathogenicity avian influenza viruses were collected in Alaska before initial detection of H5 HPAIV clade 2.3.4.4, which contained genes that had recent common ancestry with reassortant H5N2 virus PB1, nucleoprotein, and NA (N2 subtype) genes and H5N1 virus PB1, polymerase acidic, NA (N1 subtype), and nonstructural protein genes of HPAIVs (7).

We report detection of an HPAIV H5N2 subtype from wild mallard sampled in Alaska during August 2016. Influenza A virus was detected in 48/188 dabbling duck

samples collected during a live bird banding effort near Fairbanks, Alaska, during August 6–15, 2016. One sample of H5 virus from an adult mallard was identified as an HPAIV H5N2 on the basis of complete genome sequencing. We conducted comparative phylogenetic analysis of A/mallard/Alaska/AH0008887/2016(H5N2) virus, hereafter known as 8887/2016(H5N2) virus, to trace its origin and understand its genetic relationship to HPAIV H5N2 isolated in 2014–2015 (online Technical Appendix (<https://wwwnc.cdc.gov/EID/article/23/2/16-1616-Techapp1.pdf>)).

We considered 8887/2016(H5N2) virus an HPAIV on the basis of amino acid sequence at the hemagglutinin proteolytic cleavage site (PLRERRRKR/G), as shown for other Gs/GD HPAIV H5Nx subtypes in subclade 2.3.4 (http://www.offlu.net/fileadmin/home/en/resource-centre/pdf/Influenza_A_Cleavage_Sites.pdf). Homology BLAST searches showed that all genes had >99.2% nucleotide similarity with genes of H5N2 virus outbreak strains collected during late February–March 2015 (online Technical Appendix Table).

Phylogenetic analysis showed that the concatenated genome of 8887/2016(H5N2) virus formed a cluster with viruses from initial detections in the midwestern United States, including a snow goose in Missouri, a backyard poultry farm in Kansas, and a turkey farm in Minnesota (Figure). Our epidemiologic investigation data suggested that point-source introductions by indirect contact with

wild waterfowl were the most probable source of infection for these backyard poultry in Kansas and a turkey farm in Minnesota (8). This genetic cluster was supported by a maximum-likelihood bootstrap value of 80 and a Bayesian posterior probability of 1.00.

The mean time to most recent common ancestry of viruses in this genetic cluster was estimated to be the end of January 2015 (mean time to most recent common ancestry January 27, 2015, 95% Bayesian credible interval January 11–February 10, 2015). Consistent clustering of 8887/2016(H5N2) virus with other H5N2 outbreak viruses in phylogenies for each gene suggests that the 8887/2016(H5N2) virus probably evolved through genetic drift from common ancestors of outbreak viruses in the absence of further reassortment (online Technical Appendix Figure 2). The mean rate of the nucleotide substitution obtained by Bayesian analysis was 6.064×10^{-3} (95% Bayesian credible interval $4.43\text{--}7.82 \times 10^{-3}$) substitutions/site/year. In the root-to-tip regression plot of maximum-likelihood phylogeny, we found that 8887/2016(H5N2) virus fell below the regression line, which indicated sequences that are slightly less divergent than average of 2014–2015 H5N2 outbreak viruses (online Technical Appendix Figure 3).

The last reported detection during the influenza outbreak in the United States in 2015 was from a Canada goose in Michigan on June 17. There were 2 detections by

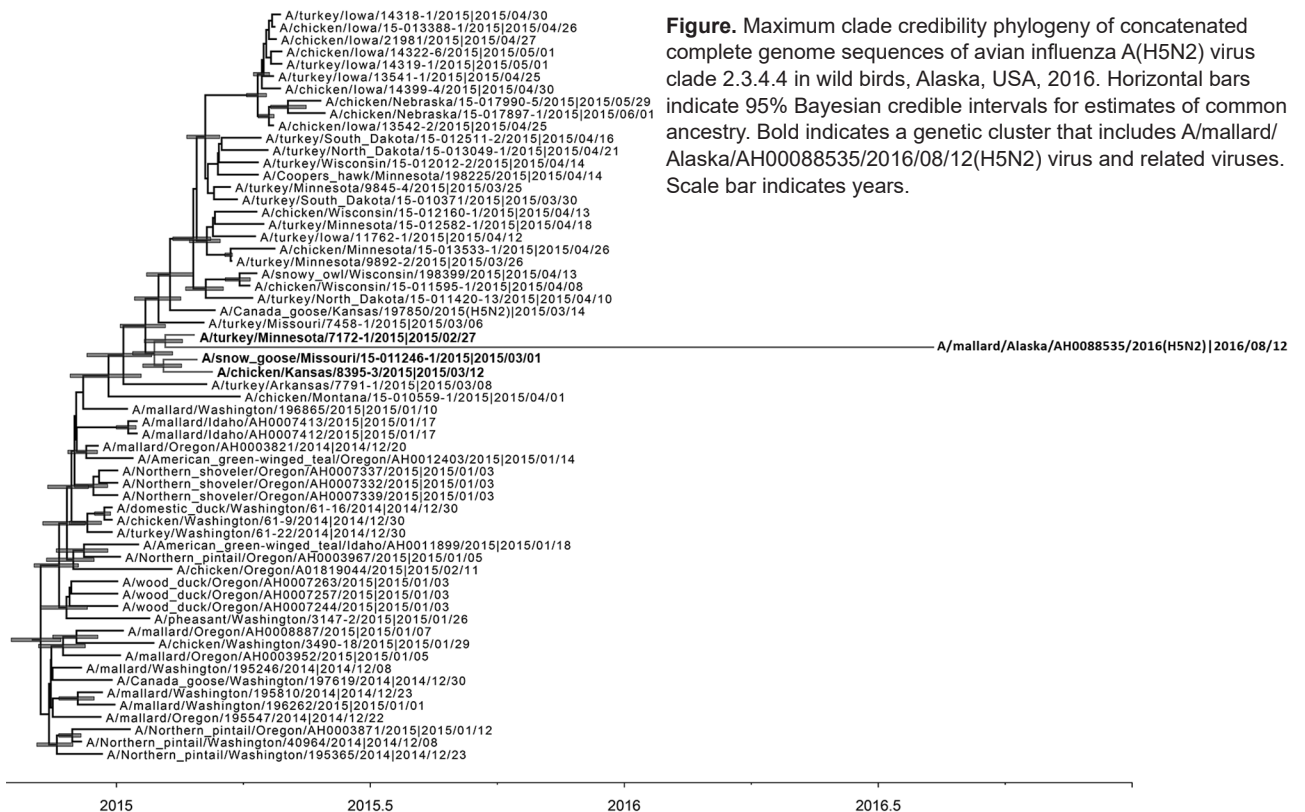


Figure. Maximum clade credibility phylogeny of concatenated complete genome sequences of avian influenza A(H5N2) virus clade 2.3.4.4 in wild birds, Alaska, USA, 2016. Horizontal bars indicate 95% Bayesian credible intervals for estimates of common ancestry. Bold indicates a genetic cluster that includes A/mallard/Alaska/AH00088535/2016/08/12(H5N2) virus and related viruses. Scale bar indicates years.

PCR (3 assays, 2 gene targets, no virus recovered, no sequence obtained) from mallards in July (bird banding effort in Utah) and November (hunter harvest in Oregon) during surveillance in 2015–2016. Sequence of the HPAIV H5N2 from a wild mallard during surveillance in 2016–2017, evidence for continued evolution of this virus lineage, widespread detections of HPAIV H5N2 in healthy wild birds (9), and lack of pathobiological effects in experimentally infected waterfowl (10) collectively provide strong evidence for maintenance of HPAIV H5N2 in wild birds in North America. Detection of HPAIV in a mallard might imply the potential for dissemination of HPAIV H5N2 during the southward fall migration of waterfowl in 2016.

Acknowledgments

We thank Michael J. Petrula and David Sinnett for collecting samples; Kerrie Franzen, Meredith Grady, Andrew Hubble for providing technical assistance; the Washington State Animal Disease Diagnostic Laboratory for their participation in wild bird surveillance activities, and the originating and submitting institution (Kagoshima University, Kagoshima, Japan) for A/crane/Kagoshima/KU1/2014(H5N8) sequences (accession no. EPI169390] from the GISAID EpiFlu Database (<http://platform.gisaid.org>).

Dr. Lee is postdoctoral researcher at the US Department of Agriculture, Athens, GA. His research interests include molecular epidemiology and host–pathogen interactions for avian influenza viruses.

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Increase in Urgent Care Center Visits for Sexually Transmitted Infections, United States, 2010–2014

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DOI: <http://dx.doi.org/10.3201/eid2302.161707>

During 2010–2014, urgent care centers saw a ≈2-fold increase in the number of visits for chlamydia and gonorrhea testing and a >3-fold increase in visits by persons with diagnosed sexually transmitted infections. As urgent care becomes more popular, vigilance is required to ensure proper management of these diseases.

Sexually transmitted infections (STIs) are the most commonly reported nationally notifiable diseases in the United States (1), and annual medical costs for these diseases are estimated to exceed \$16 billion (2). Reported rates of gonorrhea, chlamydia, and syphilis all increased from 2014 to 2015, and antimicrobial drug-resistant gonorrhea remains an important concern (3). Therefore, proper diagnosis and

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Technical Appendix

Methods for Genome Sequencing and Phylogenetic Analysis

We collected 188 wild waterfowl samples from Creamer's Field Migratory Waterfowl Refuge located in Fairbanks, Alaska, USA during August 6–15, 2016. We conducted complete genome sequencing and comparative phylogenetic analysis of A/mallard/Alaska/AH0008887/2016(H5N2) virus, hereafter 8887/2016(H5N2), to trace the origin and to estimate its evolutionary history. A sample was confirmed to be H5 positive by using matrix gene real-time reverse transcription PCR and genome sequencing.

Complete genome sequencing of 8887/2016(H5N2) virus was performed by using next-generation sequencing with Ion Chef, the Ion S5 sequencing system, and Ion Total RNA-Seq Kit v2 Library Preparation Kit (Thermo Scientific Fisher, Waltham, MA, USA) according to the manufacturer's instructions. Data were analyzed by using SeqMan NGen v. 4 (<https://www.dnastar.com/t-nextgen-seqman-ngen.aspx>). Nucleotide sequences were deposited in GenBank under accession nos. KX838896–KX838903.

For phylogenetic analysis, we retrieved and used all H5N2 highly pathogenic avian influenza virus subtype sequences identified in North America during 2014–2015 available in the Influenza Virus Resource (<https://www.ncbi.nlm.nih.gov/genome/viruses/variation/flu/>) as of September 1, 2016. Maximum-likelihood (ML) phylogenies of each gene segment and concatenated full genome were generated by using RAxML (1) and the Generalized Time Reversible nucleotide substitution model with among-site rate variation modeled by using a discrete gamma distribution.

ML phylogenies of polymerase basic 2, polymerase acidic, hemagglutinin, matrix, and nonstructural protein genes were rooted to A/Crane/Kagoshima/KU1/2014(H5N8) virus, and polymerase basic 1, nucleoprotein, and neuraminidase genes were rooted to low pathogenicity avian influenza viruses collected in Alaska that share recent common ancestry with H5N2 subtype to highly pathogenic avian influenza viruses (2). Bootstrap support values were generated by using 1,000 rapid

bootstrap replicates. To investigate the temporal signal and clocklikeness of ML phylogenies of the dataset, we performed linear regression on the root-to-tip distances of samples versus date of the isolate by using TempEst v1.5 (3).

Bayesian relaxed clock phylogenetic analysis of concatenated genome (ntax = 61) was performed by using BEAST v1.8.3 (4). We applied an uncorrelated lognormal distribution relaxed clock method, the Hasegawa–Kishino–Yano nucleotide substitution model and the Bayesian skyline coalescent prior. A Markov Chain Monte Carlo method to sample trees and evolutionary parameters was run for 1.0×10^8 generations. At least 3 independent chains were combined to ensure adequate sampling of the posterior distribution of trees. BEAST output was analyzed with TRACER v1.4 (<https://beast.bio.ed.ac.uk/tracer>) with 10% burn-in. A maximum clade credibility tree was generated for each dataset by using TreeAnnotator in BEAST. FigTree 1.4.2 (<https://tree.bio.ed.ac.uk/>) was used for visualization of trees.

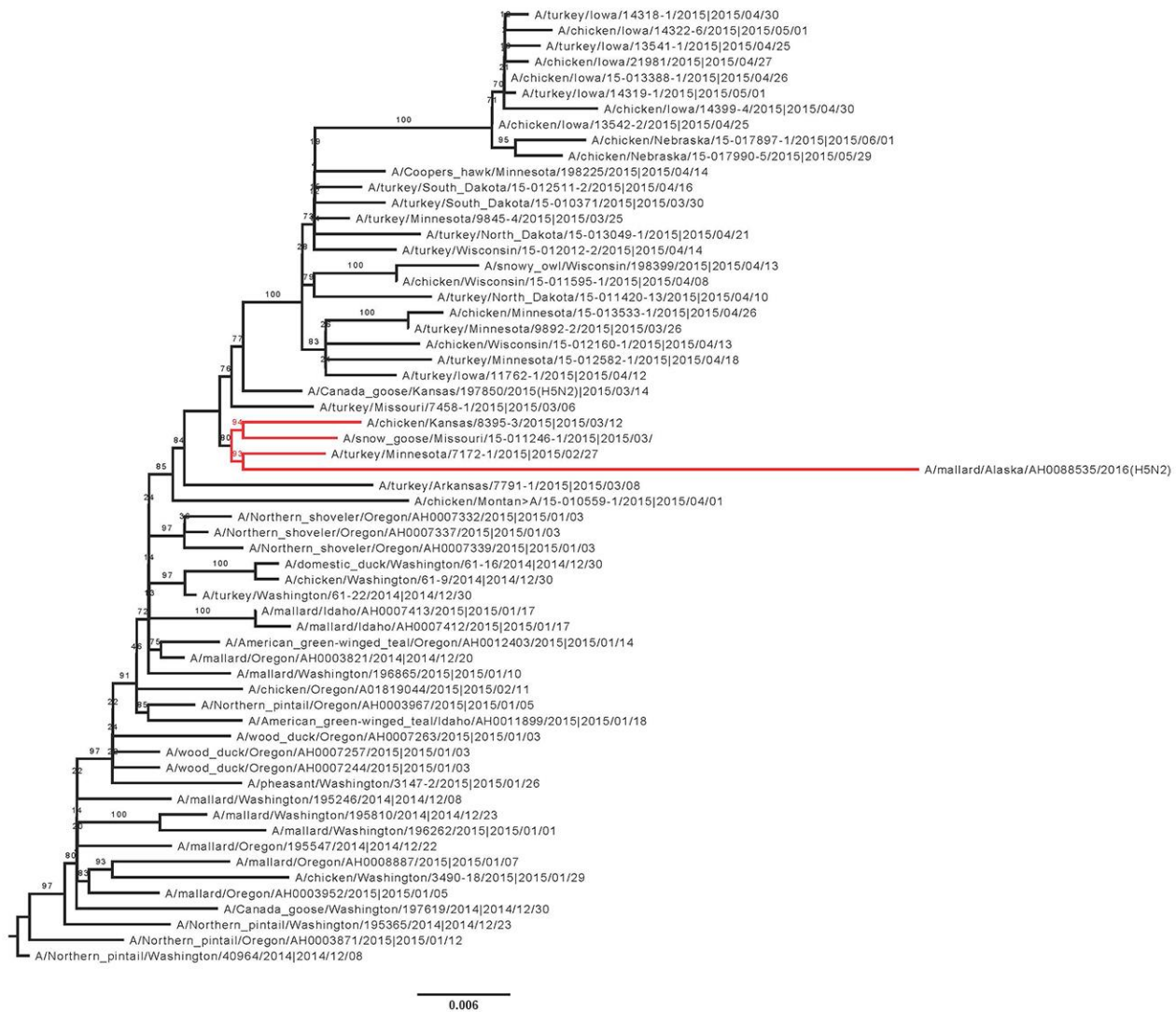
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Technical Appendix Table. Nucleotide identities between A/mallard/Alaska/AH0008887/2016(H5N2) influenza virus and nearest homologs in GenBank as of September 1, 2016

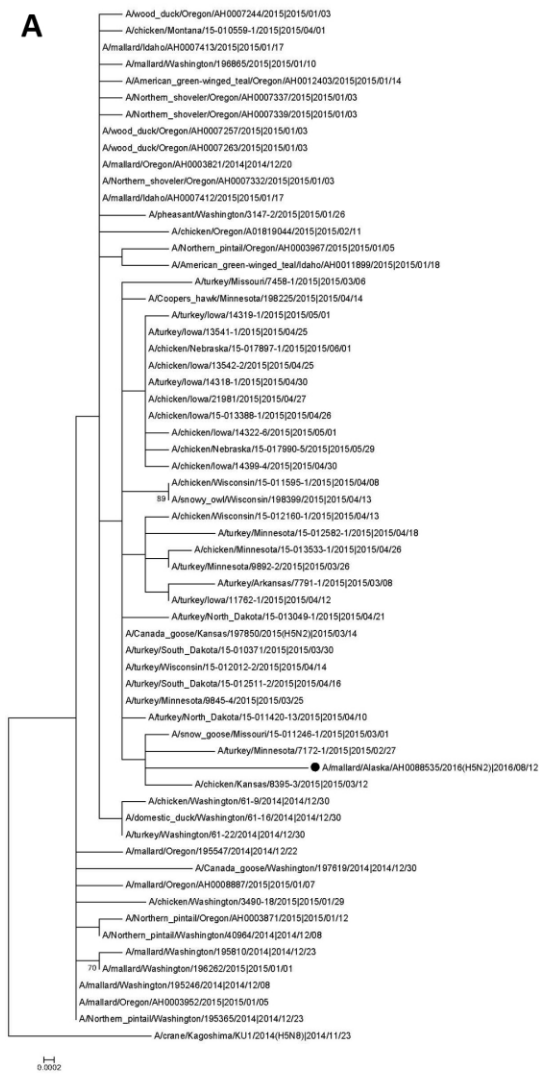
Gene*	Virus	Collection date, 2015	% Identity
PB2	A/Canada goose/Kansas/197850/2015(H5N2)	Mar 13	99.7
PB1	A/turkey/Minnesota/7172–1/2015(H5N2)	Feb 27	99.6
PA	A/turkey/Minnesota/7172–1/2015(H5N2)	Feb 27	99.7
HA	A/snow goose/Missouri/15–011246–1/2015(H5N2)	Jan 3	99.4
NP	A/turkey/Minnesota/7172–1/2015(H5N2)	Feb 27	99.5
NA	A/turkey/Missouri/7458–1/2015(H5N2)	Mar 6	99.5
MP	A/Canada goose/Kansas/197850/2015(H5N2)	Mar 13	99.6
NS	A/snow goose/Missouri/15–011246–1/2015(H5N2)	Jan 3	99.2

*HA, hemagglutinin; MP, matrix; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural; PA, polymerase acidic; PB1 polymerase basic 1; PB2, polymerase basic 2.



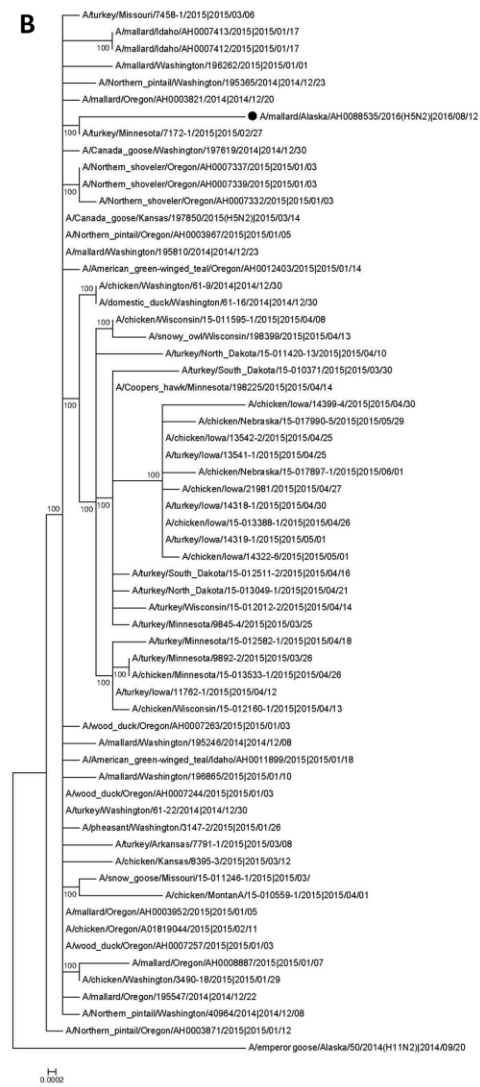
Technical Appendix Figure 1. Maximum-likelihood phylogeny of concatenated complete genome sequences of avian influenza A(H5N2) virus clade 2.3.4.4 in wild birds, Alaska, USA, 2016. Numbers along branches indicate bootstrap values >70%. Black circle indicates A/mallard/Alaska/AH0008887/2016(H5N2) virus. Red branches indicate a genetic cluster that includes the A/mallard/Alaska/AH0008887/2016(H5N2) virus and related viruses. Scale bar indicates nucleotide substitutions per site.

A



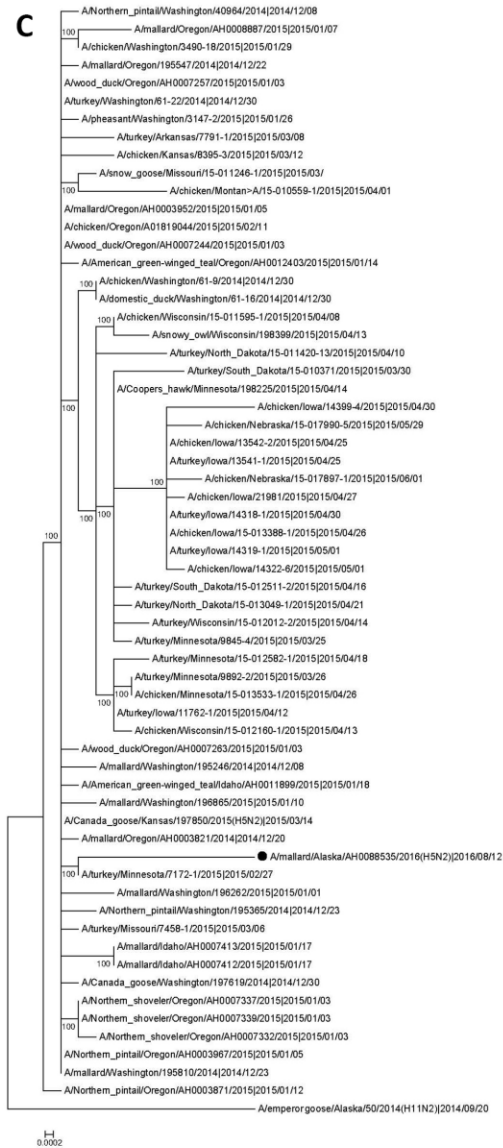
PB2 gene

B



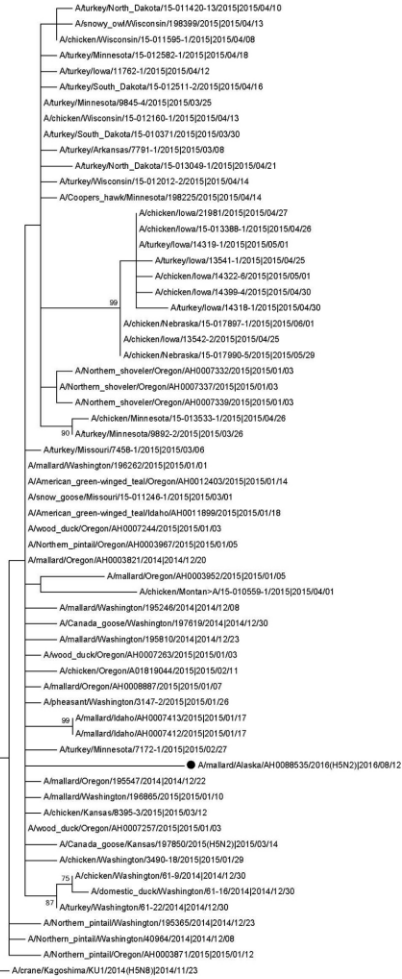
PB1 gene

C



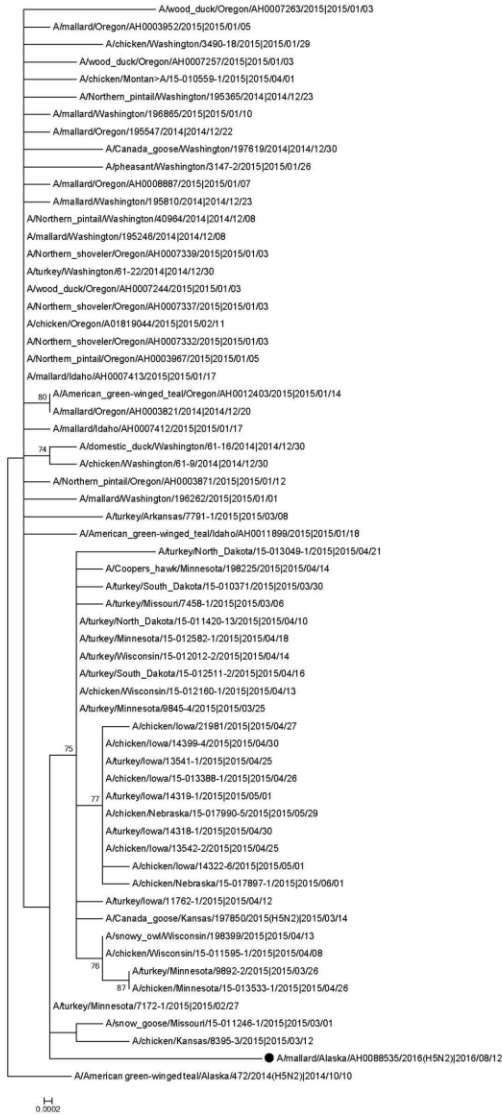
PA gene

D



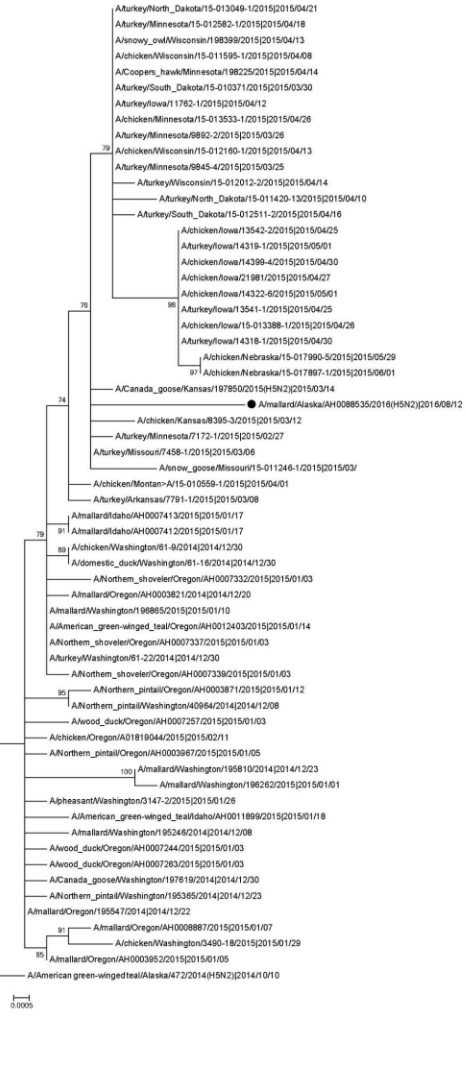
HA gene

E

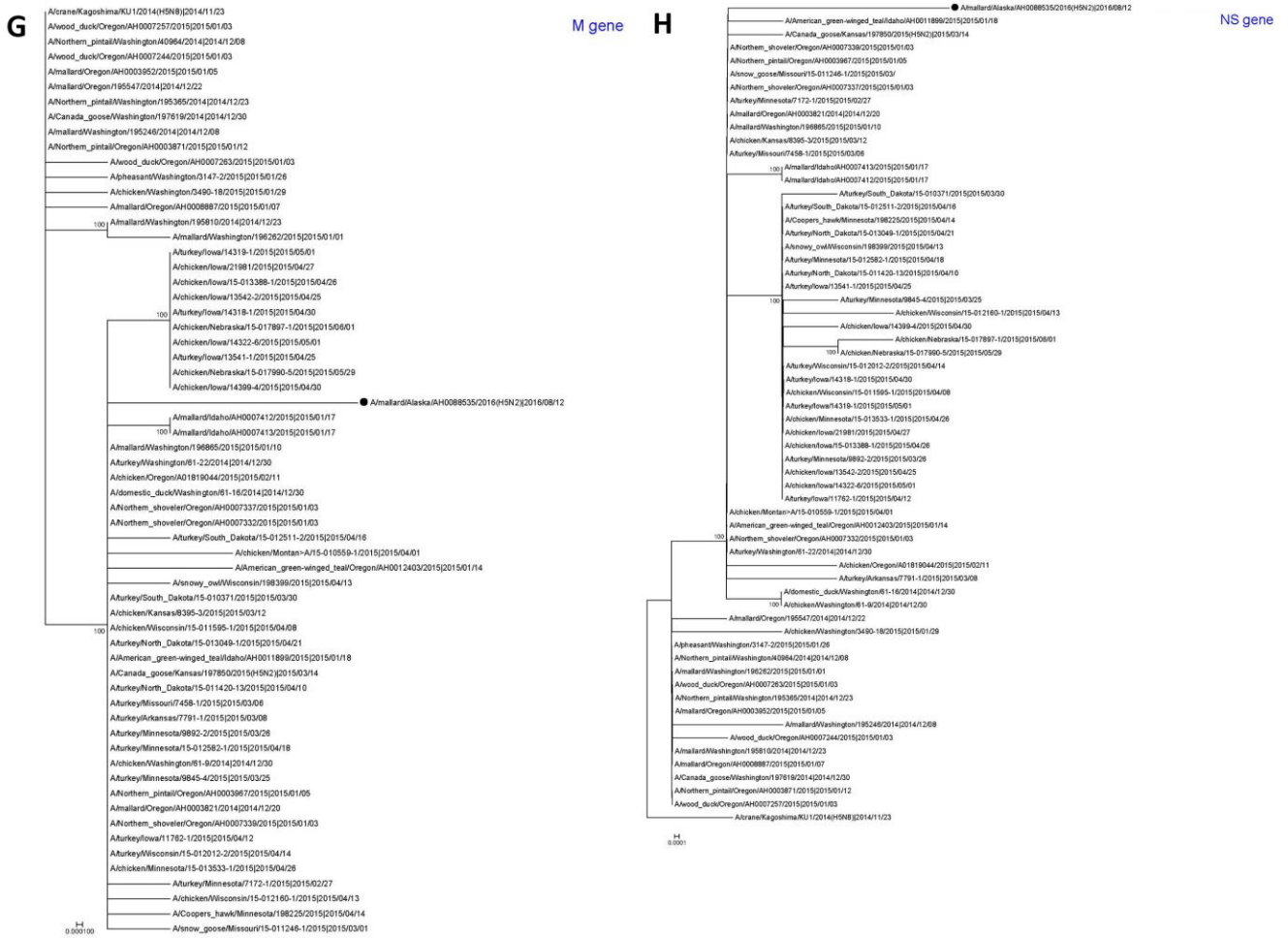


NP gene

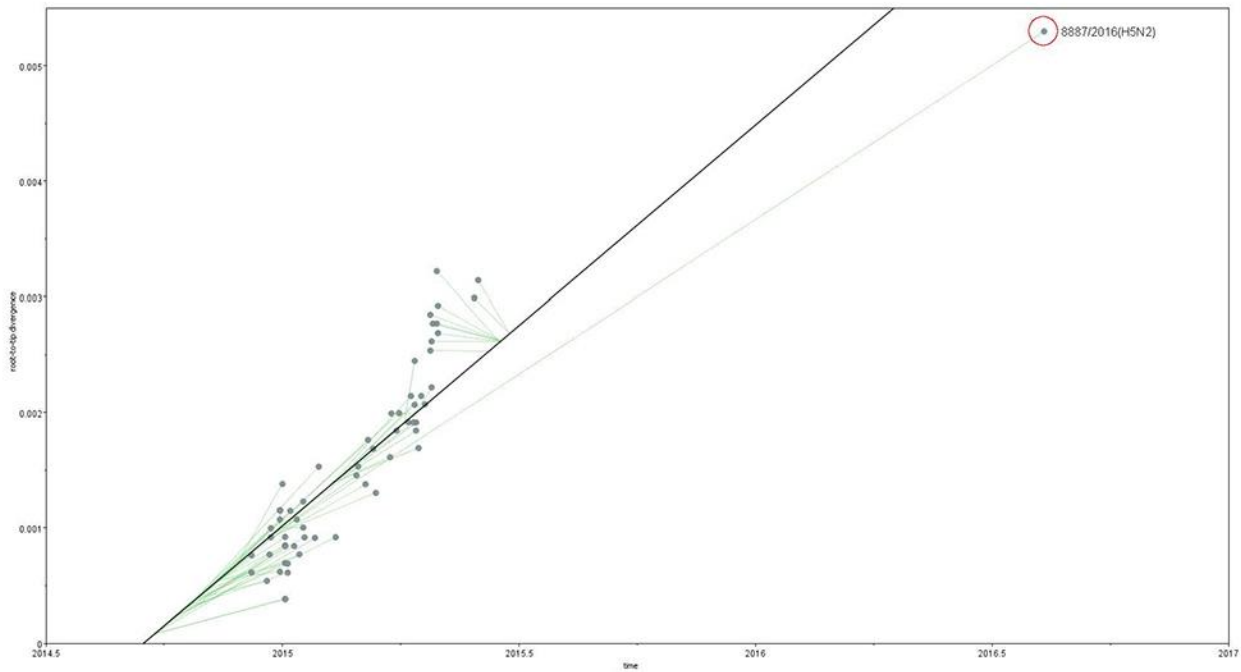
F



NA gene



Technical Appendix Figure 2. Maximum-likelihood phylogeny of A) polymerase basic 2 (PB2); B) polymerase basic 1 (PB1); C) polymerase acidic (PA); D) hemagglutinin (HA); E) nucleoprotein (NP); F) neuraminidase (NA); G) matrix (M), and H) nonstructural (NS) protein genes of avian influenza A(H5N2) virus clade 2.3.4.4 in wild birds, Alaska, USA, 2016. Numbers along branches indicate bootstrap values >70%. Black circle indicates A/mallard/Alaska/AH0008887/2016(H5N2) virus. Scale bars indicate nucleotide substitutions per site.



Technical Appendix Figure 3. Root-to-tip regression plot, with ancestor traces shown, of maximum-likelihood phylogeny of concatenated complete genome sequences of avian influenza A(H5N2) virus clade 2.3.4.4 in wild birds, Alaska, USA, 2016. Red circle indicates A/mallard/Alaska/AH0008887/2016(H5N2) virus.