

# Avian Influenza A(H7N2) Virus in Human Exposed to Sick Cats, New York, USA, 2016

**Atanaska Marinova-Petkova, Jen Laplante, Yunho Jang, Brian Lynch, Natosha Zanders, Marisela Rodriguez, Joyce Jones, Sharmi Thor, Erin Hodges, Juan A. De La Cruz, Jessica Belser, Hua Yang, Paul Carney, Bo Shu, LaShondra Berman, Thomas Stark, John Barnes, Fiona Havers, Patrick Yang, Susan C. Trock, Alicia Fry, Larisa Gubareva, Joseph S. Bresee, James Stevens, Demetre Daskalakis, Dakai Liu, Christopher T. Lee, Mia Kim Torchetti, Sandra Newbury, Francine Cigel, Kathy Toohey-Kurth, Kirsten St. George, David E. Wentworth, Stephen Lindstrom, C. Todd Davis**

An outbreak of influenza A(H7N2) virus in cats in a shelter in New York, NY, USA, resulted in zoonotic transmission. Virus isolated from the infected human was closely related to virus isolated from a cat; both were related to low pathogenicity avian influenza A(H7N2) viruses detected in the United States during the early 2000s.

Avian influenza viruses occasionally cross the species barrier, infecting humans and other mammals after exposure to infected birds and contaminated environments. Unique among the avian influenza A subtypes, both low pathogenicity and highly pathogenic H7 viruses have demonstrated the ability to infect and cause disease in humans (1,2). In the eastern and northeastern United States, low pathogenicity avian influenza (LPAI) A(H7N2) viruses

circulated in live bird markets periodically during 1994–2006 (3) and caused poultry outbreaks in Virginia, West Virginia, and North Carolina in 2002 (4). During an outbreak in Virginia in 2002, human infection with H7N2 virus was serologically confirmed in a culler with respiratory symptoms (5). In 2003, another human case of H7N2 infection was reported in a New York resident (6); although the source of exposure remains unknown, the isolated virus was closely related to viruses detected in live bird markets in the region. Because of the sporadic nature of these and other zoonotic infections with influenza H7 viruses throughout the world, the World Health Organization (WHO) recommended development of several candidate vaccine viruses for pandemic preparedness purposes, including 2 vaccines derived from North American lineage LPAI viruses, A/turkey/Virginia/4529/2002 and A/New York/107/2003 (7).

## The Study

On December 19, 2016, the New York City Department of Health and Mental Hygiene collected a respiratory specimen from a veterinarian experiencing influenza-like illness after exposure to sick domestic cats at an animal shelter in New York, NY, USA. The specimen tested positive for influenza A but could not be subtyped. Specimen aliquots were shipped to the Wadsworth Center, New York State Department of Health (Albany, NY, USA), and to the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA). Next-generation sequencing performed at the New York State Department of Health generated a partial genomic sequence (6 of 8 influenza A virus gene segments) that aligned most closely with North American lineage LPAI A(H7N2) viruses. North American lineage H7 real-time reverse transcription PCR (rRT-PCR) testing and diagnostic sequence analysis performed at CDC confirmed the sample to be positive for influenza A(H7N2) virus. Virus isolation was attempted by inoculating the sample in 10-day-old embryonated chicken eggs and MDCK CCL-34 and CRFK (Crandell-Rees Feline Kidney) cell lines (American Type Culture Collection). A/New York/108/2016 was successfully isolated from eggs but not from MDCK or CRFK cells. Codon complete sequencing of the egg-isolated virus (GISAID accession nos. EPI944622–9; <http://www.gisaid.org>) showed no nucleotide changes compared with the hemagglutinin (HA) and neuraminidase (NA) gene segments sequenced directly from the clinical specimen. The virus was nearly identical (99.9%) to a virus isolated

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (A. Marinova-Petkova, Y. Jang, B. Lynch, N. Zanders, M. Rodriguez, J. Jones, S. Thor, E. Hodges, J.A. De La Cruz, J. Belser, H. Yang, P. Carney, B. Shu, L. Berman, T. Stark, J. Barnes, F. Havers, P. Yang, S.C. Trock, A. Fry, L. Gubareva, J.S. Bresee, J. Stevens, D.E. Wentworth, S. Lindstrom, C.T. Davis); New York State Department of Health, Albany, New York, USA (J. Laplante, K. St. George); New York City Department of Health and Mental Hygiene, Long Island City, New York, USA (D. Daskalakis, D. Liu, C.T. Lee); US Department of Agriculture, Ames, Iowa, USA (M.K. Torchetti); University of Wisconsin, Madison, Wisconsin, USA (S. Newbury, F. Cigel, K. Toohey-Kurth)

DOI: <https://doi.org/10.3201/eid2312.170798>

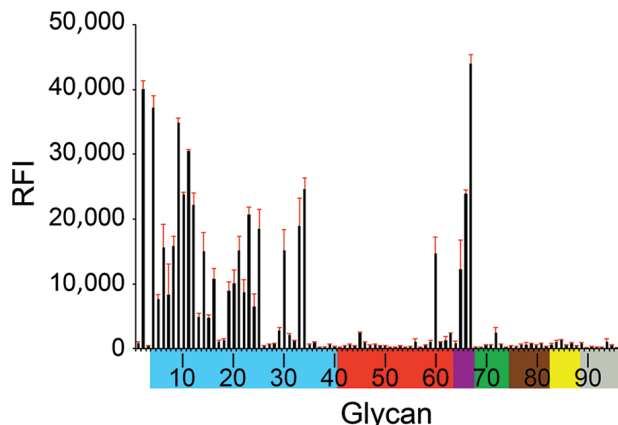


Figure 1. Receptor binding specificity of A/New York/108/2016 (H7N2) influenza virus isolated from a human who experienced influenza-like illness after exposure to sick domestic cats at an animal shelter in New York, NY, USA, 2016. Figure indicates glycan microarray analysis. Colored bars represent glycans that contain  $\alpha$ -2,3 sialic acid (SA) (blue),  $\alpha$ -2,6 SA (red),  $\alpha$ -2,3/ $\alpha$ -2,6 mixed SA (purple), N-glycolyl SA (green),  $\alpha$ -2,8 SA (brown),  $\beta$ -2,6 and 9-O-acetyl SA (yellow), and non-SA (gray). Error bars reflect SE in the signal for 6 independent replicates on the array. RFI, relative fluorescence intensity.

from a cat, A/feline/New York/16-040082-1/2016, from a New York shelter where the veterinarian had worked; the cat died of its illness. Phylogenetic analysis of the cat and human viruses showed that their genomes were closely related to LPAI A(H7N2) viruses that were circulating in the northeastern United States in the early 2000s (online Technical Appendix Figure, <https://wwwnc.cdc.gov/EID/article/23/12/17-0798-Techapp1.pdf>).

Analysis of the HA gene segments revealed that A/New York/108/2016 and A/feline/New York/16-040082-1/2016 were phylogenetically related to H7N2 viruses isolated from poultry in the eastern United States (New York, Virginia, Pennsylvania, North Carolina, Massachusetts) during 1996–2005, including 2 influenza A(H7N2) WHO-recommended candidate vaccine viruses. Although the internal protein coding gene segments (polybasic 1 and 2, polyacidic, nucleoprotein, matrix, nonstructural) were distant to sequences available in databases (average nucleotide identity to the closest genetic relative was 97.6%), analysis indicated that they were of LPAI virus origin and lacked known mammalian adaptive substitutions. The longer branch lengths of the internal protein coding gene segments highlighted the scarcity of sequence data available for contemporary H7N2 viruses in the United States.

Similar to well-characterized H7N2 viruses, such as A/turkey/Virginia/4529/2002 and A/New York/107/2003, A/New York/108/2016 had deletion of amino acids 212–219 in the mature HA protein (H7 numbering), known as the 220-loop of the HA receptor binding domain (8). Such deletion has been previously shown to enhance binding and

infectivity of H7 viruses to the mammalian respiratory tract and increase direct contact transmission between mammals (9). Glycan microarray analysis showed that A/New York/108/2016 bound preferentially to  $\alpha$ -2,3 avian-like receptors but also showed binding to the  $\alpha$ -2,6 glycan with internal sialoside (LSTb, glycan #60), as well as to glycans with mixed  $\alpha$ -2,3/ $\alpha$ -2,6 receptors (Figure 1). Strong binding to the LSTb glycan has been previously reported for North America H7N2 viruses of avian origin (8,9) and 2013 human H7N9 viruses (10). The role of the LSTb glycan binding remains unknown; it has been identified only in human milk (11).

Additional molecular characterization of the HA1 protein showed 20 aa differences between A/New York/108/2016 and A/turkey/Virginia/4529/2002 (26 aa in both HA1 and HA2; Figure 2). The substitution A125S resulted in a gain of glycosylation in the HA protein of A/New York/108/2016, previously correlated with increased replication efficiency and wider tissue distribution of A/Netherlands/219/2003 (H7N7) (12). The substitution of T183I was shown in other avian influenza viruses (e.g., H5N1) to enhance binding to mammalian sialic acid receptors (13). Four of the 20 aa changes were in residues associated with antibody recognition at antigenic site B (E177G, S180N, T183I, and S188N) and antigenic site C (R269G).

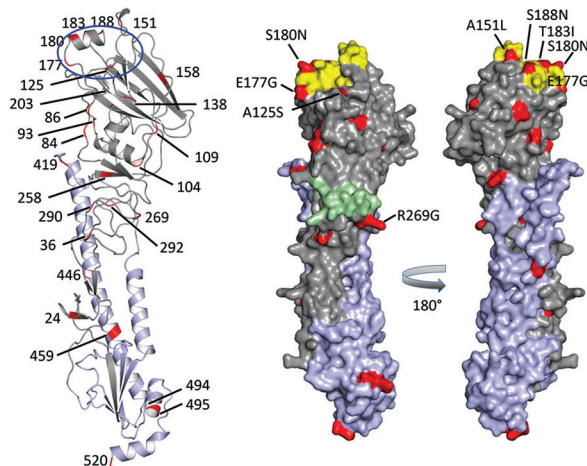


Figure 2. Receptor binding specificity of A/New York/108/2016 (H7N2) influenza virus isolated from a human who experienced influenza-like illness after exposure to sick domestic cats at an animal shelter in New York, NY, USA, 2016. Figure shows A/New York/108/2016 hemagglutinin (HA) monomer structure. HA1 is shown in gray, HA2 in light purple, amino acid changes in comparison with reference virus A/turkey/Virginia/4529/2002 (H7N2) in red. On the cartoon view (left), all amino acid changes in the HA protein are labeled. The location of the receptor binding site (blue circle) includes the 120-loop and the 180-helix. The 220-loop is missing due to deletion of amino acids 212–219 in the mature HA protein (H7 numbering). On the surface model (right), only amino acid substitutions adjacent to the antigenic sites and receptor binding site are labeled. Antigenic site B is yellow, antigenic site C green.

**Table.** Hemagglutination inhibition testing of influenza A(H7) virus isolated from cat and human in New York, NY, USA, 2016, and reference viruses\*

Antigens	Subtype	Ferret antisera					Normal ferret serum
		$\alpha$ -Gs/NE	$\alpha$ -Tk/MN	$\alpha$ -Tk/VA	$\alpha$ -NY/107	$\alpha$ -NY/108	
Reference							
A/goose/Nebraska/17097-4/11	H7N9	160	80	160	80	<10	<10
A/turkey/Minnesota/0141354/09	H7N9	20	80	20	20	<10	<10
A/turkey/Virginia/4529/02	H7N2	40	10	160	640	10	<10
A/New York/107/03	H7N2	40	20	160	640	10	<10
A/New York/108/16†	H7N2	40	10	80	80	320	<10
Test							
A/feline/New York/16-040082-1/16	H7N2	40	10	80	80	320	<10

\*Gray shading indicates homologous titers.  $\alpha$ -, reference antiserum. Gs/NE, A/goose/Nebraska/17097-4/11; Tk/MN, A/turkey/Minnesota/0141354/09; Tk/VA, A/turkey/Virginia/4529/02; NY/107, A/New York/107/03; NY/108, A/New York/108/16.

†Virus isolated from human (veterinarian who experienced influenza-like illness after exposure to sick domestic cats at an animal shelter).

To determine the effect of these differences on antigenicity, we assessed the relationships in a 2-way hemagglutination inhibition assay, using a panel of ferret antisera raised to related H7 viruses (Table). The results showed that A/New York/108/2016 and A/feline/New York/16-040082-1/2016 reacted with  $\alpha$ -A/turkey/Virginia/4529/2002 postinfection ferret antiserum (2-fold reduction of the hemagglutination inhibition titer compared with the A/turkey/Virginia/4529/2002 homologous titer) and  $\alpha$ -A/New York/107/2003 antiserum (8-fold reduction compared with the A/New York/107/2003 homologous titer). These data suggest that the A/turkey/Virginia/4529/2002 candidate vaccine virus would provide cross protection if vaccination against the 2016 H7N2 viruses was needed. Both A/turkey/Virginia/4529/2002 and A/New York/107/2003, however, reacted poorly with the antiserum raised against A/New York/108/2016.

A 20-aa deletion in the NA stalk region, considered a genetic marker of poultry-adapted viruses (14), was also identified in the human and feline H7N2 viruses. No genetic markers known to reduce susceptibility to the NA inhibitor class of antiviral drugs were identified in the NA gene. Results of the NA inhibition assay indicated that the H7N2 viruses were susceptible to 4 NA inhibitors: oseltamivir, zanamivir, peramivir, and laninamivir (data not shown).

## Conclusions

The circulation of an influenza A(H7N2) virus at the animal–human interface, especially among common companion animals such as domestic cats, is of public health concern. Moreover, from an epidemiologic perspective, it is essential to understand the current distribution of LPAI A(H7N2) viruses in both avian and feline hosts. The US Department of Agriculture and state departments of agriculture have conducted routine avian influenza surveillance in live bird markets; 132,000–212,000 tests for avian influenza were performed annually during 2007–2014 (15), but LPAI A(H7N2) viruses were not detected. The acquisition of many genetic changes throughout the genome of the human and cat H7N2 viruses we report, however, suggests

onward evolution of the virus since it was last detected in poultry and wild birds. We found that the human virus bound to  $\alpha$ -2,6–linked sialic acid receptors, which are more common in mammals, yet retained  $\alpha$ -2,3–linked sialic acid binding, indicating that it has dual receptor specificity; this information can be used in pandemic risk assessment of zoonotic viruses. Although human infections with LPAI A(H7N2) viruses have occurred previously, we know of no other reported instances of direct transmission from a cat to a human.


Dr. Marinova-Petkova is a microbiologist with the Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA. Her research interests include studying influenza viruses at the animal–human interface, influenza virus evolution, and animal models for risk assessment of zoonotic pathogens.

## References

1. Belser JA, Bridges CB, Katz JM, Tumpey TM. Past, present, and possible future human infection with influenza virus A subtype H7. *Emerg Infect Dis.* 2009;15:859–65. <http://dx.doi.org/10.3201/eid1506.090072>
2. World Health Organization. Influenza at the human-animal interface. 2016 [cited 2017 Feb 12]. [http://www.who.int/influenza/human\\_animal\\_interface/Influenza\\_Summary\\_IRA\\_HA\\_interface\\_12\\_19\\_2016.pdf?ua=1](http://www.who.int/influenza/human_animal_interface/Influenza_Summary_IRA_HA_interface_12_19_2016.pdf?ua=1)
3. Senne DA, Pederson JC, Panigrahy B. Live-bird markets in the northeastern United States: a source of avian influenza in commercial poultry. In: Schrijver RS and Koch G, editors. *Proceedings of the Frontis Workshop on Avian Influenza: Prevention and Control.* Wageningen (the Netherlands): Springer; 2003. p. 19–24.
4. Senne DA, Holt TJ, Akey BL. An overview of the 2002 outbreak of low-pathogenic H7N2 avian influenza in Virginia, West Virginia and North Carolina. In: Schrijver RS and Koch G, editors. *Proceedings of the Frontis Workshop on Avian Influenza: Prevention and Control.* Wageningen (the Netherlands): Springer; 2003. p. 41–7.
5. Centers for Disease Control and Prevention (CDC). Update: influenza activity—United States, 2003–04 season. *MMWR Morb Mortal Wkly Rep.* 2004;53:284–7.
6. Ostrowsky B, Huang A, Terry W, Anton D, Brunagel B, Traynor L, et al. Low pathogenic avian influenza A (H7N2) virus infection in immunocompromised adult, New York, USA, 2003. *Emerg Infect Dis.* 2012;18:1128–31. <http://dx.doi.org/10.3201/eid1807.111913>

7. Pappas C, Matsuoka Y, Swayne DE, Donis RO. Development and evaluation of an influenza virus subtype H7N2 vaccine candidate for pandemic preparedness. *Clin Vaccine Immunol.* 2007;14:1425–32. <http://dx.doi.org/10.1128/CVI.00174-07>
8. Yang H, Chen L-M, Carney PJ, Donis RO, Stevens J. Structures of receptor complexes of a North American H7N2 influenza hemagglutinin with a loop deletion in the receptor binding site. *PLoS Pathog.* 2010;6:e1001081. <http://dx.doi.org/10.1371/journal.ppat.1001081>
9. Belser JA, Blixt O, Chen LM, Pappas C, Maines TR, Van Hoeven N, et al. Contemporary North American influenza H7 viruses possess human receptor specificity: implications for virus transmissibility. *Proc Natl Acad Sci U S A.* 2008;105:7558–63. <http://dx.doi.org/10.1073/pnas.0801259105>
10. Yang H, Carney PJ, Chang JC, Villanueva JM, Stevens J. Structural analysis of the hemagglutinin from the recent 2013 H7N9 influenza virus. *J Virol.* 2013;87:12433–46. <http://dx.doi.org/10.1128/JVI.01854-13>
11. Weinstein J, de Souza-e-Silva U, Paulson JC. Sialylation of glycoprotein oligosaccharides *N*-linked to asparagine. Enzymatic characterization of a Gal  $\beta$  1 to 3(4)GlcNAc  $\alpha$  2 to 3 sialyltransferase and a Gal  $\beta$  1 to 4GlcNAc  $\alpha$  2 to 6 sialyltransferase from rat liver. *J Biol Chem.* 1982;257:13845–53.
12. Fouchier RAM, Schneeberger PM, Rozendaal FW, Broekman JM, Kemink SAG, Munster V, et al. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proc Natl Acad Sci U S A.* 2004;101:1356–61. <http://dx.doi.org/10.1073/pnas.0308352100>
13. Yang ZY, Wei CJ, Kong WP, Wu L, Xu L, Smith DF, et al. Immunization by avian H5 influenza hemagglutinin mutants with altered receptor binding specificity. *Science.* 2007;317:825–8. <http://dx.doi.org/10.1126/science.1135165>
14. Matrosovich M, Zhou N, Kawaoka Y, Webster R. The surface glycoproteins of H5 influenza viruses isolated from humans, chickens, and wild aquatic birds have distinguishable properties. *J Virol.* 1999;73:1146–55.
15. Myers TJ. Overview of avian influenza surveillance in the United States. US Department of Agriculture, Animal and Plant Health Inspection Service. 2015 [cited 2017 Feb 12]. [https://www.aphis.usda.gov/animal\\_health/animal\\_dis\\_spec/poultry/downloads/mtg/presentations/we\\_3\\_myers\\_ai\\_surveillance\\_overview.pdf](https://www.aphis.usda.gov/animal_health/animal_dis_spec/poultry/downloads/mtg/presentations/we_3_myers_ai_surveillance_overview.pdf)

Address for correspondence: C. Todd Davis, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop D30, Atlanta, GA 30329-4027, USA; email: [ctdavis@cdc.gov](mailto:ctdavis@cdc.gov)



**@CDC\_EIDJournal**

**Follow the EID journal on Twitter and get the most current information from Emerging Infectious Diseases.**

# Avian Influenza A(H7N2) Virus in Human Exposed to Sick Cats, New York, USA, 2016

## Technical Appendix

### Methods

#### Influenza A Confirmation and Subtyping of the Clinical Specimen

RNA was extracted from the clinical specimen using Qiamp viral RNA purification kit (QIAGEN, Hilden, Germany) at Wadsworth Center, and MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostics Corporation, Indianapolis, IN, USA), at the Centers for Disease Control and Prevention (CDC). Influenza A virus was confirmed by using rRT-PCR with primers and probe for detection of universal influenza A Matrix gene (CDC Laboratory Support for Influenza Surveillance, Centers for Disease Control and Prevention, Atlanta, GA, USA); and subtyped using specific primers and probe for North American lineage influenza A(H7) viruses (primer and probe sequences available upon request). The A(H7N2) subtype was further confirmed by Sanger sequencing analysis of PCR products amplified using a single step RT-PCR reaction with H7 and N2-specific primers (available upon request). Illumina next-generation sequencing of the viral RNA was performed using MiSeq analysis (Illumina, San Diego, CA, USA) and sequence data was analyzed with IRMA (*1*).

#### Virus isolation

Virus isolation was attempted in Madin Darby Canine Kidney (MDCK) and Crandell-Rees Feline Kidney (CRFK) cell lines (ATCC), and in 10-day-old embryonated chicken eggs (*2*). Cell cultures were incubated at 35°C for 72 hours and checked for cytopathic effect twice daily. Inoculated eggs were chilled after 48 hours of incubation at 35°C. Presence of influenza A virus in the allantoic fluids of eggs, but not cell-culture supernatants, was confirmed by positive reaction hemagglutination with 0.5% suspension of turkey erythrocytes.

### **Viral Genome Sequencing, Phylogenetic Analysis, and HA Monomer Protein Structure Modeling**

Codon complete genome sequencing of virus isolated in embryonated chicken eggs was performed using MiSeq analysis. Gene sequences were submitted to GISAID with the following accession numbers: PB2, EPI944626; PB1, EPI944627; PA, EPI944625; HA, EPI944629; NP, EPI944622; NA, EPI944628; MP, EPI944624; and NS, EPI944623. Reference sequences for the phylogenetic reconstruction were retrieved from the GenBank and GISAID databases (3,4). Codon complete genome sequences were aligned via MUSCLE (5) and HA sequences were trimmed to the start of the mature H7 HA protein sequence using BioEdit v7.0 (6). Neighbor-joining phylogenetic trees (Jukes-Cantor model) with 1,000 bootstrap replicates were constructed using MEGA 5.05 (7). The model of HA monomer structure was generated using SWISS – Model (8) with 3M5G as the starting model. All the structural figures were generated using MacPyMOL (9).

### **Antigenic Characterization**

Hemagglutination inhibition (HI) testing was performed by using selected subtype H7 WHO candidate vaccine viruses and CDC reference viruses of the North American lineage, as well as postinfection ferret antisera produced against H7 viruses of the North American lineage (see Table in main article). Turkey erythrocytes at 0.5% concentration were used for the HI test (10). All antisera used in the HI test were treated with receptor-destroying enzyme (Denka Seiken, Tokyo, Japan) according to the manufacturer's recommendations, and used at 1:10 starting dilution.

### **Glycan Microarray Analysis**

Glycan microarray slides were produced under contract for CDC using a glycan library generously provided by the Consortium for Functional Glycomics ([www.functionalglycomics.org](http://www.functionalglycomics.org)), funded by National Institute of General Medical Sciences grant GM62116 (Technical Appendix Table, glycans used for analyses in these experiments). Virus preparations were diluted in phosphate-buffered saline (PBS) with 2% (wt/vol) bovine serum albumin to an HA titer of 128. Virus suspensions were applied to the slides, and the slides were incubated in a closed container (at 4°C) subjected to gentle agitation for 1.5 hours. Unbound virus was washed off with brief sequential rinses in PBS with 0.05% Tween 20 (PBS-T) and PBS. The slides were then immediately incubated with ferret serum raised against A/New York/108/2016 (30 min); a biotinylated anti-ferret IgG antibody (Rockland) in combination with

streptavidin-Alexa Fluor488 conjugate (30 min) (Thermo Fisher, Waltham, MA, USA), with brief PBS-T/PBS washes being performed after each incubation. After the final PBS-T/PBS washes, the slides were washed briefly in deionized water, dried by a gentle stream of nitrogen gas, and immediately subjected to imaging. Fluorescence intensities were detected using an Innoscan 1100AL scanner (Innopsys, Carbonne, France). Image analyses were carried out using ImaGene 9 image analysis software (BioDiscovery, El Segundo, CA, USA).

## References

1. Shepard SS, Meno S, Bahl J, Wilson MM, Barnes J, Neuhaus E. Viral deep sequencing needs an adaptive approach: IRMA, the iterative refinement meta-assembler. *BMC Genomics*. 2016;17:708. <http://dx.doi.org/10.1186/s12864-016-3030-6>
2. Shortridge KF, Butterfield WK, Webster RG, Campbell CH. Isolation and characterization of influenza A viruses from avian species in Hong Kong. *Bull World Health Organ*. 1977;55:15–20.
3. Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. GenBank. *Nucleic Acids Res*. 2005;33:D34–8. <http://dx.doi.org/10.1093/nar/gki063>
4. Bogner P, Capua I, Lipman DJ, Cox NJ, et al. A global initiative on sharing avian flu data. *Nature*. 2006;442:981 <http://platform.gisaid.org>. <http://dx.doi.org/10.1038/442981a>
5. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*. 2004;32:1792–7. <http://dx.doi.org/10.1093/nar/gkh340>
6. Hall TA. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser*. 1999;41:95–8.
7. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*. 2011;28:2731–9. <http://dx.doi.org/10.1093/molbev/msr121>
8. Biasini M, Bienert S, Waterhouse A, Arnold K, Studer G, Schmidt T, et al. SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. *Nucleic Acids Res*. 2014;42(W1):W252-8. <http://dx.doi.org/10.1093/nar/gku340>
9. DeLano WL. The PyMol Molecular Graphics Systems. 2002. [cited 2017 May 5]. [www.pymol.org](http://www.pymol.org)
10. Palmer DFDW, Coleman MT, Schild GC. Advanced laboratory techniques for influenza diagnosis. Washington, DC: US Department of Health, Education and Welfare, 1975.

**Technical Appendix Table.** Glycan microarray for H7N2 viruses\*

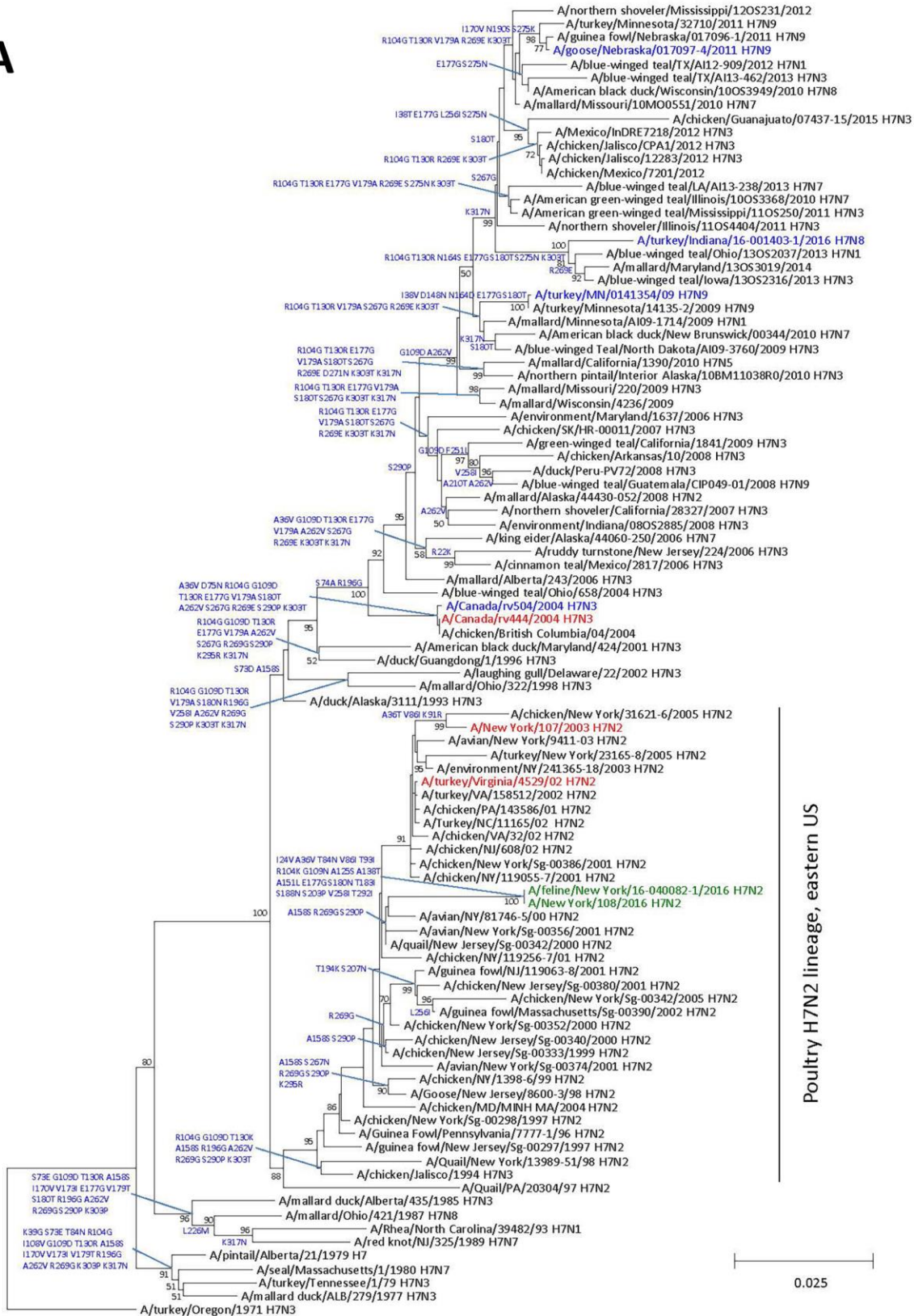
No.	Structure
1	Neu5A $\alpha$
2	Neu5A $\alpha$
3	Neu5A $\alpha$
4	Neu5A $\alpha$ 2-3(6-O-Su)Gal $\beta$ 1-4GlcNAc $\beta$
5	Neu5A $\alpha$ 2-3Gal $\beta$ 1-3[6OSO3]GalNAc $\alpha$
6	Neu5A $\alpha$ 2-3Gal $\beta$ 1-4[6OSO3]GlcNAc $\beta$
7	Neu5A $\alpha$ 2-3Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)[6OSO3]GlcNAc $\beta$ -propyl-NH <sub>2</sub>
8	Neu5A $\alpha$ 2-3Gal $\beta$ 1-3[6OSO3]GlcNAc-propyl-NH <sub>2</sub>
9	Neu5A $\alpha$ 2-3Gal $\beta$ 1-3(Neu5A $\alpha$ 2-3Gal $\beta$ 1-4)GlcNAc $\beta$
10	Neu5A $\alpha$ 2-3Gal $\beta$ 1-3(Neu5A $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-6)GalNAc $\alpha$
11	Neu5A $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3(Neu5A $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc $\beta$
12	Neu5A $\alpha$ (2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-2)-Man $\alpha$ (1-3)-[Neu5A $\alpha$ (2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-2)-Man $\alpha$ (1-6)]-Man $\beta$ (1-4)-GlcNAc $\beta$ (1-4)-GlcNAc $\beta$
13	Neu5A $\alpha$ 2-3Gal $\beta$
14	Neu5A $\alpha$ 2-3Gal $\beta$ 1-3GalNAc $\alpha$
15	Neu5A $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$
16	Neu5A $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$
17	Neu5A $\alpha$ 2-3Gal $\beta$ 1-4Glc $\beta$
18	Neu5A $\alpha$ 2-3Gal $\beta$ 1-4Glc $\beta$
19	Neu5A $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$
20	Neu5A $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$
21	Neu5A $\alpha$ 2-3GalNAc $\beta$ 1-4GlcNAc $\beta$
22	Neu5A $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$
23	Neu5A $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$
24	Neu5A $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$
25	Neu5A $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-3GlcNAc $\beta$
26	Neu5A $\alpha$ 2-3Gal $\beta$ 1-3GalNAc $\alpha$
27	Gal $\beta$ 1-3(Neu5A $\alpha$ 2-3Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-6)GalNAc $\alpha$
28	Neu5A $\alpha$ 2-3Gal $\beta$ 1-3(Fuc $\alpha$ 1-4)GlcNAc $\beta$
29	Neu5A $\alpha$ 2-3Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$
30	Neu5A $\alpha$ 2-3Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$
31	Neu5A $\alpha$ 2-3Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$
32	Neu5A $\alpha$ 2-3Gal $\beta$ 1-3[Fuc $\alpha$ 1-4]GlcNAc $\beta$ 1-3Gal $\beta$ 1-4[Fuc $\alpha$ 1-3]GlcNAc $\beta$
33	Neu5A $\alpha$ 2-3Gal $\beta$ 1-3[Fuc $\alpha$ 1-3]GlcNAc $\beta$ 1-3Gal $\beta$ 1-4[Fuc $\alpha$ 1-3]GlcNAc $\beta$
34	Neu5A $\alpha$ 2-3Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$
35	Neu5A $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-4GlcNAc $\beta$
36	Neu5A $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-4GlcNAc $\beta$
37	Neu5A $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-4Glc $\beta$
38	Gal $\beta$ 1-3GalNAc $\beta$ 1-4(Neu5A $\alpha$ 2-3)Gal $\beta$ 1-4Glc $\beta$
39	Fuc $\alpha$ 1-2Gal $\beta$ 1-3GalNAc $\beta$ 1-4(Neu5A $\alpha$ 2-3)Gal $\beta$ 1-4Glc $\beta$
40	Fuc $\alpha$ 1-2Gal $\beta$ 1-3GalNAc $\beta$ 1-4(Neu5A $\alpha$ 2-3)Gal $\beta$ 1-4Glc $\beta$
41	Neu5A $\alpha$ 2-6Gal $\beta$ 1-4[6OSO3]GlcNAc $\beta$
42	Neu5A $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3(Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc $\beta$
43	Neu5A $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3(Neu5A $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc $\beta$
44	Neu5A $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3[Neu5A $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6]Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc $\beta$
45	Neu5A $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3[Neu5A $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6]Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc $\beta$
46	Neu5A $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3[Neu5A $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-6]GalNAc $\alpha$
47	Neu5A $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3[Neu5A $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-6]GalNAc $\alpha$
48	Neu5A $\alpha$ 2-6GalNAc $\alpha$
49	Neu5A $\alpha$ 2-6Gal $\beta$
50	Neu5A $\alpha$ 2-6Gal $\beta$ 1-4Glc $\beta$
51	Neu5A $\alpha$ 2-6Gal $\beta$ 1-4Glc $\beta$
52	Neu5A $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$
53	Neu5A $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$
54	Neu5A $\alpha$ 2-6GalNAc $\beta$ 1-4GlcNAc $\beta$
55	Neu5A $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3GalNAc $\alpha$
56	Neu5A $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$



No.	Structure
57	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3GalNAc $\alpha$
58	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$
59	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$
60	Gal $\beta$ 1-3(Neu5Ac $\alpha$ 2-6)GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc $\beta$ -Sp10
61	Neu5Ac $\alpha$ 2-6[Gal $\beta$ 1-3]GalNAc $\alpha$
62	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-6[Gal $\beta$ 1-3]GalNAc $\alpha$
63	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-6[Gal $\beta$ 1-3]GalNAc $\alpha$
64	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3(Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc $\beta$
65	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3(Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc $\beta$
66	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3(Neu5Ac $\alpha$ 2-6)GalNAc $\alpha$
67	Neu5Ac $\alpha$ 2-3(Neu5Ac $\alpha$ 2-6)GalNAc $\alpha$
68	Neu5Gc $\alpha$
69	Neu5Gc $\alpha$ 2-3Gal $\beta$ 1-3(Fuc $\alpha$ 1-4)GlcNAc $\beta$
70	Neu5Gc $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$
71	Neu5Gc $\alpha$ 2-3Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$
72	Neu5Gc $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$
73	Neu5Gc $\alpha$ 2-6GalNAc $\alpha$
74	Neu5Gc $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$
75	Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$
76	Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$
77	Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-4Glc $\beta$
78	Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4Glc $\beta$
79	Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-4Glc $\beta$
80	Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4Glc $\beta$
81	Neu5Ac $\alpha$ 2-8Neu5Ac $\beta$ -Sp17
82	Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-8Neu5Ac $\beta$
83	Neu5Ac $\beta$ 2-6GalNAc $\alpha$
84	Neu5Ac $\beta$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$
85	Neu5Gc $\beta$ 2-6Gal $\beta$ 1-4GlcNAc
86	Gal $\beta$ 1-3(Neu5Ac $\beta$ 2-6)GalNAc $\alpha$
87	[9NAc]Neu5Ac $\alpha$
88	[9NAc]Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$
89	Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$
90	Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-3GlcNAc $\beta$
91	Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3[Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6]Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc $\beta$
92	GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-3GlcNAc $\beta$
93	GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4GlcNAc $\beta$
94	Gal $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-3GlcNAc $\beta$
95	Gal $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$
96	Gal $\beta$ 1-3GalNAc $\alpha$

\*Colors represent glycans that contain  $\alpha$ -2,3 sialic acid (SA) (blue),  $\alpha$ -2,6 SA (red),  $\alpha$ -2,3/ $\alpha$ -2,6 mixed SA (purple), N-glycolyl SA (green),  $\alpha$ -2,8 SA (brown),  $\beta$ -2,6 and 9-O-acetyl SA (yellow), and non-SA (gray).

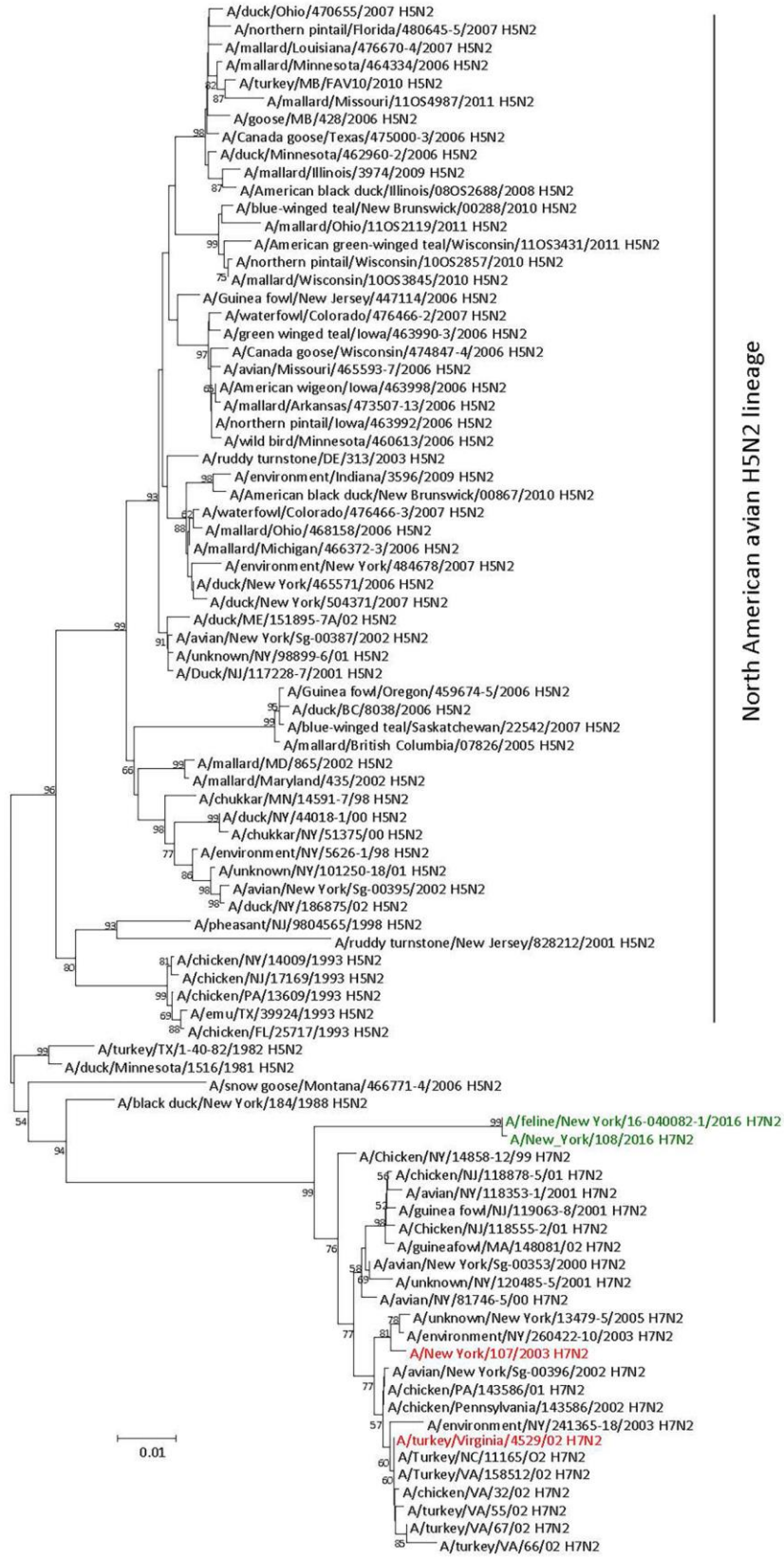
A



North American wild bird-origin H7 lineage

Poultry H7N2 lineage, eastern US

B



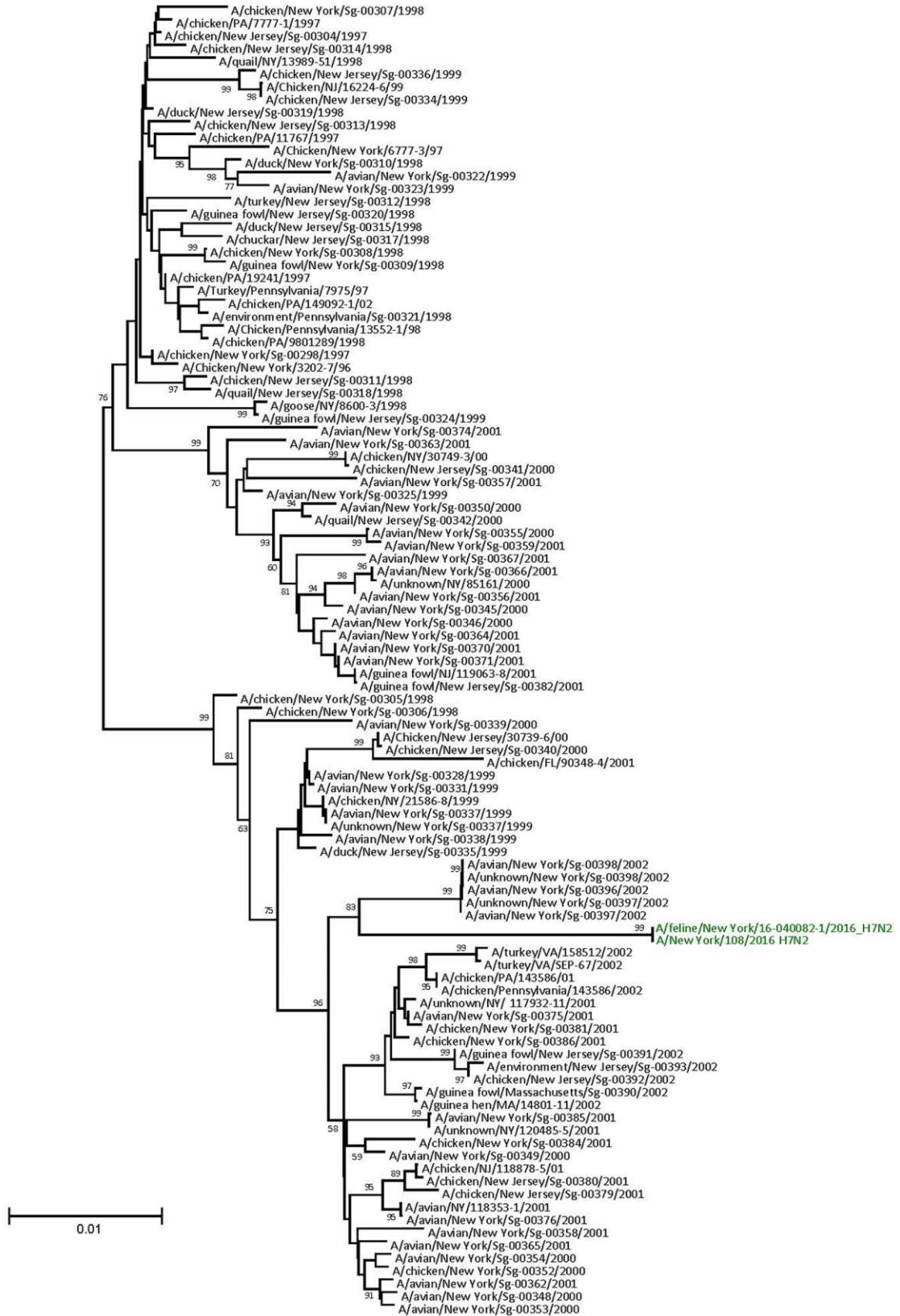
North American avian H5N2 lineage

Poultry H7N2 lineage, eastern US

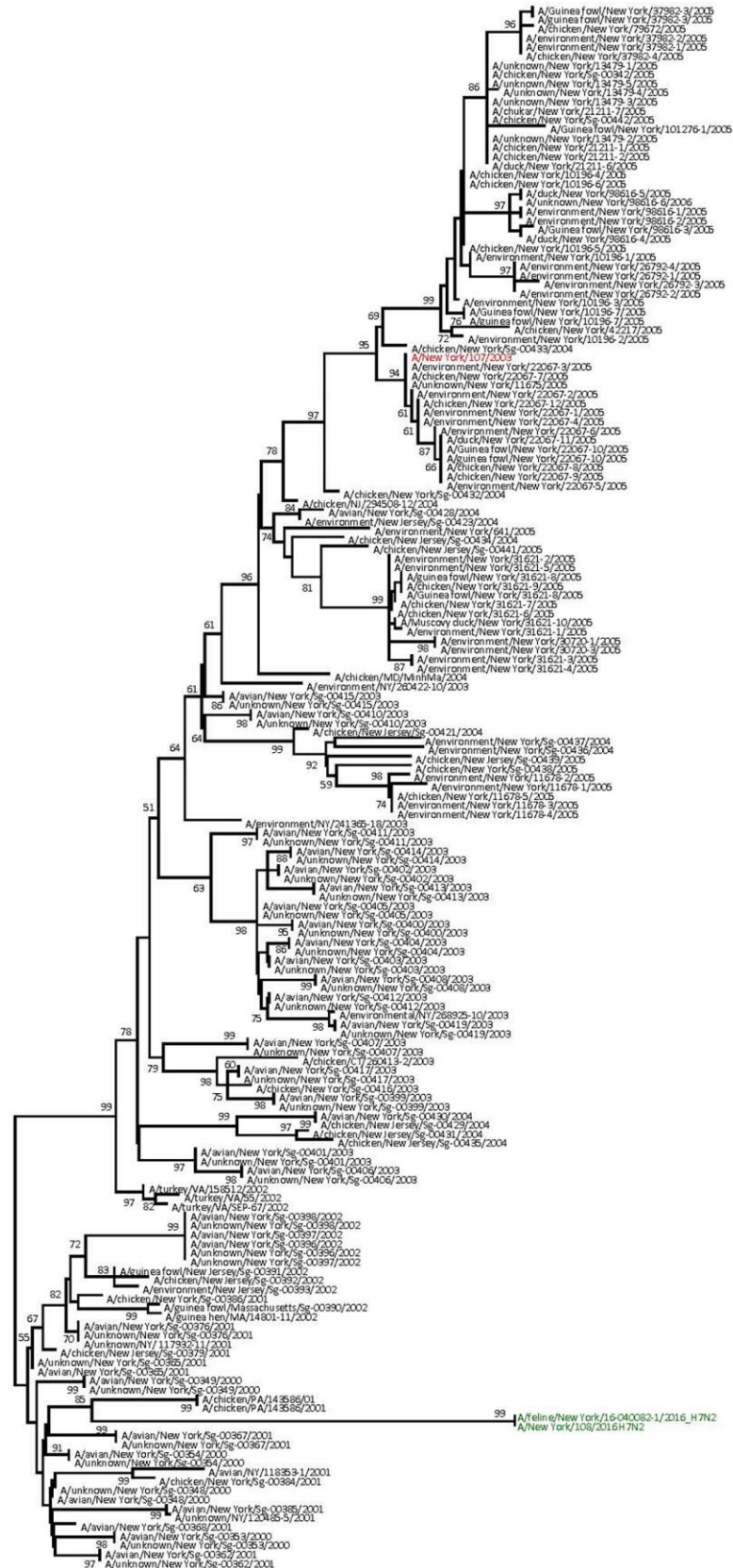
C



D

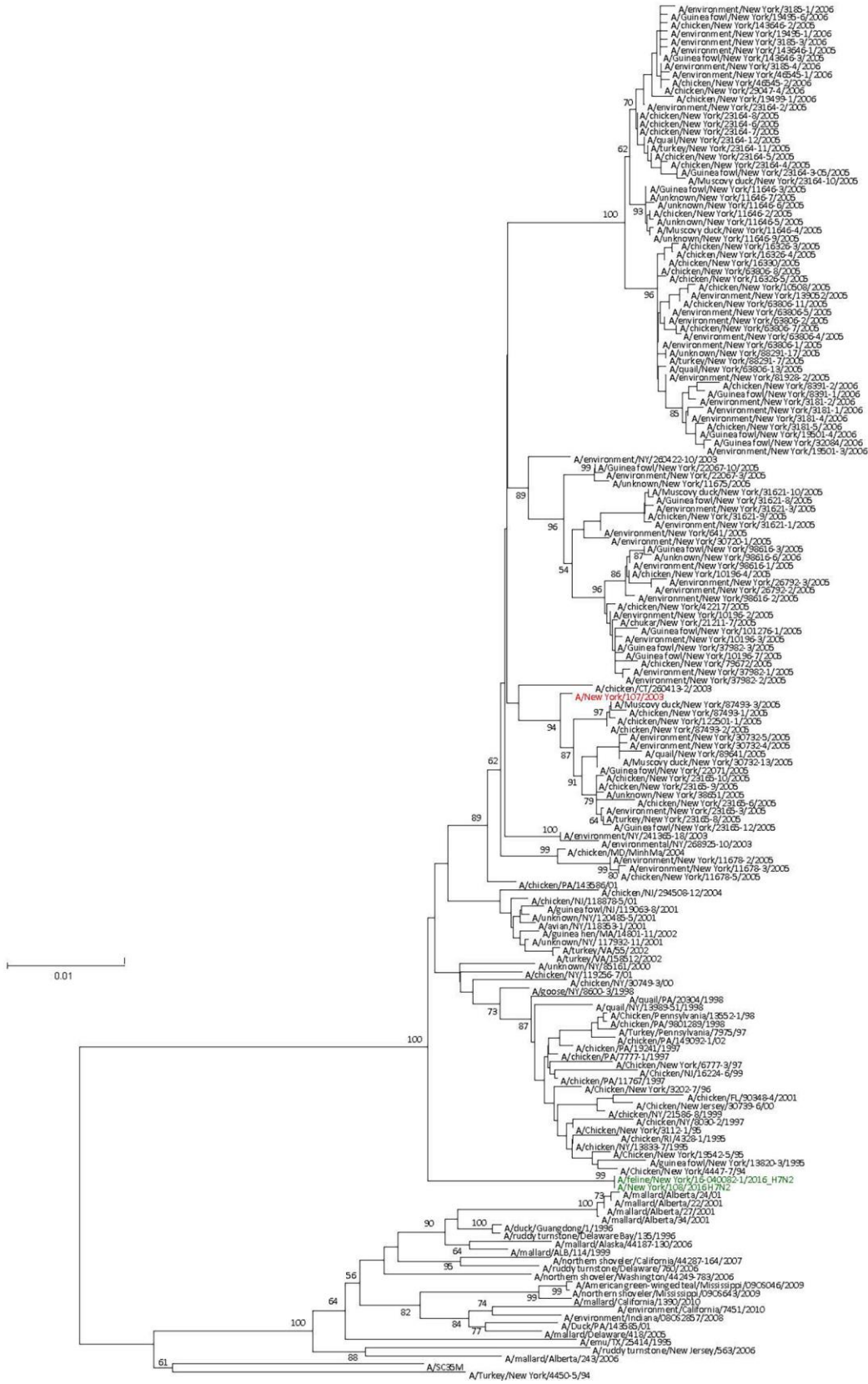


E

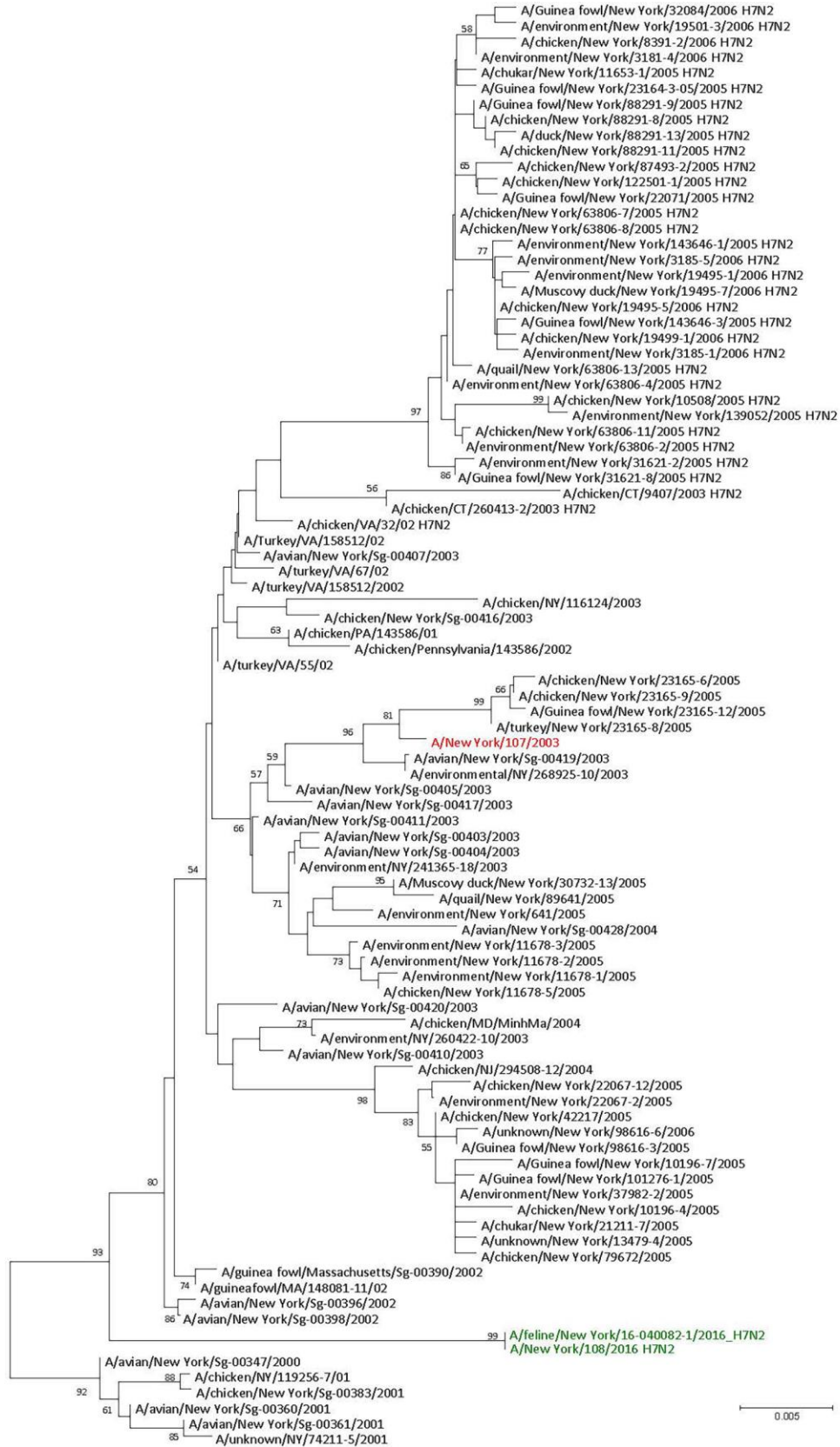


0.005

F

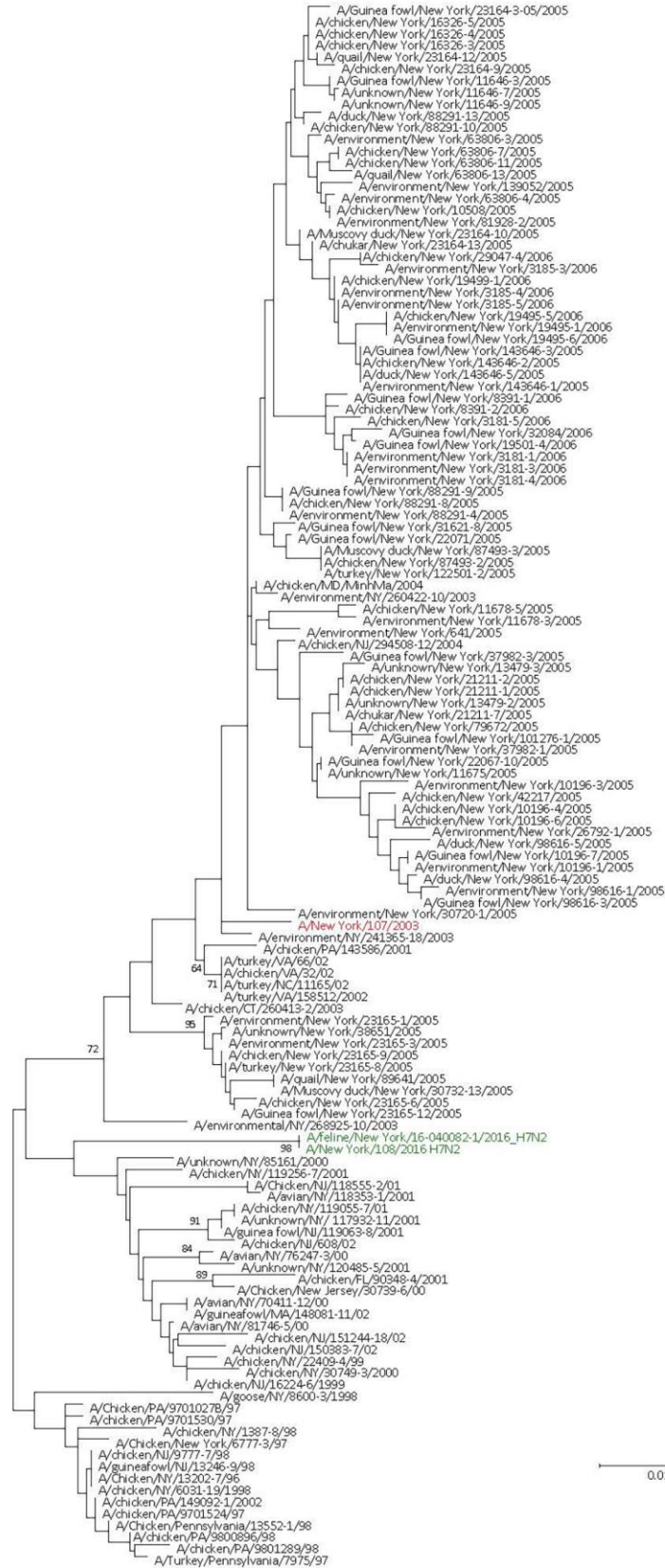


G





H



**Technical Appendix Figure.** Neighbor-joining phylogenetic trees of the (A) HA, (B) NA, and (C–H) internal genes. The human and feline H7N2 isolates are green. All candidate vaccine viruses are red and HI reference viruses are blue. Amino acid differences were calculated based on A/turkey/Virginia/4529/2002; bootstraps >50 generated from 1,000 replicates are shown at branch nodes. The scale bar represents nucleotide substitutions per site.