

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), 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

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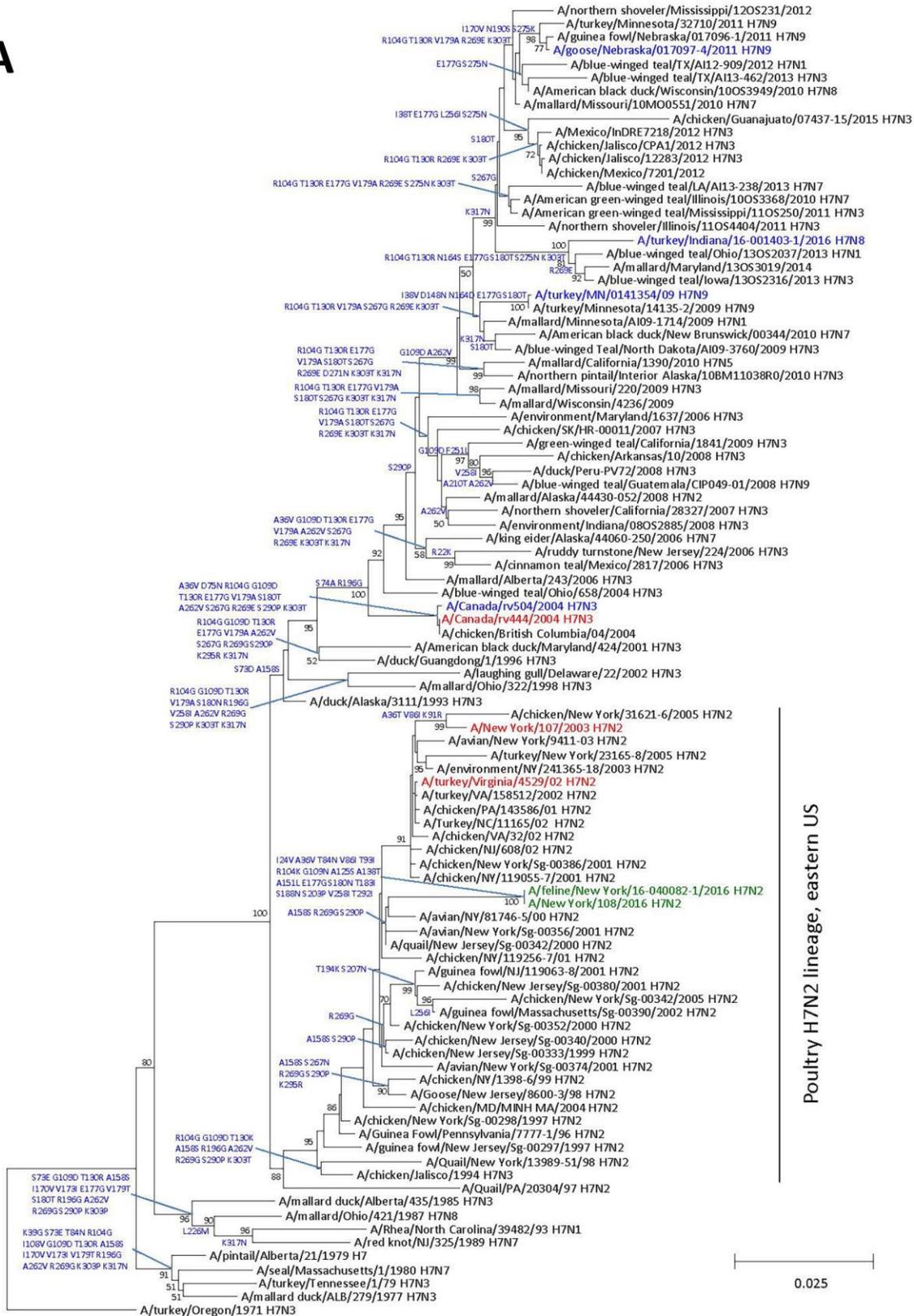
Technical Appendix Table. Glycan microarray for H7N2 viruses*

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

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

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

A

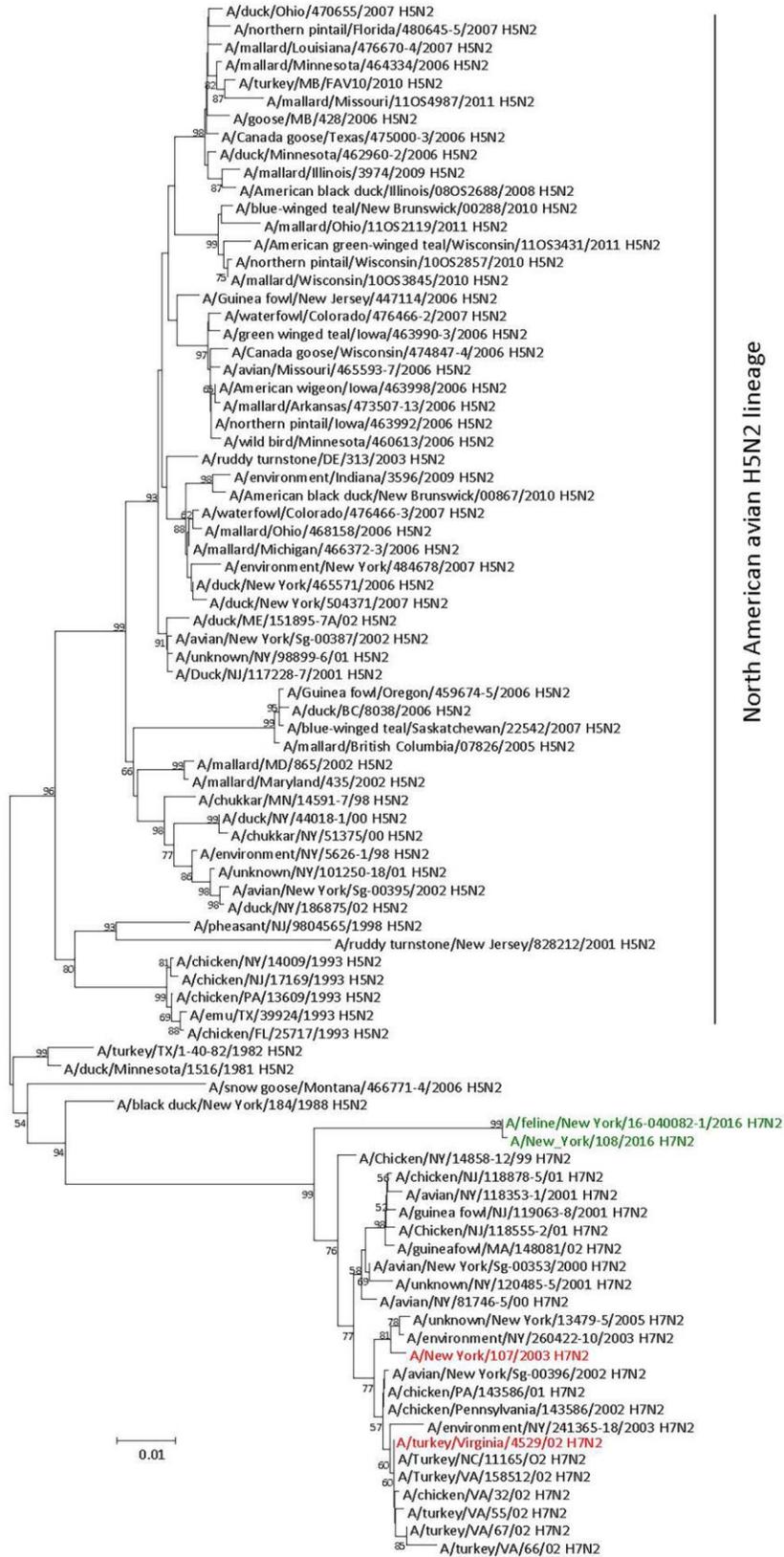


North American wild bird-origin H7 lineage

Poultry H7N2 lineage, eastern US

0.025

B

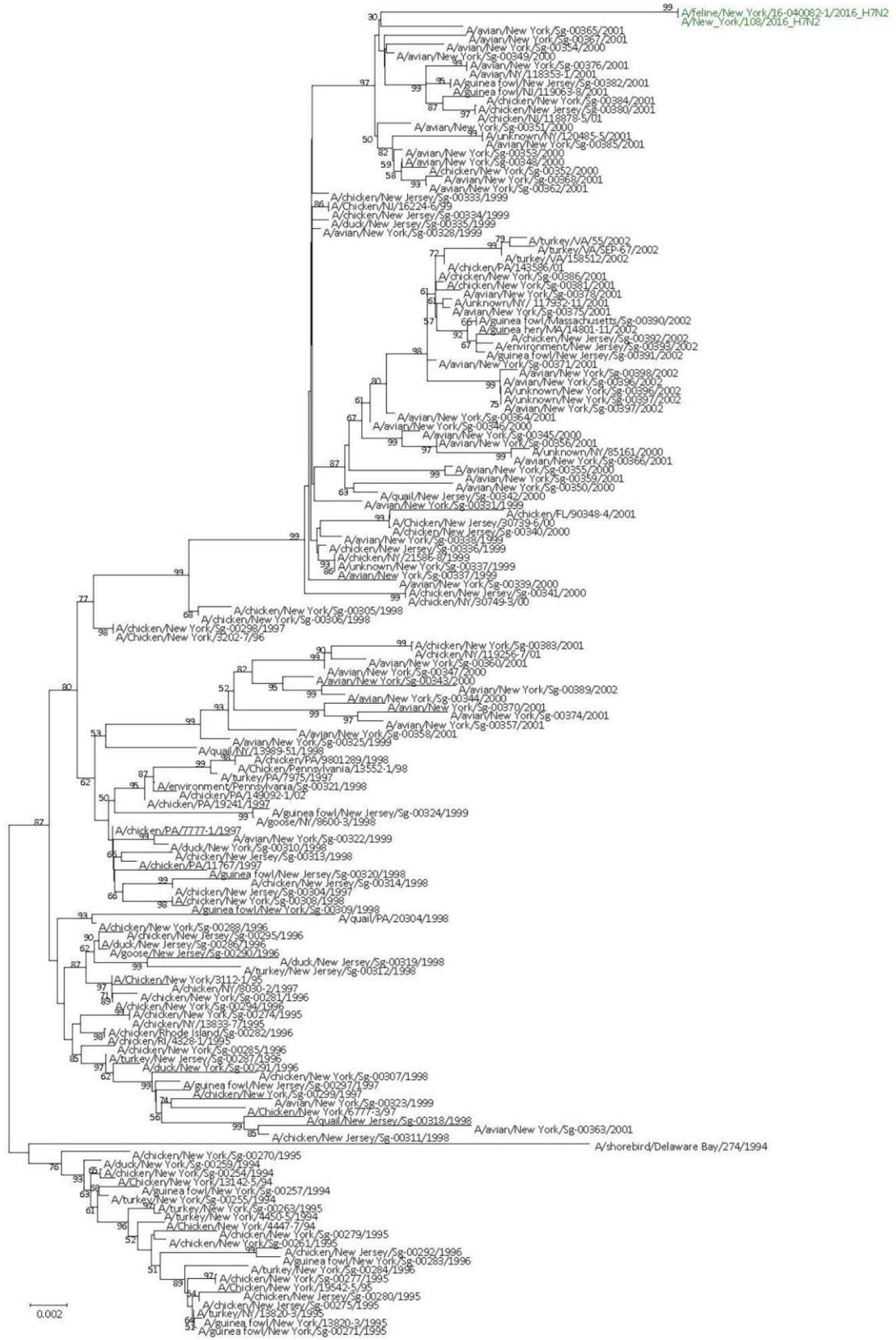


North American avian H5N2 lineage

Poultry H7N2 lineage, eastern US

0.01

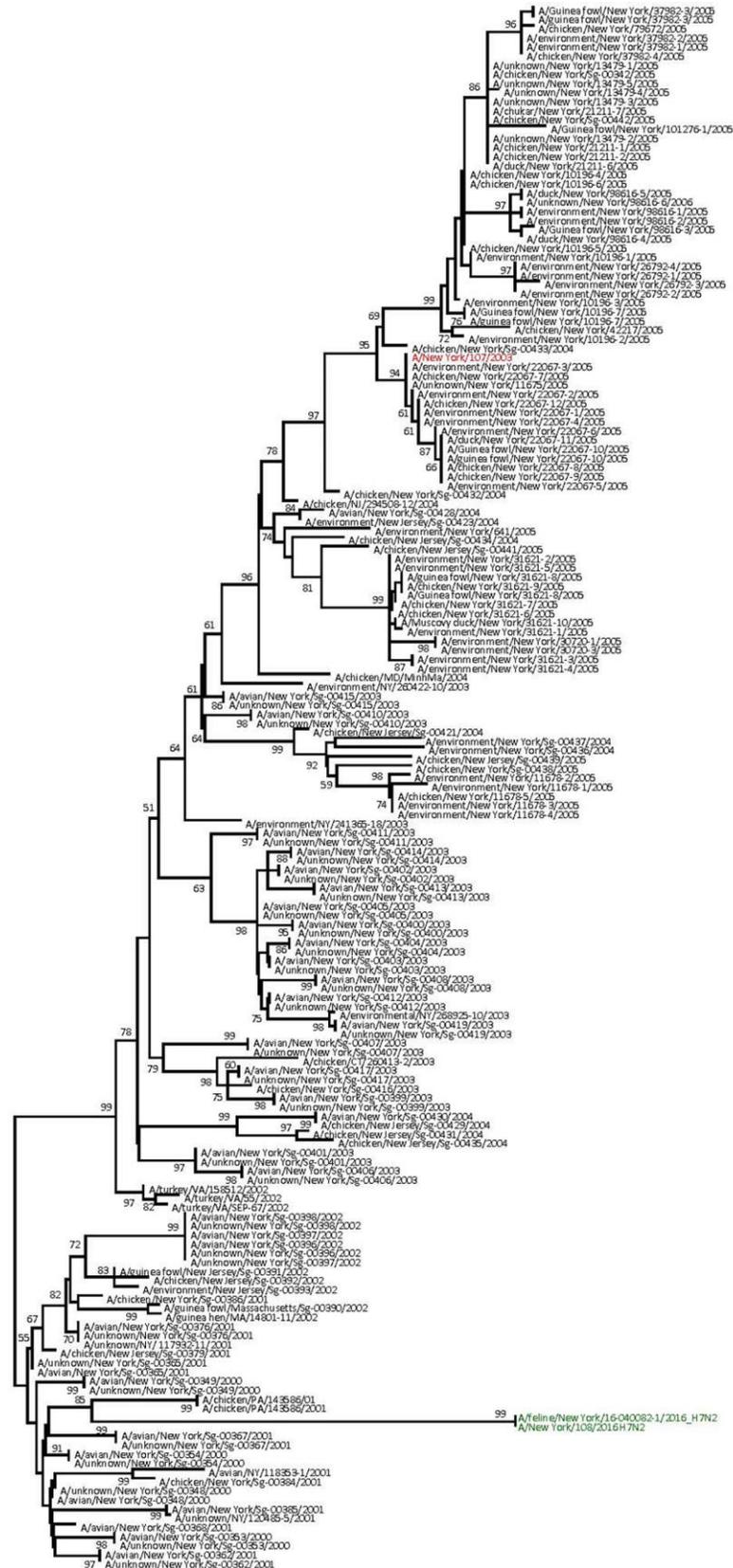
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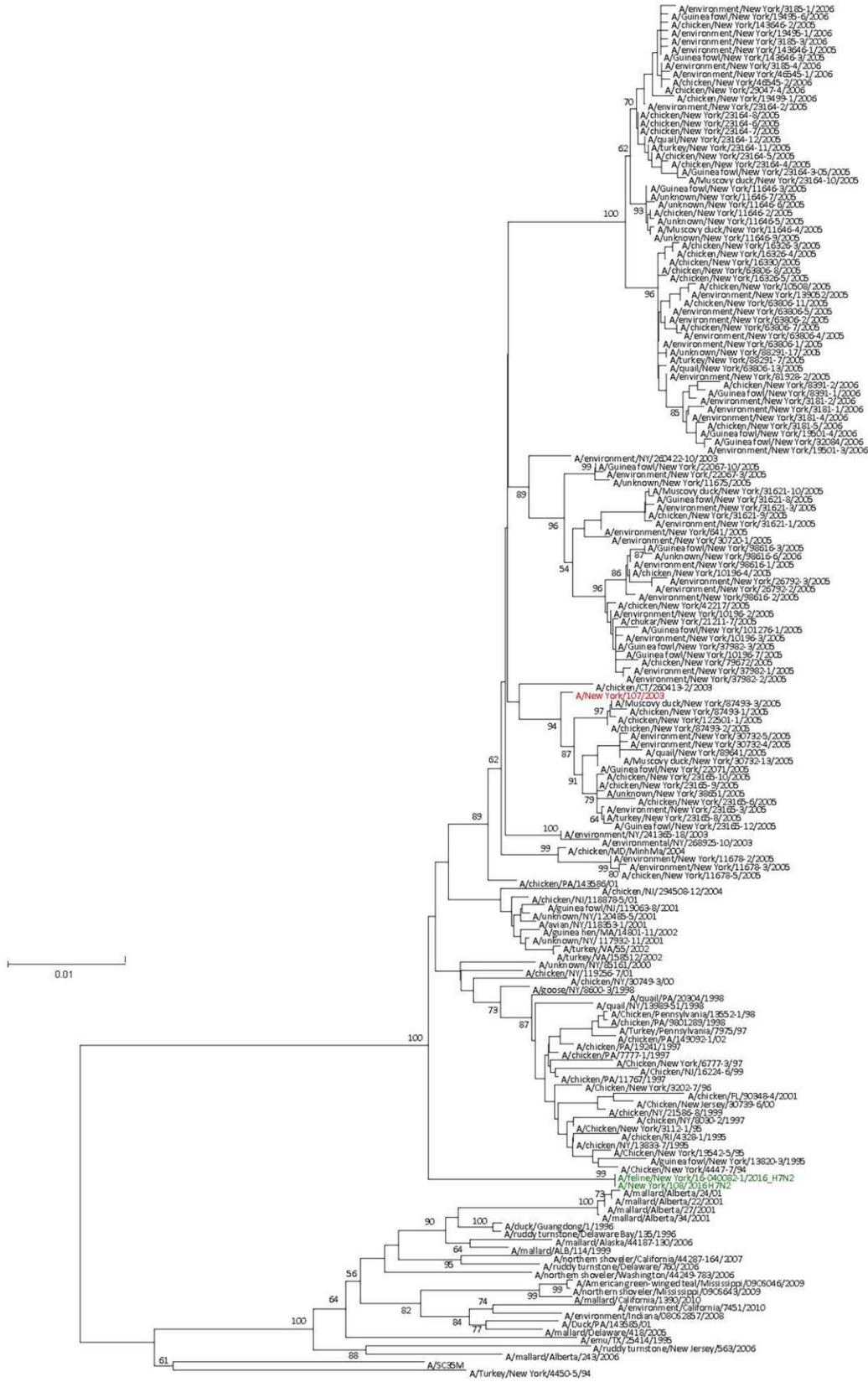
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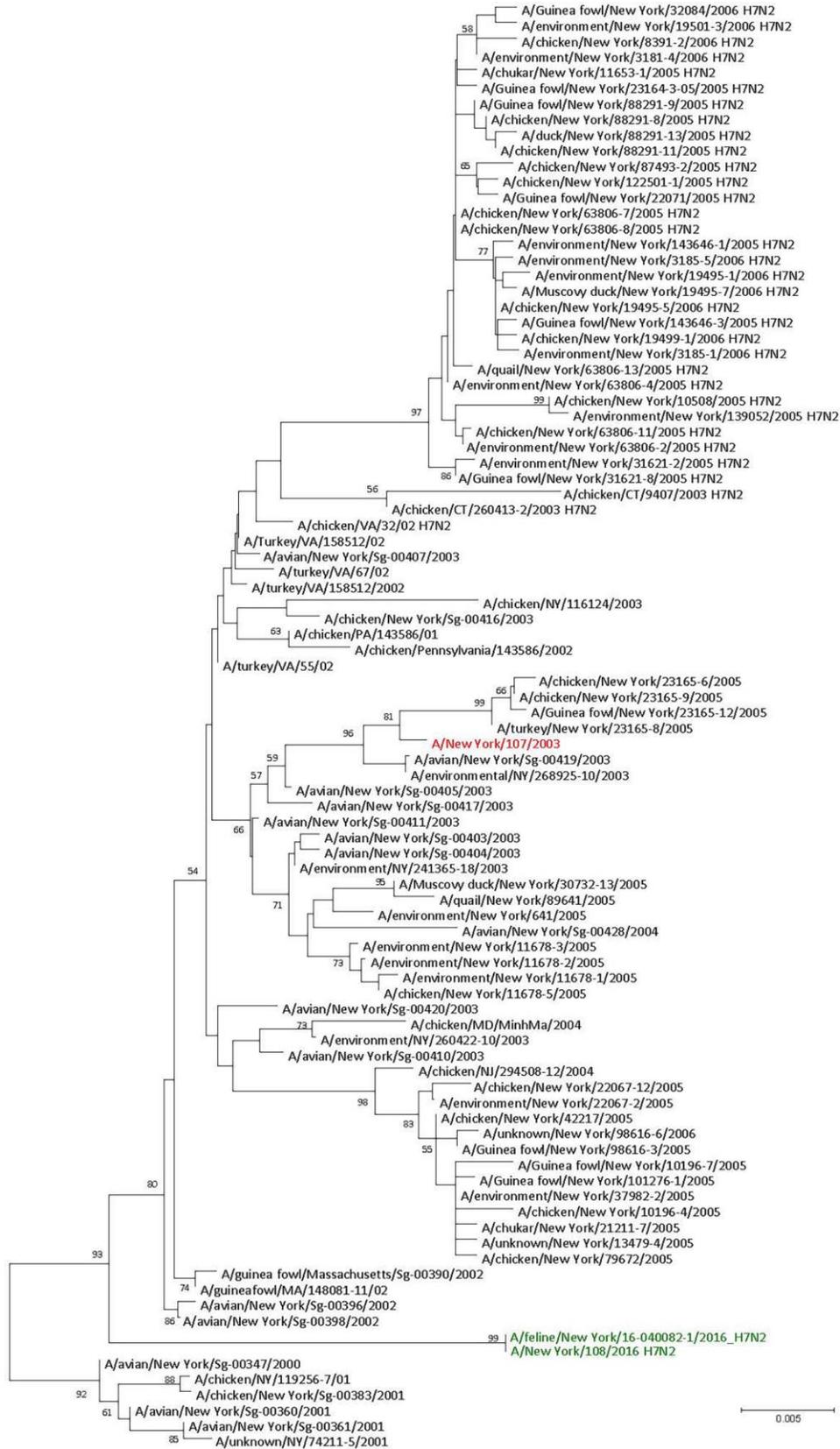
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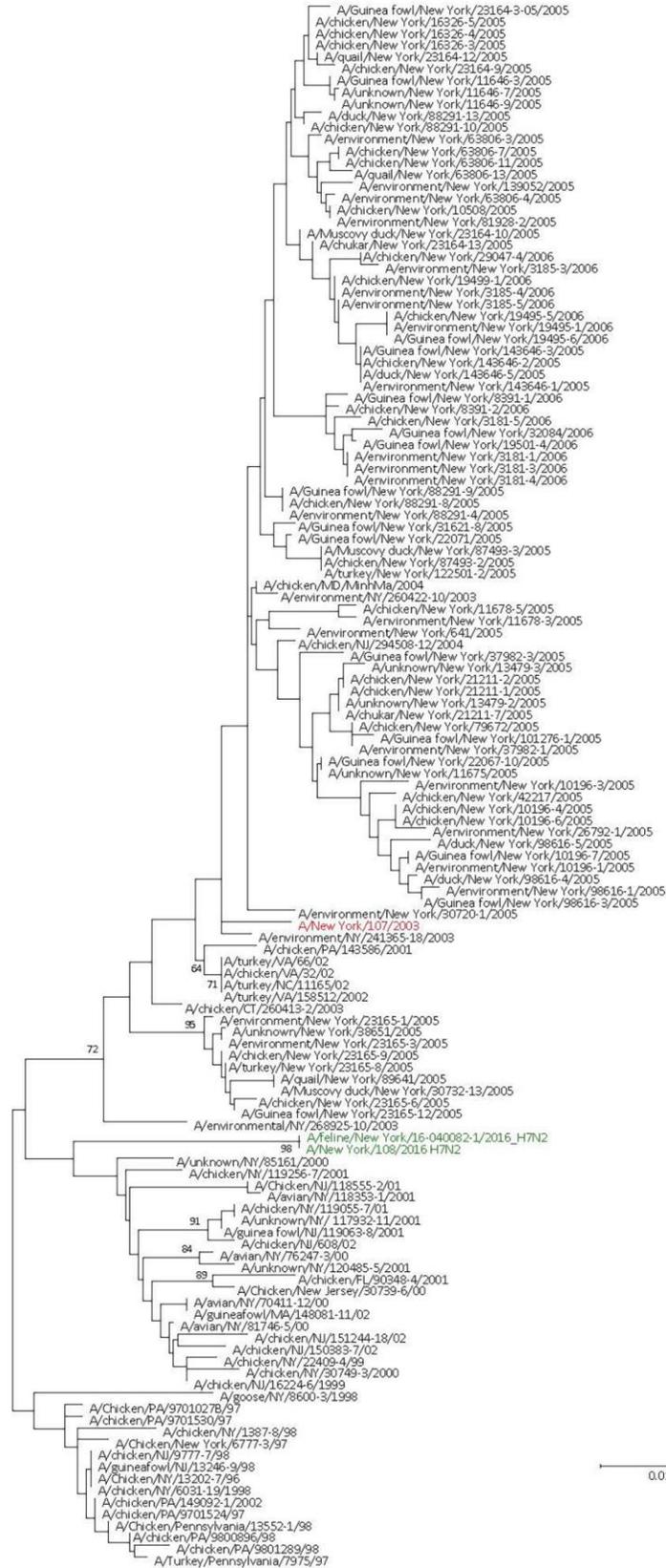
F



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.