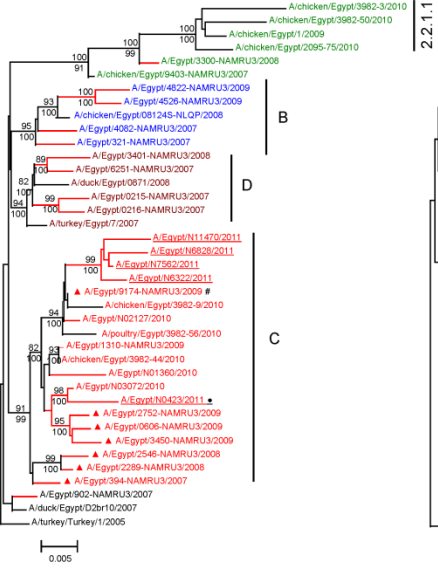


Microevolution of Highly Pathogenic Avian Influenza A(H5N1) Viruses Isolated from Humans, Egypt, 2007–2011

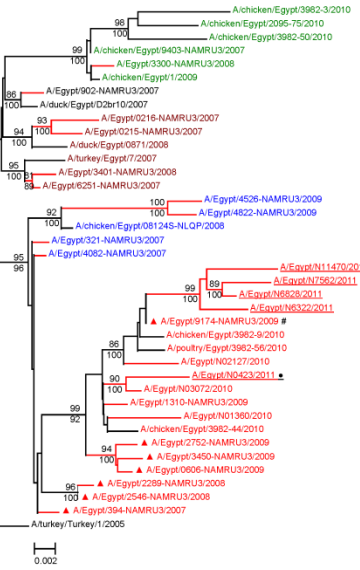
Technical Appendix 2

Technical Appendix 2 Figure 1 (next page). Phylogenetic relationships among highly pathogenic avian influenza A(H5N1) viruses isolated from humans, Egypt, 2007–2011. Trees were built on the basis of representative viral genomes from the 90 isolates analyzed in this study (Technical Appendix 2 Figure 2). Bootstrap values >79 generated from 1,000 neighbor-joining replicates are shown above branches, and Bayesian posterior probabilities are shown below the branches at relevant nodes. The trees were rooted to the *A/turkey/Turkey/1/2005* genes. The viruses are labeled and colored according to the clade 2.2 subgroups of their HA sequences (i.e., B, D, C, and 2.2.1.1); branch colors in all trees are used similarly to those in the HA tree. Red branches denote human viruses. Triangles, variant viruses with clade 2.2.1-C HA lacking unique genetic signatures in the internal genes (as shown in the Table in the main text); underlining, viruses isolated in 2011; dots, isolate from the Aswan governorate; hash mark, NS gene reassortant. HA, hemagglutinin; NA, neuraminidase; PB2, polymerase basic 2 gene; PB1, PB 1 gene; PA, polymerase acidic gene; NP, nucleoprotein; M, matrix gene; NS, nonstructural gene. Scale bar indicates nucleotide substitutions per site.

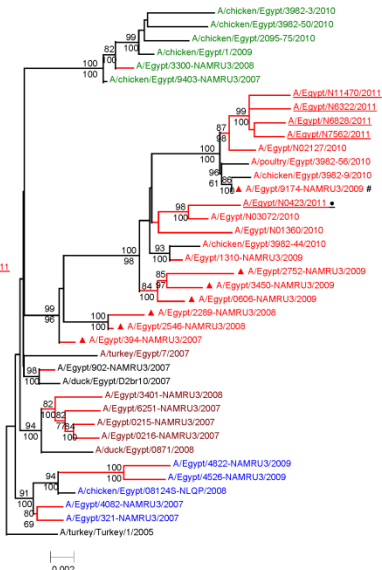
HA



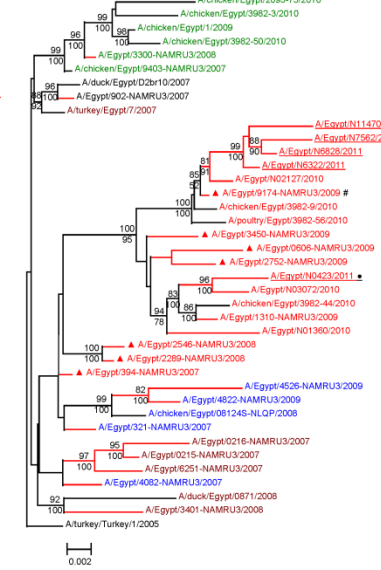
NA



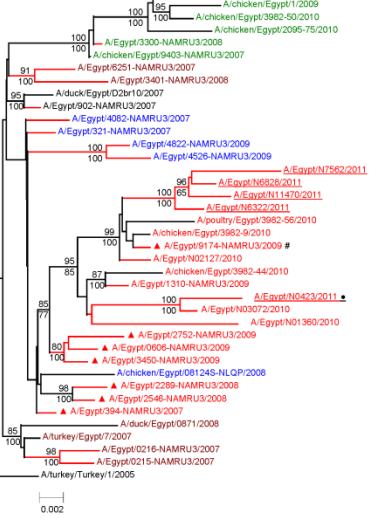
PB2



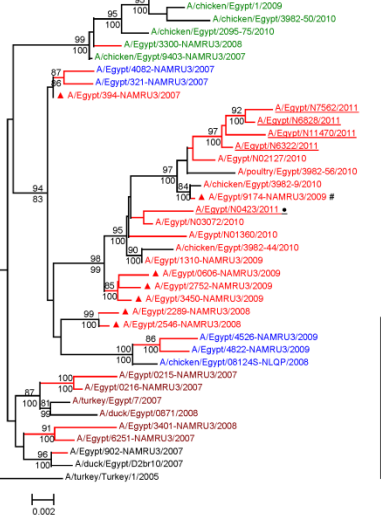
PB1



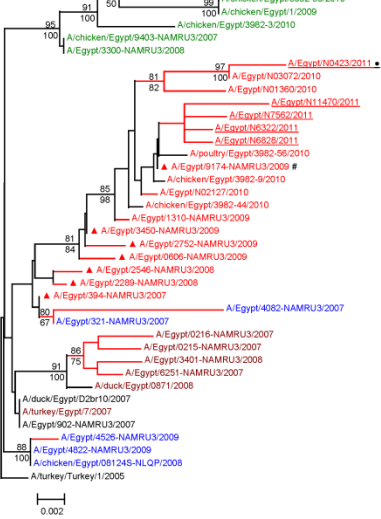
PA



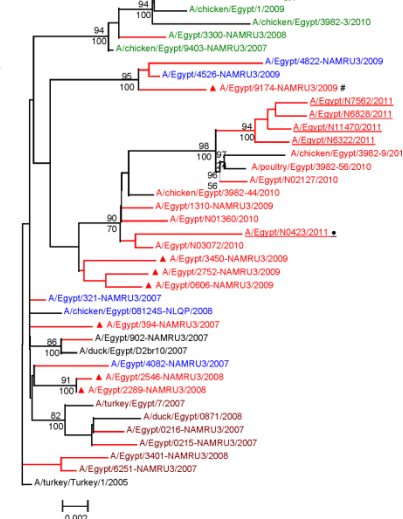
NP



M

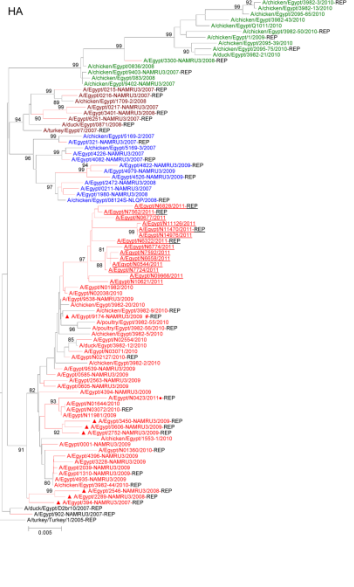


NS

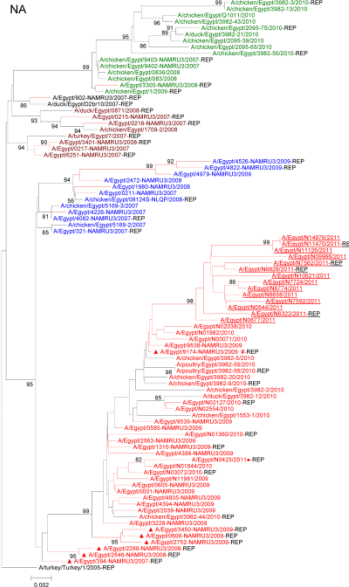


Technical Appendix 2 Figure 2 (next page). Phylogenetic trees of 90 viral genomes analyzed in a study of highly pathogenic avian influenza A(H5N1) viruses isolated from humans, Egypt, 2007–2011. The sequences of the 8 viral genes were aligned and trees were constructed as described for Technical Appendix 2 Figure 1. Annotation conventions and abbreviated terms are similar to those used for Technical Appendix 2 Figure 1. Sequences labeled Representative sequences, labeled as REP, were included in Technical Appendix 2 Figure 1. Scale bars indicate nucleotide substitutions per site.

HA



NA



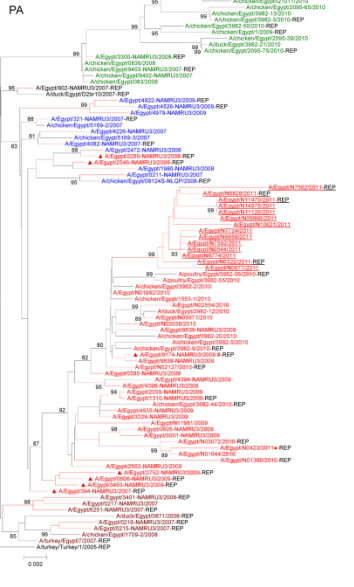
PB2



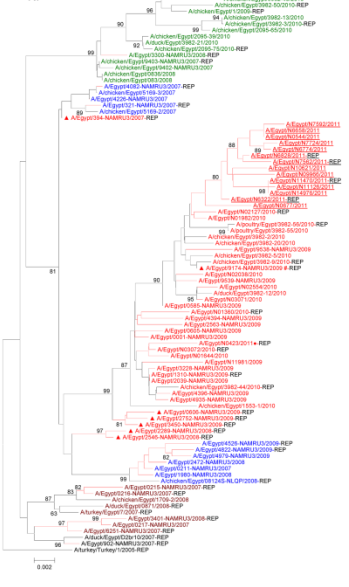
PB1



PA



NP



M



NS



Technical Appendix 2 Figure 3. Phylogeny of the complete genomes of highly pathogenic avian influenza A(H5N1) viruses in Egypt, 2007–2011. The sequences of the internal genes belonging to the 90 viruses analyzed in this study were aligned with representative viruses from other countries from different lineages, and trees were built in MEGA5 (www.megasoftware.net), using the neighbor-joining method based on a maximum composite likelihood model. Bootstrap values >79 generated from 1,000 neighbor-joining replicates are shown on the branches. PB2, polymerase basic 2 gene; PB1, PB 1 gene; PA, polymerase acidic gene; NP, nucleoprotein; M, matrix gene; NS, nonstructural gene. Scale bars indicate nucleotide substitutions per site.

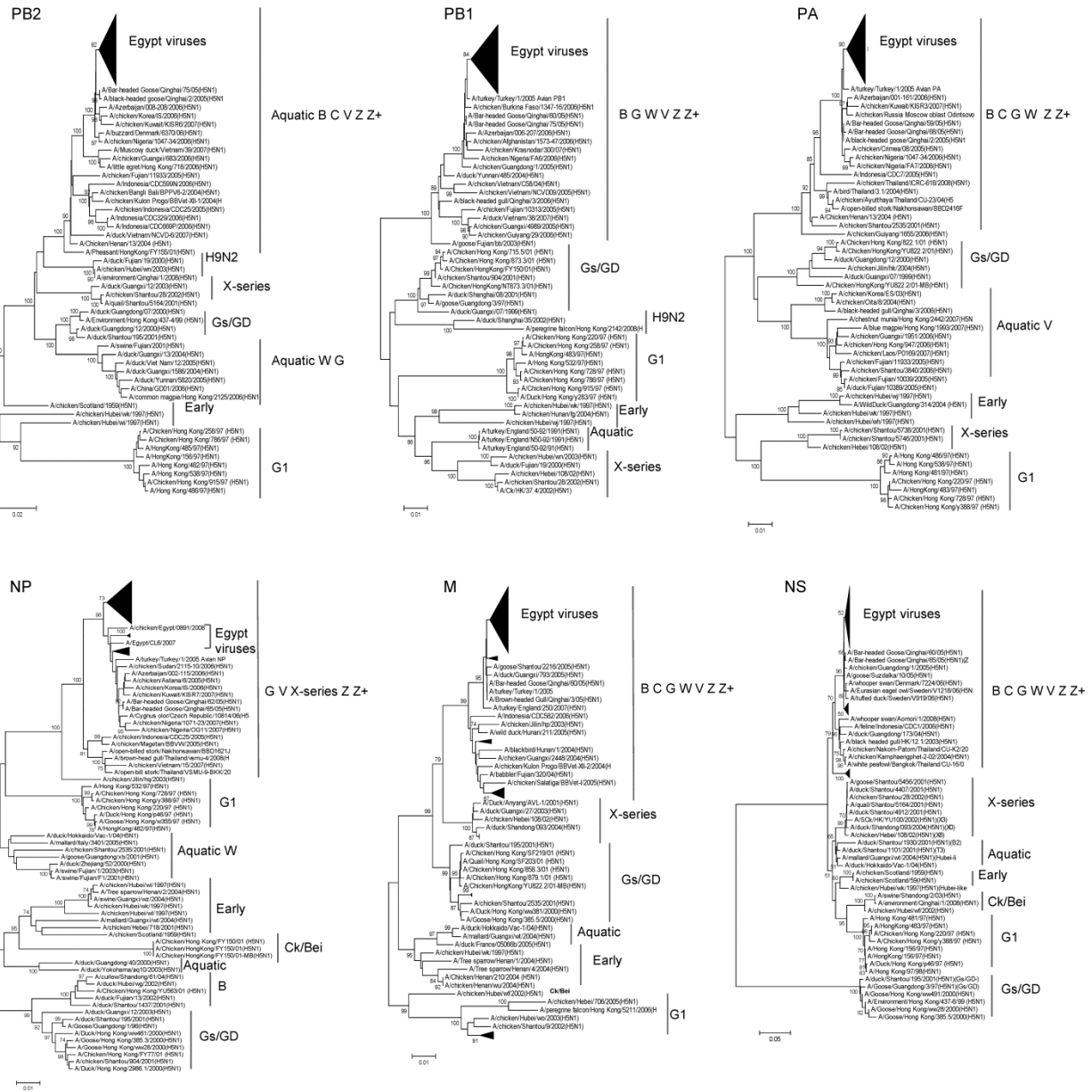


Table . Antigenic analysis of avian influenza A(H5N1) viruses isolated from humans in Egypt during January–September 2011*

Antigen	Clade/ Egypt group	Reference ferret antisera clade, reference antigen													
		2.2.1, group											2.2.1.1, ck/ EG	2.2.1.1, EG/ 3300	2.3.4, ANH/1
		1, VN/04	2.2, ws/MG	2.2.1, tk/TK	A, eg/EG	B, EG/ 321	C, EG/ 9539	C, EG/ 3072	C, EG/ 2289	C, EG/ 2546	D, EG/ 0215				
Reference antigens															
A/Vietnam/1203/2004	1	160	10	10	5	10	5	10	5	10	5	5	5	5	
A/ws/MG/244/2005	2.2	5	1280	640	320	160	5	1280	640	160	80	20	5	5	
A/turkey/Turkey/1/2005	2.2.1	5	1280	2560	320	160	80	1280	640	5120	160	10	5	5	
A/eg/Egypt/1162-N3/06	2.2.1-A	5	2560	1280	640	320	40	1280	640	160	160	20	5	5	
A/Egypt/321-N3/2007	2.2.1-B	5	640	320	160	160	20	640	160	40	20	5	5	5	
A/Egypt/9539-N3/2009	2.2.1-C	5	640	640	160	320	320	2560	2560	640	160	5	5	10	
A/Egypt/N03072/2010	2.2.1-C	5	1280	1280	320	320	320	2560	2560	640	160	10	5	10	
A/Egypt/2289-N3/2008	2.2.1-C	5	2560	2560	640	640	1280	5120	5120	2560	1280	640	5	320	
A/Egypt/2546-N3/2008	2.2.1-C	5	2560	2560	320	320	640	5120	5120	1280	640	320	5	320	
A/Egypt/0215-N3/2007	2.2.1-D	5	1280	640	320	160	40	640	320	160	320	10	10	5	
A/ck/Egypt/9403N3/07	2.2.1.1	5	5	5	5	80	5	5	5	5	5	640	5	5	
A/Egypt/3300-N3/2008	2.2.1.1	5	5	5	5	10	5	5	80	10	10	320	2560	5	
A/Anhui/1/2005	2.3.4	5	40	40	5	20	5	10	10	20	10	10	5	640	
Test antigens															
A/Egypt/N0544/2011	2.2.1-C	5	160	320	80	40	80	640	640	160	5	5	5	5	
A/Egypt/N6322/2011	2.2.1-C	5	320	320	40	40	80	640	640	160	20	5	5	5	
A/Egypt/N6658/2011	2.2.1-C	5	640	1280	160	80	320	1280	1280	320	40	5	5	5	
A/Egypt/N0677/2011	2.2.1-C	5	320	320	10	40	80	640	640	160	10	5	5	5	
A/Egypt/N6774/2011	2.2.1-C	5	320	320	5	40	160	640	640	160	10	5	5	5	
A/Egypt/N6828/2011	2.2.1-C	5	160	160	10	20	10	320	320	80	10	5	5	5	
A/Egypt/N7562/2011	2.2.1-C	5	80	160	20	20	40	320	320	80	20	5	5	5	
A/Egypt/N7592/2011	2.2.1-C	5	640	640	80	80	160	1280	1280	160	10	5	5	5	
A/Egypt/N7724/2011	2.2.1-C	5	640	1280	320	160	640	1280	1280	640	80	5	5	80	

*Bold indicates homologous titer.