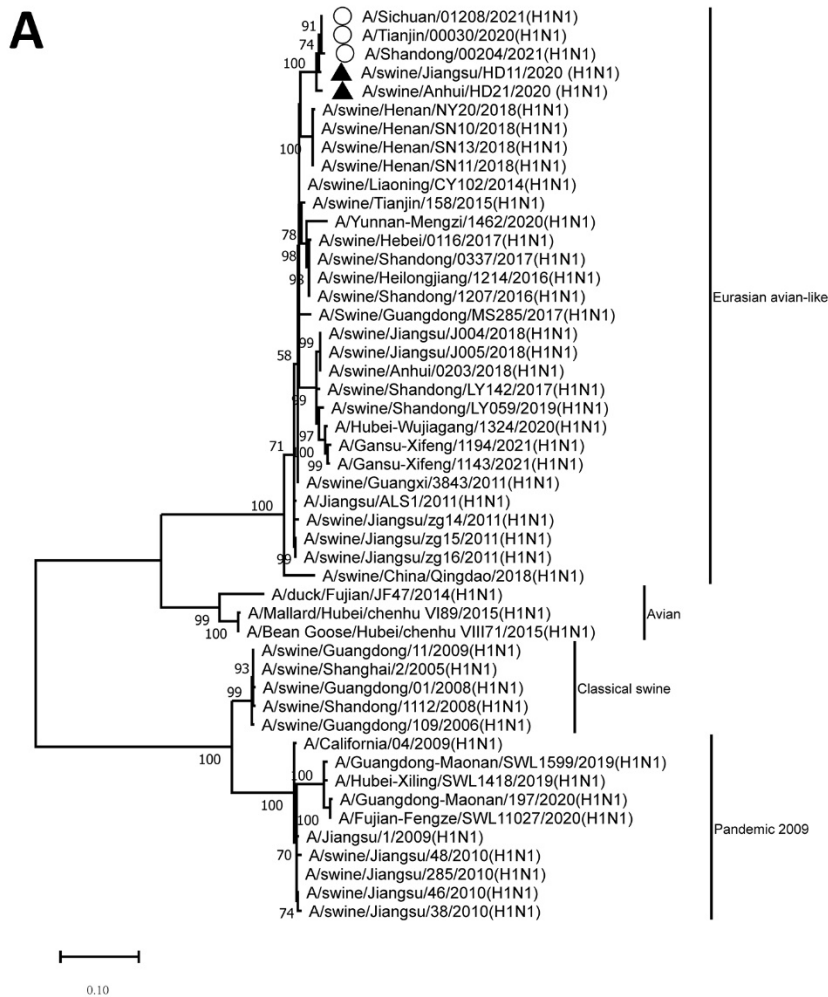
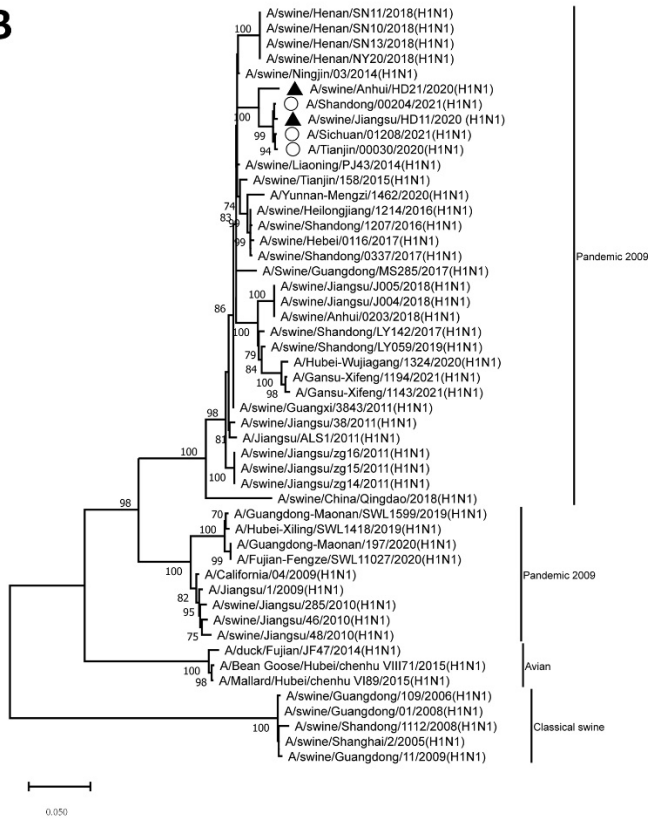
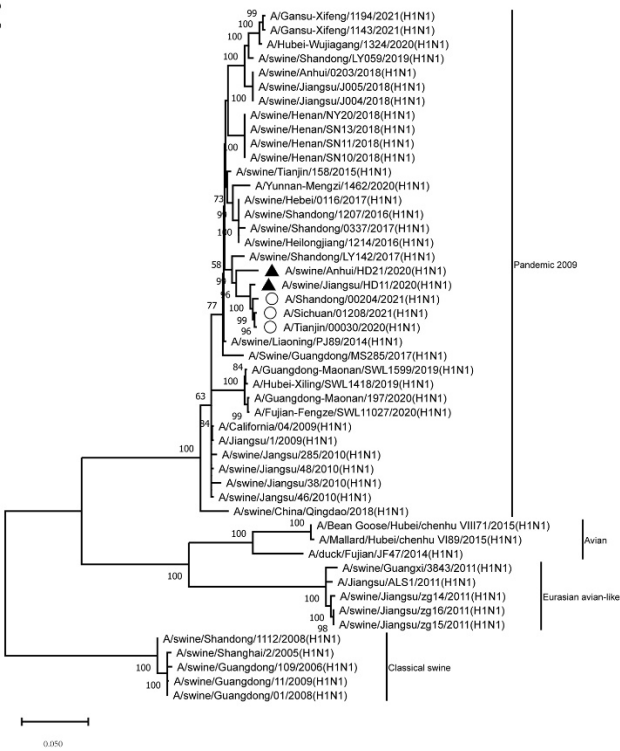
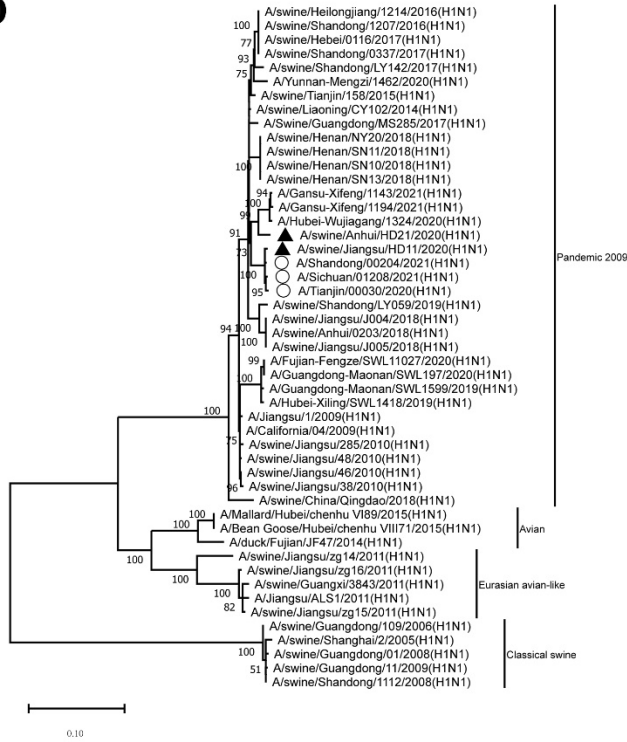
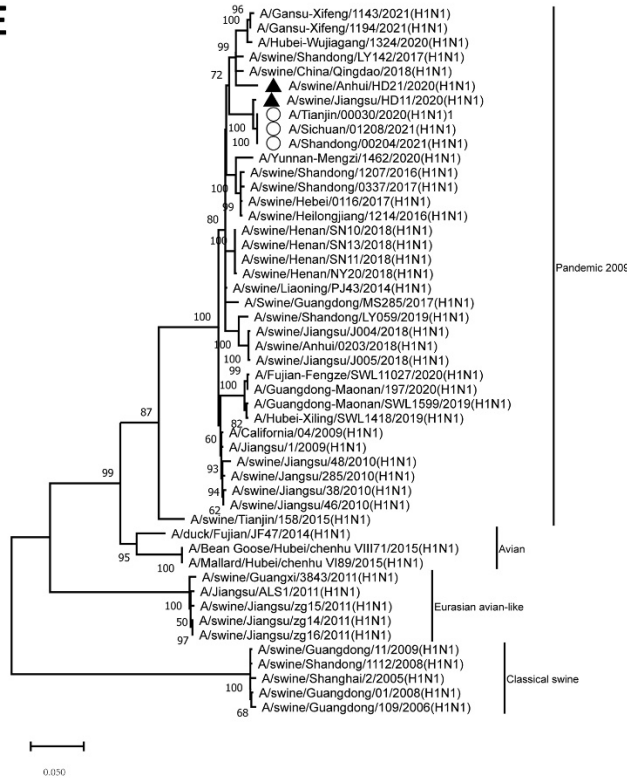


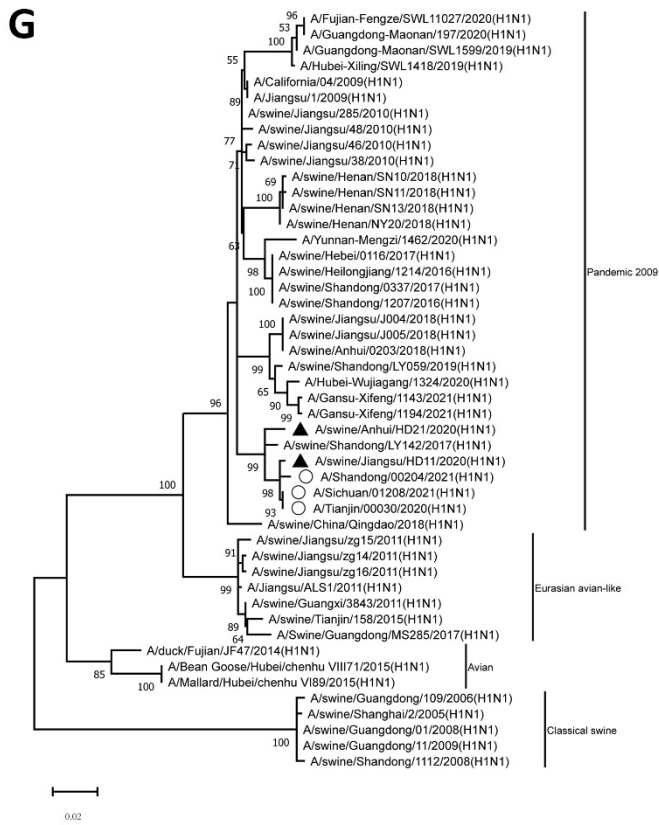
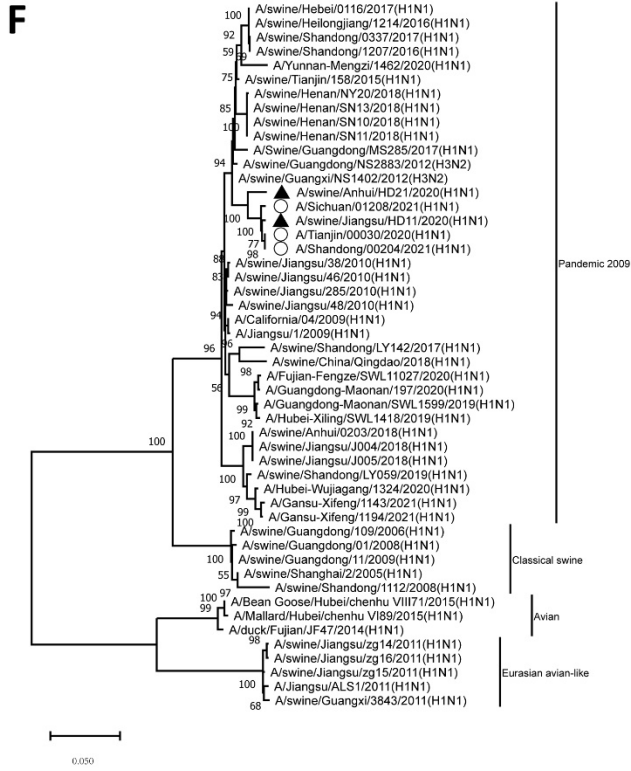
Zoonotic Threat of G4 Genotype Eurasian Avian-Like Swine Influenza A(H1N1) Viruses, China, 2020

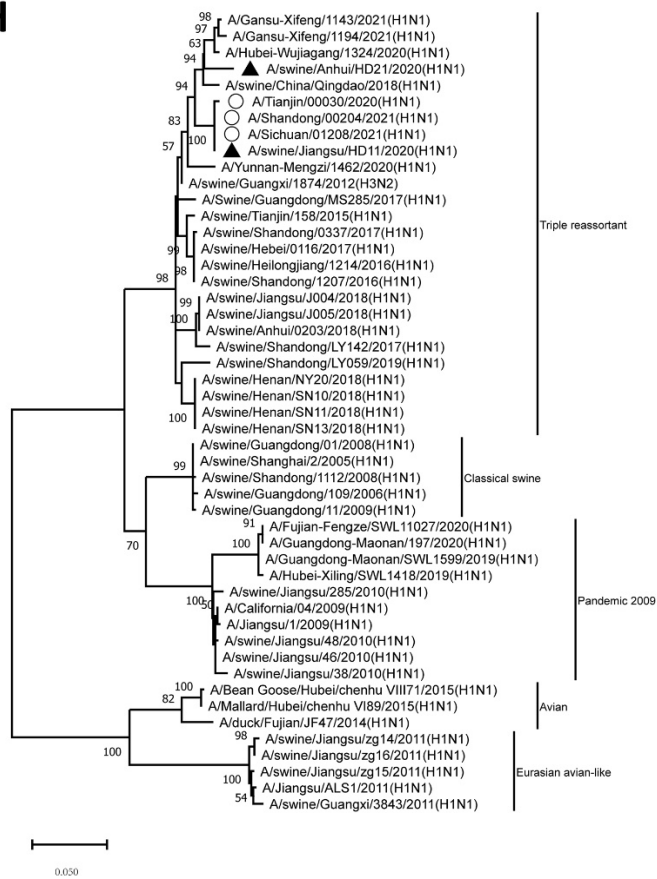
Appendix



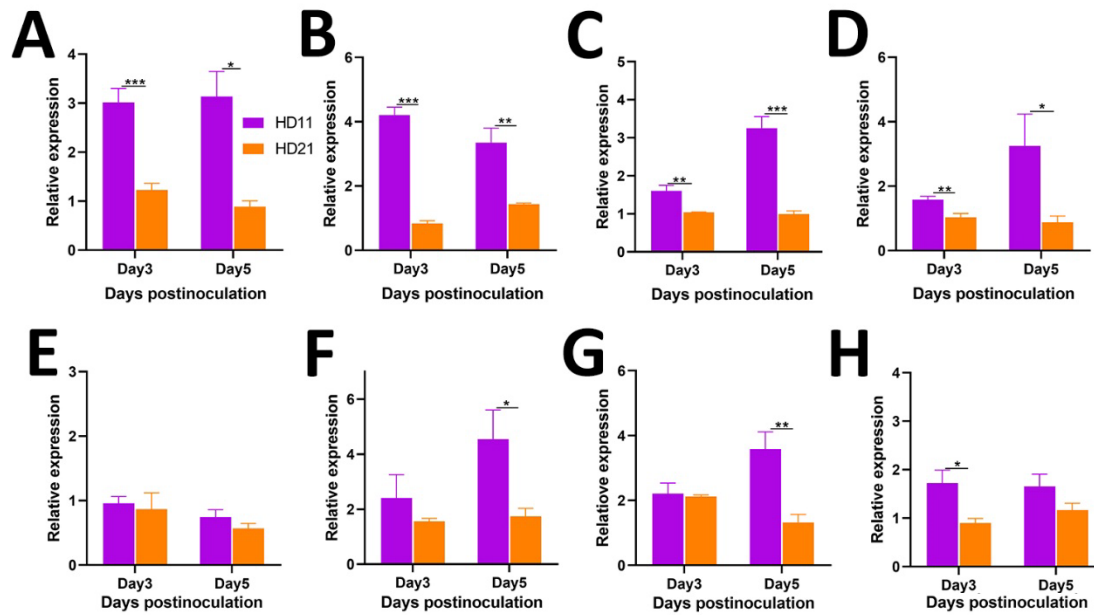
B**C**

D**E**



H

Black triangles indicate G4 EA H1N1 swine isolates of A/swine/Jiangsu/HD11/2020(H1N1) [HD11] and A/swine/Anhui/HD21/2020(H1N1) [HD21] in this study. Reference sequences of Eurasian avian-like, Pandemic 2009, Classical swine, Triple reassortant and avian H1N1 subtype IAV lineages were retrieved from the GISAID platform (<http://www.gisaid.org>), especially those human viruses showing consistent homology with HD11 and HD21 in all 8 genes; hollow circles indicate those strains. Each maximum-likelihood tree was generated using the MEGA software version 11 (<http://www.megasoftware.net>). We evaluated tree topology by bootstrap resampling method with 1,000 replicates. Scale bar indicates nucleotide substitutions per site.



Appendix Figure 2. The expression level of inflammatory cytokines induced by 2 G4 Eurasian avian-like H1N1 swine isolates in mouse lungs. Groups of six 6-week-old BABL/c mice were intranasally inoculated with $10^{6.0}$ EID₅₀ A/swine/Jiangsu/HD11/2020(H1N1) [HD11] or A/swine/Anhui/HD21/2020(H1N1) [HD21]. On 3 and 5 days postinoculation, 3 mice from each group were euthanized to collect the lungs for homogenate preparation. We evaluated relative mRNA expression levels of IL-6 (A), IL-10 (B), IFN-β (C), INF-γ (D), TNF-α (E), MX1 (F), CXCL-10 (G), and CXCL-11 (H) in the infected mouse lungs by the real-time quantitative RT-PCR method. All values were normalized to GAPDH and expressed as fold change against controls. Values were shown as mean±SD from 3 independent determinations. (*, p<0.05; **, p<0.01; ***, p<0.001).