Automated classification of influenza A virus gene sequences detected in U.S. swine to evolutionary origin

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**ABSTRACT**

Influenza A viruses (IAV) in swine are classified as H1N1, H1N2, or H3N2 subtype, with diversity of the 8 genes reflecting introductions from non-swine hosts and subsequent antigenic drift and shift. Here, we curated a dataset and present a pipeline that assigns evolutionary lineage and genetic clade to query gene segments.

Influenza A virus (IAV) is a negative-sense, single-stranded, enveloped RNA virus of the *Orthomyxoviridae* family. Although only H1N1, H1N2, and H3N2 subtypes are endemic in swine around the world, much diversity can be found in the genes coding for major surface proteins, hemagglutinin (HA) and neuraminidase (NA), and in the other 6 internal gene segments. This diversity is the result of bidirectional transmission between swine and humans, the occasional transmission of an avian virus into swine, followed by periods of antigenic drift and shift.

Swine IAV emerged coincident with the 1918 Spanish flu, and genes derived from this lineage are classified as classical-swine H1N1 (1). In the late 1990s, triple-reassortant H3N2 viruses were identified containing gene segments derived from seasonal human H3N2 (HA, NA, and PB1), avian IAV (PB2 and PA), and the classical H1N1 swine IAV (NP, M, and NS) (2, 3). The HA persisted, evolving into phylogenetic clades that are detected to present day (Cluster-IV (C-IV) clades A-F) (4). The triple-reassortant H3N2 viruses also reassorted with classical-swine H1N1 viruses, driving diversification and new genetic clades of H1N1 and H1N2 viruses (5), but preserving the triple reassortant internal gene (TRIG) constellation. Genetically distinct human seasonal H1 also spilled into and established in swine in the early 2000s (6, 7). In 2009, a virus with NA and M genes from Eurasian-avian H1N1 swine in addition to TRIG and classical-swine lineage genes emerged in swine, and infected humans as a pandemic (H1N1pdm09). Although sharing common ancestors, the human H1N1pdm09 genes were phylogenetically distinct from contemporary swine IAV in the U.S.. Via reverse zoonoses, the H1N1pdm09 continues to contribute to genetic diversity in swine, particularly the internal gene segments (8, 9). More recently, a human H3N2 virus was transmitted to swine, H3.2010.1, this virus is distinct from the H3N2 lineage C-IV viruses (10). In the U.S., HA genes are paired with N2 genes derived from the 1998 or 2002 human seasonal origin (11), or an N1 gene from the classical-swine lineage or pandemic-lineage (12, 13). In 2018, a live-attenuated influenza virus (LAIV) vaccine became commercially available in the U.S. (14). The LAIV uses HA (H1 and H3) and NA (N1 and N2) expressed on a TRIG internal gene backbone, with all components isolated from swine in the 1990s. These LAIV genes are distinct from contemporary IAV and reassorted viruses have been detected with vaccine-derived internal genes (annotated as LAIV), and surface genes (annotated as H3 Cluster-I, H1 gamma2-beta-like, N2 LAIV-98, N1 LAIV-Classical). Thus, interspecies transmission episodes and the processes of antigenic shift and drift have led to approximately 16 distinct HA clades, 4 NA lineages, and 3 internal gene lineages (15, 16).

We generated reference gene datasets and an analytical pipeline that assigns query HA to genetic clade and NA and internal IAV genes to evolutionary lineages that are commonly found in IAV from North American swine. The pipeline can aid in the detection of interspecies transmission or reassorted viruses containing gene segments derived from avian, human, or other sources. Users need (i) the reference datasets; and (ii) a FASTA with query sequences from any IAV gene segment. The pipeline (Fig. 1) processes query sequences by: (i) identification to one of 8 segments using BLASTn; (ii) alignment of queries to reference gene segment dataset; (iii) the inference of a maximum likelihood tree; (iv) classification to evolutionary lineage or genetic clade of queries using patristic distance extracted from the inferred tree; and (v) generation of a summary classification file. The reference dataset for each gene includes outgroup sequences allowing the pipeline to flag sequences that are not contemporary circulating U.S. swine IAV.

**Data availability.** Gene segment sequences were extracted from the Influenza Research Database (XXX) (17). The pipeline and reference gene sets are provided on GitHub (flu-crew/nn\_patristic\_classifier), and DockerHub (j23414/nn\_patristic\_classifier).

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**FIGURE LEGEND**

**Figure 1.** The nearest neighbor patristic classifier pipeline (A) and an inferred maximum likelihood tree generated on query and reference PB2 sequences (B). The PB2 gene example demonstrates the genetic lineages of contemporary influenza A virus circulating in United States swine populations: the H1N1 pandemic 2009 (red) and LAIV PB2 genes (orange) are monophyletic clades nested within the TRIG lineage (purple), human seasonal (grey), and classical-swine (blue) lineage PB2 genes are separate monophyletic clades. The tree is midpoint rooted for clarity; branch lengths are drawn to scale; and the scale bar indicates the number of nucleotide substitutions per site.