

United States Department of Agriculture

Animal and Plant Health Inspection Service

Influenza A Virus in Swine Surveillance

Veterinary Services Fiscal Year 2023 Quarterly Report

April 2024

Surveillance Summary for Fourth Quarter Fiscal Year 2023: July 1 to September 30, 2023

Report Summary¹

- This report covers the fourth quarter (Q4) of fiscal year (FY) 2023, from July 1 to September 30, 2023.
 - There were 1,925 samples submitted for influenza A virus (IAV) surveillance in swine from 1.665 accessions.
 - H1N1 was the predominant subtype reported in USDA data.
 - o Over the past 8 quarters, H1N1 was the predominant subtype in all regions.
 - The Agricultural Research Service (ARS) characterized 182 isolates with published sequences in GenBank by phylogenetic analysis.
 - The National Veterinary Services Laboratory's (NVSL) Diagnostic Virology Laboratory (DVL) provided 21 total isolates to four governmental entities.

Key Points

- All IAV-S submissions are voluntary and based on clinical case submissions to veterinary diagnostic labs. These data are not a statistically representative sampling of the U.S. swine population.
- Due to the voluntary nature of this surveillance, the information in this report cannot be used to determine regional and/or national incidence, prevalence, or other epidemiological measures, but it may help identify IAV-S trends.
- The report provides data from both national and regional levels.
- Limited accessions from a region can skew data and lead to misinterpretation. Therefore, less inference can be applied to results from Regions 3, 4, and 5.
- Where relevant, this report includes previous years' data for historical perspective.

Introduction

This report, based on data received into the database as of April 8, 2024, provides a brief update on the status of national surveillance for influenza A virus in swine (IAV-S) for producers, swine practitioners, diagnosticians and the public. Summaries in this report may differ from those provided in past reports due to the regular addition of data from participating laboratories. The IAV-S surveillance program is voluntary and, as a result, the accessions and samples submitted represent a subset of the swine population. The surveillance system is not representative of the total U.S. domestic swine population; therefore, the data cannot be used to determine IAV-S prevalence or other epidemiologic measures in the swine population; however, the data may help identify influenza trends in swine. Program guidance indicates samples should

¹ In November 2016, VS modernized the process that prepares and stages laboratory results data for reporting. Consequently, VS recognizes there is a small difference in previously reported summary numbers for IAV-S surveillance. The results in this report reflect updated and corrected numbers achieved with the modernized data process.

only be collected from animals displaying influenza-like illness. Reporting months are based on the month the sample was collected. When the submitter does not report relevant information, data are recorded as "unknown."

A laboratory accession generally represents a set of samples collected at a single premises on a single day and subsequently received at the laboratory. While a nasal swab or lung tissue sample represents a single animal within the herd, a single oral fluid sample may represent one to two pens of animals in a herd. A positive sample status is based on the screening real-time reverse transcriptase polymerase chain reaction (rRT-PCR) on one or more samples within the accession. The subtype result is based on rRT-PCR-based subtyping assays. Virus isolation (VI) and sequencing in the National Animal Health Laboratory Network (NAHLN) labs are only attempted on rRT-PCR positives meeting criteria, with sequences deposited into GenBank, the public sequence database. On a monthly basis, USDA NVSL also performs whole genome sequencing (WGS) on a selected subset of virus isolates received into the repository through the surveillance program and deposits those sequences into GenBank. On a quarterly basis, a phylogenetic analysis is performed by USDA's Agricultural Research Service (ARS) National Animal Disease Center (NADC) influenza researchers; phylogenetic analyses are based on all successful USDA surveillance sequencing results deposited into GenBank.

Program Updates

Information on IAV-S and the IAV-S surveillance program, as well as previous IAV-S quarterly reports, can be found at:

https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/swine-disease-information/influenza-a-virus

The focus of IAV-S surveillance remains on acquiring and analyzing contemporary viruses from sick swine for ongoing genetic studies. The NAHLN has several submission options to ensure that unusual viruses identified by methods other than standardized NAHLN testing processes can be submitted into the program. An updated version of the IAV-S NAHLN testing guidelines and instruction sheet can be found at:

- Algorithm: https://www.aphis.usda.gov/animal-health/animal-dis-spec/swine/downloads/appendix-c-testing-g-uidelines.pdf
- Instructions: https://www-author.aphis.usda.gov/animal_health/animal_dis_spec/swine/downloads/iav-s-algorithm-instructions.pdf

IAV-S isolates can be requested from the NVSL repository by following the instructions found at:

 Isolate request: https://www.aphis.usda.gov/animal_health/lab_info_services/downloads/OrderingIAV-SRepositoryIsolates.pdf

IAV-S Surveillance Objectives

<u>USDA's National Surveillance Plan for Swine Influenza Virus in Pigs (July 2010)</u> describes the current surveillance system for IAV in swine in detail. The surveillance objectives are to:

- 1. Monitor genetic evolution of endemic IAV in swine to better understand endemic and emerging influenza virus ecology;
- 2. Make influenza isolates from swine available for research and establish a data management system to facilitate genetic analysis of these isolates and related information; and
- 3. Select proper isolates for the development of relevant diagnostic reagents, updated diagnostic assays, and vaccine seed stock products.

Objective 1. Monitoring Genetic Evolution of Endemic IAV in Swine to Better Understand Endemic and Emerging Influenza Virus Ecology

Objective 1 is met through the submission of diagnostic laboratory samples to the surveillance system, collection of the viruses that are isolated from the samples, and analysis of the hemagglutinin (HA) and neuraminidase (NA) sequences that are generated at the NAHLN laboratories. Each month, selected viruses undergo whole genome sequencing by the NVSL. Phylogenic analysis of the genetic sequences submitted through the surveillance program is provided through an interagency agreement with the USDA's Agricultural Research Service (ARS) National Animal Disease Center (NADC).

National Surveillance Data Summary

From FY2010 through FY2015, the total number of accessions and samples submitted increased. In FY2016, based on historical data for successful virus isolation, cycle threshold (Ct) maximum values for different sample types were established to try to improve the efficiency of the surveillance program while reducing the required resources. If lung/nasal samples have a Ct value of 25 or less and oral fluid samples have a Ct value of 20 or less, virus isolation and sequencing will be attempted. If there is something unique related to the virus, like it is causing high mortality, but the samples have higher than the established maximum Ct values, they will still enter the surveillance stream. These changes resulted in decreased laboratory accessions and samples; however, they also yielded a higher percentage of accessions resulting in a virus isolate that could be sequenced and analyzed.

In FY2023, a total of 5,811 samples were tested from 4,710 accessions (Figure 1). Figure 2 shows the overall trends in rRT-PCR and VI positive accessions and subtyped accessions.

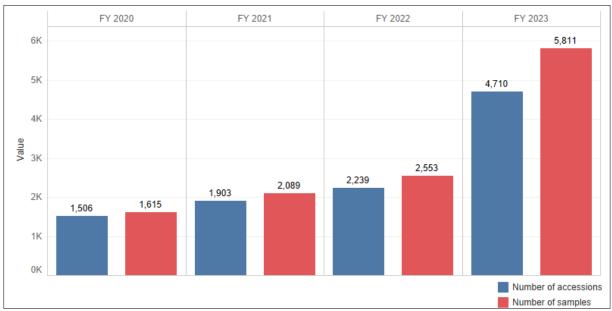


Figure 1. Number of IAV-S laboratory accessions and samples tested in swine FY2020 through Q4 FY2023

Figure 3 shows the number and distribution of subtype detections in Q4 FY2023. A total of 177 samples were subtyped, including H1N1 (n=65), H1N2 (n=64), H3N2 (n=46), H3N1 (n=0), and mixed subtype (n=2).

Figure 4 breaks down accessions by rRT-PCR subtype for FY2019 through Q4 FY2023. H1N1 has been the predominant subtype detected from 2020 through Q4 FY2023. H1N2 predominated in 2019. It is important to note that there is wide genetic diversity within each subtype.

Figure 5 displays the number of times VI was attempted in blue, the number of successful VI attempts in purple, and the number of sequenced viral isolates submitted to <u>GenBank</u> in green. Since the implementation of the June 2016 program modifications, most VIs attempted now yield a virus with the sequences submitted to <u>GenBank</u> for analysis.

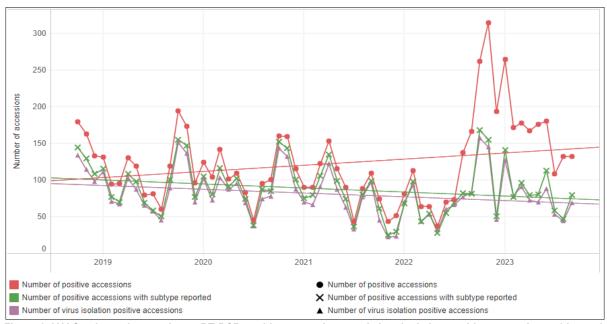


Figure 2. IAV-S subtyped accessions, rRT-PCR positive accessions, and virus isolation positive accessions with trend lines over time FY2019 through Q4 FY2023

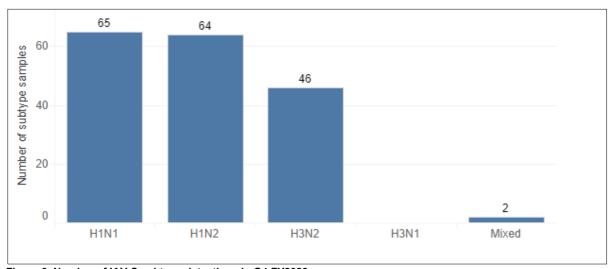


Figure 3. Number of IAV-S subtype detections in Q4 FY2023

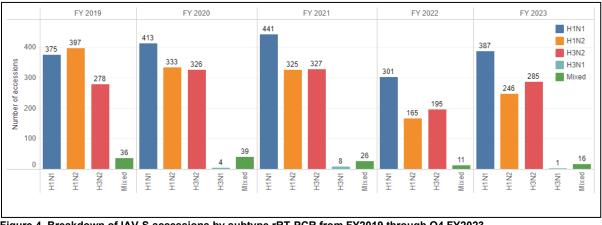


Figure 4. Breakdown of IAV-S accessions by subtype rRT-PCR from FY2019 through Q4 FY2023

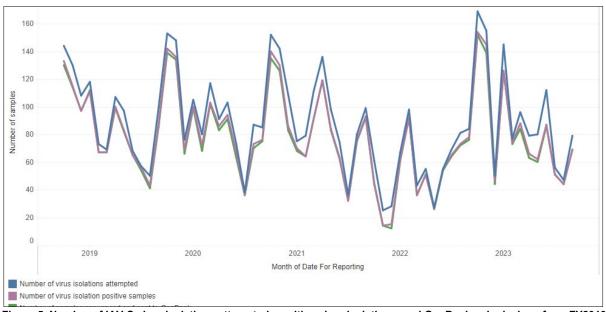


Figure 5. Number of IAV-S virus isolations attempted, positive virus isolations, and GenBank submissions from FY2019 through Q4 FY2023

Laboratory accessions were evaluated by age-class for the fourth quarter in FY2023. The most common subtype isolated among the nursery and grow/finish classes was H1N2. Subtype H3N2 was the most common subtype among the suckling group. H1N1 was the most common subtype among the unknown class and there were no positive accessions from the sow/boar class (Table 1). Table 2 displays the number of IAV-S positive accessions by specimen and viral subtype. All sample types yielded above 50% success for virus isolation and submission to GenBank.

Table 1. Number of positive accessions tested for IAV-S by age class and viral subtype, Q4 FY2023

·					
Age Class (group)	Number of H1N1	Number of H1N2	Number of H3N1	Number of H3N2	Number of Mixed
Suckling	11	12	0	16	1
Nursery	25	27	0	17	1
Grow/Finish	19	20	0	12	0
Sow/Boar	0	0	0	0	0
Not Recorded/Unknown	10	5	0	1	0

Table 2. Number of positive accessions* tested for IAV-S by specimen type and by viral subtype, Q4 FY2023

Specimen Type (group)	Number of accessions with subtype reported	Percent of subtyped accessions with positive virus isolation	Number of H1N1	Number of H1N2	Number of H3N1	Number of H3N2	Number of Mixed	Number of samples sequenced and sent to GenBank
Lung	164	90%	59	62	0	41	2	147
Nasal or Nasal Swab	12	100%	5	0	0	5	0	12
Oral Fluids	2	50%	1	1	0	0	0	1
Other Specimens	1	100%	0	1	0	0	0	1

^{*}Accessions may include samples with multiple specimen types. In these cases, individual accessions are counted in more than one specimen type category.

Regional surveillance data

In this section, we present IAV-S surveillance data across five different regions of the United States (Figure 6). These regions are based on former USDA administrative districts only and do not represent specific industry distributions. Submissions are voluntary, as is providing any identifying information beyond State of animal origin with the submission. Therefore, regional and/or national incidence, prevalence, or other epidemiological measures cannot be determined from this data.



Figure 6. A map of the regions for national IAV-S surveillance

Summary of Regional Data from ARS

Table 3 lists the predominant HA/NA phylo-type pairs by region from October 2022 through September 2023, with predominant being defined as comprising at least 10% of a region's HA/NA pairs. The total number column displays the total number of isolates that were phylo-typed for each region during that time. Historically, region 5 submits substantially fewer accessions than the other regions.

Figure 7 shows the distribution of rRT-PCR subtyped accessions across the five regions for Q1 FY2022 through Q4 FY2023. Over the last 8 quarters, H1N1 was the predominant subtype in all regions with the exception of Region 4, where H1N2 was the predominant subtype.

Table 3. Summary of predominant IAV-S HA/NA* phylo-types by US region for the 1-year window from October 2022 through September 2023

Region	Total number	Predominant HA/NA subtypes
1	91	H1N1 (H1-Alpha-del / N1-Classical) (n=39) H3N2 (H3-Cluster IV-A / N2-2002B) (n=20) H1N2 (H1-Delta2 / N2-1998B) (n=10)
2+	787	H1N1 (H1-Gamma-c3 / N1-Classical) (n=199) H1N2 (H1-Delta2 / N2-1998B) (n=150) H3N2 (H3-2010.1 / N2-2002B) (n=142)
3	78	H1N1 (H1-Gamma-c3 / N1-Classical) (n=16) H1N1 (H1-Alpha-del / N1-Pandemic) (n=11) H1N2 (H1-Delta2 / N2-1998B) (n=9)
4	71	H3N2 (H3-2010.1 / N2-2002B) (n=17) H1N2 (H1-Delta2 / N2-1998B) (n=12) H1N2 (H1-Alpha-del / N2-2002B) (n=9)
5**	12	H1N1 (H1-Alpha-del / N1-Classical) (n=4) H3N2 (H3-Other-Human / N2-Human-like) (n=3) H3N2 (H3-2010.1/ N2-2002B) (n=2)
All	1,039	H1N1 (H1-Gamma-c3 / N1-Classical) (n=222) H1N2 (H1-Delta2 / N2-1998B) (n=182) H3N2 (H3-2010.1 / N2-2002B (n=177)

^{*}HA/NA pairs included if they compromise over 10% from a region

^{*} Most diversity of all regions

^{**} Low participation

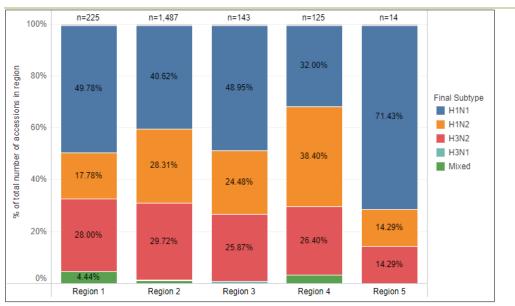


Figure 7. Distribution of IAV-S rRT-PCR subtyped accessions across five US regions for Q1 FY2022 through Q4 FY2023

Phylogenetic analysis of sequences from the IAV-S surveillance system

Phylogenetic analysis of gene sequences of IAV in swine is conducted to further examine the genetic changes that occur in HA and NA genes of this rapidly changing virus. Through collaboration with ARS, a dataset of 182 isolates with published sequences in GenBank was characterized by phylogenetic analysis for the Q4 FY2023 report. This analysis provides information on the genetic diversity and evolutionary patterns of IAV in swine and allows for inferences about population and/or vaccine immunity.²

The following series of bar charts parse the data into an approximately 2-year window by quarters and region, describing virus subtypes (Figure 8) and phylogenetic clades of H1, H3, N1 and N2 subtypes (Figures 9-12). Regional charts depicting various combinations of HA and NA are available in Appendix 1.

Figure 8 demonstrates the four subtypes H1N1, H1N2, H3N1 and H3N2 across the five regions. Regions 1, 2, and 3 reported the most submissions, with a mixture of mostly H1N1, H1N2, and H3N2 subtypes. Limited accessions from a region can skew data and lead to misinterpretation, therefore less inference can be applied to results from regions 4 and 5.

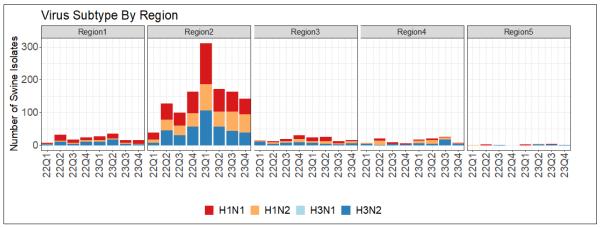


Figure 8. Temporal distribution of Influenza A virus of swine subtype by region for Q1 FY2022 to Q4 FY2023

² The ARS dataset is comprised of IAV-S surveillance isolate sequences from Genbank. This represents only a subset of the complete IAV-S surveillance dataset that includes PCR diagnostic test-based results as well as sequencing results. Therefore, ARS dataset results, such as subtype percentages, differ from the complete IAV-S dataset results provided in other sections of this report.

National phylogenetic HA gene information

HA genes from H1 subtype viruses are classified into phylogenetic clade alpha, beta, gamma, delta1, delta2, or pandemic H1N1 2009 (H1N1pdm09) based on a previously <u>published nomenclature system</u>. Similarly, H3 subtype viruses are classified as Cluster IV, Cluster IV-A, Cluster IV-B, Cluster IV-C, Cluster IV-D, Cluster IV-E, Cluster IV-F, 2010.1, 2010.2, or human-like. In Q4 FY2023, the predominant H1 HA genes were alphadel, gamma-c3, and delta2, representing 59% of all H1 HA detections (Figure 9); 2010.1 and IV-A, representing 29% of all H3 HA detections, were the predominant H3 HA genes (Figure 10).

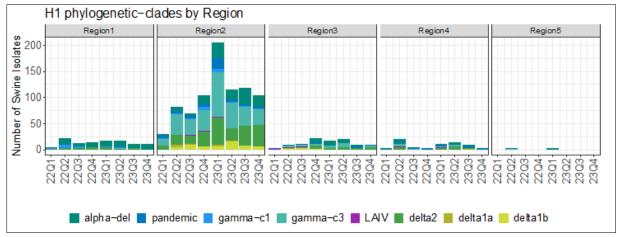


Figure 9. Temporal distribution of IAV-S H1 phylogenetic clades by region for Q1 FY2022 to Q4 FY2023

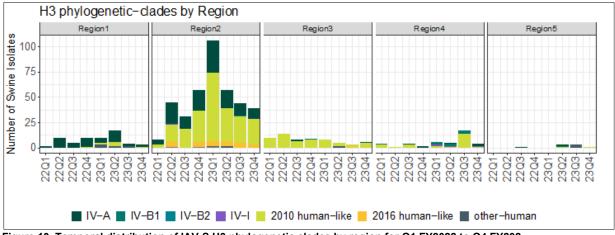


Figure 10. Temporal distribution of IAV-S H3 phylogenetic clades by region for Q1 FY2022 to Q4 FY202

National phylogenetic NA gene information

In Q4 FY2023, N1-Classical was the predominant N1 phylogenetic-clade (Figure 11), representing approximately 84.4% of the Q4 FY2023 N1 collection (n=64). In Q4 FY2023, the predominant N2 phylogenetic-clade was 2002B-lineage (Figure 12), representing approximately 48.3% of the Q4 FY2023 N2 collection (n=118).

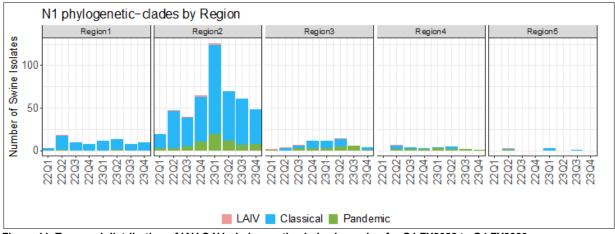


Figure 11. Temporal distribution of IAV-S N1 phylogenetic-clades by region for Q1 FY2022 to Q4 FY2023

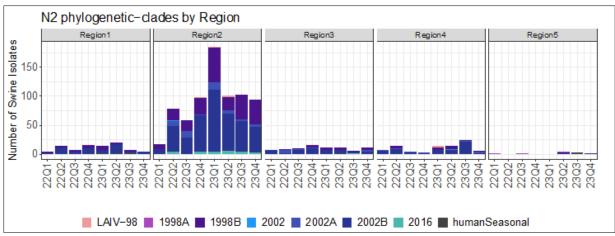


Figure 12. Temporal distribution of IAV-S N2 phylogenetic-clades by region for Q1 FY2022 to Q4 FY2023

Representative HA genes

Six months of IAV-S data, April 2023 – September 2023, were used by the NADC to identify circulating HA clades. An objective algorithm was used to identify the best-matched field strain housed in the USDA IAV-S virus repository (Table 4).

Table 4. IAV-S Surveillance NADC Representative HA genes*

Strain	GenBank	Global Clade	US Clade
A/swine/Illinois/A02861420/2023	OR067115	1A.1.1.3	alpha-del
A/swine/Kansas/A02861399/2023	OQ996083	1A.3.3.2	pandemic
A/swine/Minnesota/A02978529/2023	OR397555	1A.3.3.3-c3	gamma-c3
A/swine/Iowa/A02861398/2023	OR067111	1B.2.1	delta2
A/swine/Ohio/A02861370/2023	OQ954445	1B.2.2.2	delta1b
A/swine/lowa/A02861530/2023	OR293269	1990.4.a	IV-A
A/swine/Iowa/A02861558/2023	OR293293	2010.1	2010.1
A/swine/Ohio/A02685126/2023	OR345507	2010.2	2010.2

^{*6-}month HA1 objective algorithm and best-matched field strain in the repository was identified.

[•] April 2023 to September 2023 USDA HA data downloaded (n = 280 H1, n = 125 H3) and a phylogenetic tree was inferred. For each HA clade, an objective representative selection was made using PARNAS (https://github.com/flu-crew/parnas).

[•] The 5 H1 selections cover 88% of observed diversity; the 3 H3 selections cover ~64% of observed diversity.

[•] Clades were required to have a detection rate of at least 2% to be considered for selection (n >= 9).

[•] Omitted 1B.2.2.1 (n=2), 1A.3.3.3-c1 (n=3), 1990.4.i (n=1), 1990.4.b1 (n=4), 1990.4.b2 (n=1), Other-Human-2020 (n=5)

Representative NA genes

Six months of IAV-S data, April 2023 – September 2023, were used by the NADC to identify circulating NA clades. An objective algorithm was used to identify the best-matched field strain housed in the USDA IAV-S virus repository (Table 5).

Table 5. IAV-S Surveillance NADC Representative NA genes*

Strain	GenBank	Global Clade	US Clade
A/swine/lowa/A02861468/2023	OR144393	N1.P	Pandemic
A/swine/Iowa/A02861446/2023	OR067152	N1.C.2.1	Classical
A/swine/lowa/A02861421/2023	OR067118	N1.C.3.2	Classical
A/swine/Iowa/A02861522/2023	OR293260	2002B	2002B
A/swine/lowa/A02861395/2023	OQ996082	1998B	1998B
A/swine/Indiana/A02861538/2023	OR293280	2016	2016
A/swine/Illinois/A02861526/2023	OR293264	2002A	2002A

^{*6-}month NA1 objective algorithm and best-matched field strain in the repository was identified.

[•] April 2023 to September 2023 USDA NA data downloaded (n = 142 N1, n = 263 N2) and a phylogenetic tree was inferred. For each NA clade, an objective representative selection was made using PARNAS (https://github.com/flu-crew/parnas).

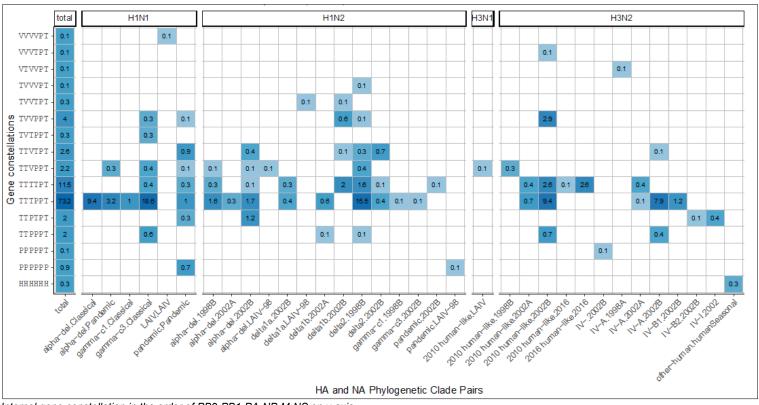
[•] The 3 N1 selections cover 79% of observed diversity; the 4 N2 selections cover ~67% of observed diversity.

[•] Clades were required to have a detection rate of at least 2% to be considered for selection (n >= 9).

[•] Omitted N1.C.3.1 (n=5), 2002 (n=1), Human-like (n=5)

Gene constellations

The most dominant internal gene constellations for October 2022 to September 2023 were TTTPPT (73.5%), TTTTPT (11.5%), and TVVPPT (4.0%). Out of 695 strains with completed whole genome sequencing that were analyzed, 38% were H1N1, 30.6% were H1N2, 31.2% were H3N2, and 0.2% were H3N1 with 16 unique gene constellations and 35 unique HA/NA pairs (Figure 13). Of observed constellations, 9.9% had at least one vaccine gene. Figure 14 displays the change in whole genome patterns over the last 12 months. It shows the gene constellation percentage differences from the current timeframe of interest, Q1 FY2023- Q4 FY2023, against the prior timeframe of interest, Q1 FY2022- Q4 FY2022.

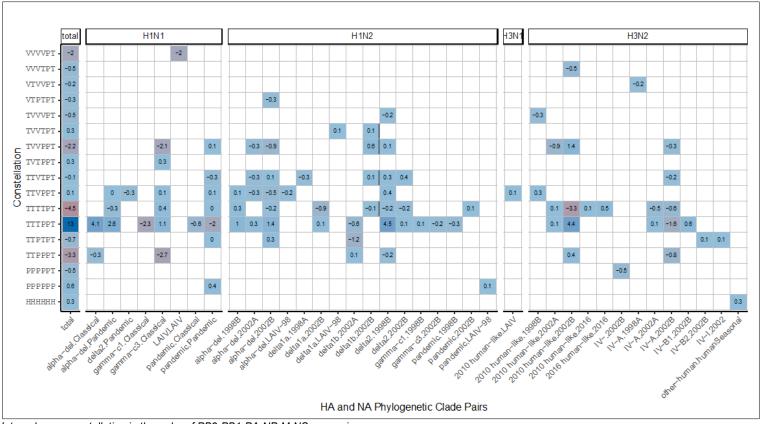


Internal gene constellation in the order of PB2-PB1-PA-NP-M-NS on y-axis

T=TRIG: P=Pandemic: V=Vaccine: H=Human-seasonal

Figure 13. IAV-S gene constellations by HA and NA phylogenetic clade pairs for October 2022 to September 2023

Influenza A Virus in Swine Surveillance Quarterly Report for Fiscal Year 2023, Quarter 4



Internal gene constellation in the order of PB2-PB1-PA-NP-M-NS on y-axis

T=TRIG; P=Pandemic; V=Vaccine; H=Human-seasonal

Figure 14. IAV-S gene constellations percentage differences for (Current [Q1 FY2023 to Q4 FY2023] - Prior [Q1 FY2022 to Q4 FY2022])

Objective 2. Make Influenza Isolates from Swine Available for Research and Establish a Data Management System to Facilitate Genetic Analysis of these Isolates and Related Information

A primary goal of the IAV swine surveillance program is to share selected virus isolates obtained through the surveillance system with public health, animal health, and academic researchers to facilitate genetic analysis and research on viruses of interest. The NVSL Diagnostic Virology Laboratory maintains a repository of the viruses submitted into the surveillance system and provides these viruses upon request.

In Q4 FY2023, the NVSL Diagnostic Virology Laboratory provided a total of 21 isolates to four governmental entities. NVSL received 196 isolates into the repository in Q4 FY2023. Table 6 reports the total number of virus isolates received into the repository each year from FY2014 through Q4 of FY2023. Table 7 reports the total number of isolates by subtype available in the repository for sharing.

IAV-S isolates can be requested from the NVSL repository by following the instructions found at: https://www.aphis.usda.gov/animal_health/lab_info_services/downloads/OrderingIAV-SRepositoryIsolates.pdf

Table 6. IAV-S isolates received in NVSL repository by fiscal year

Fiscal Year	Number of Isolates
FY2023	1,035
FY2022	641
FY2021	1,108
FY2020	1,074
FY2019	1,055
FY2018	994
FY2017	844
FY2016	1,046
FY2015	883
FY2014	765
TOTAL	9,445

Table 7. Total number of subtyped IAV-S isolates collected from 2009- FY2023 and available through the NVSL repository

Subtype	Number of Isolates
H3N2	3,169
H3N1	26
H1N1	4,024
H1N2	3,544
Mixed	304
TOTAL	11,067

Objective 3. Select Proper Isolates for Development of Relevant Diagnostic Reagents, Updating Diagnostic Assays, and Vaccine Seed Stock Products

USDA makes IAV-S isolates available in the public domain for further research. ARS-NADC conducts research on isolates obtained from the repository and sequences generated from the surveillance system. Genetic sequencing from the USDA program that is reported to GenBank is available for private corporations, government entities, academia, and other scientific community partners for research and vaccine strain selection and efficacy testing. NVSL and ARS staff are consulted as subject matter experts when necessary.

Conclusion

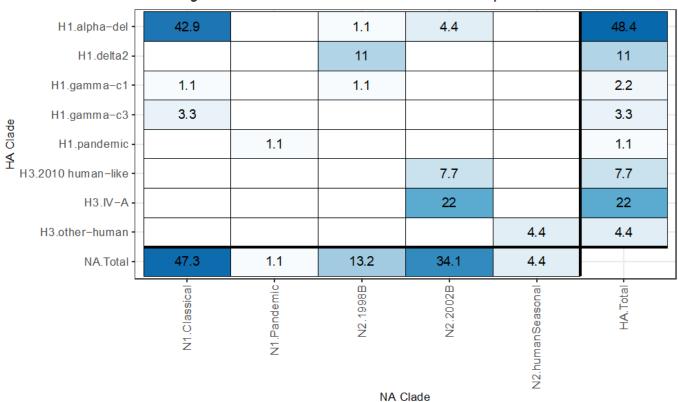
The IAV-S voluntary surveillance system in swine continues to provide insight into the genetic makeup of circulating influenza A virus in limited populations of commercial pigs. Genetic information and virus isolates are made publicly available for further research and possible vaccine strain selection and efficacy testing. Influenza A virus in swine remains a dynamic virus with high levels of genetic variability in the hemagglutinin and neuraminidase genes.

Appendix 1. Regional Charts of IAV-S HA and NA Combinations by Percentage

The following charts present the percentages of combinations of IAV-S HA and NA by region based on ARS-NADC phylogenetic analyses. The results are reported from October 2022 through September 2023 for regions 1 through 3, October 2022 through August 2023 for region 4, and November 2022 through July 2023 for region 5. These "heat maps" represent the percentage of combinations by using a color gradient where a deeper gradient color represents a greater percentage occurrence for a particular HA-NA combination. HA clusters are listed on the left vertical axis of the chart and NA clusters are listed on the bottom horizontal axis. Line up the HA cluster with the corresponding NA cluster to determine the occurrence of that particular combination.

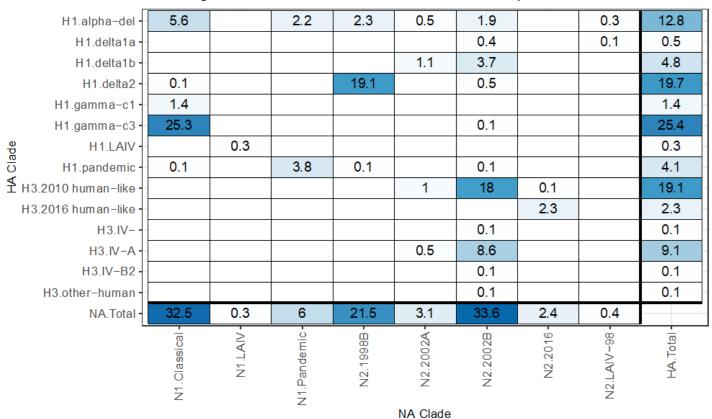


Percentage of HA and NA combinations - Oct 2022 to Sep 2023





Percentage of HA and NA combinations - Oct 2022 to Sep 2023



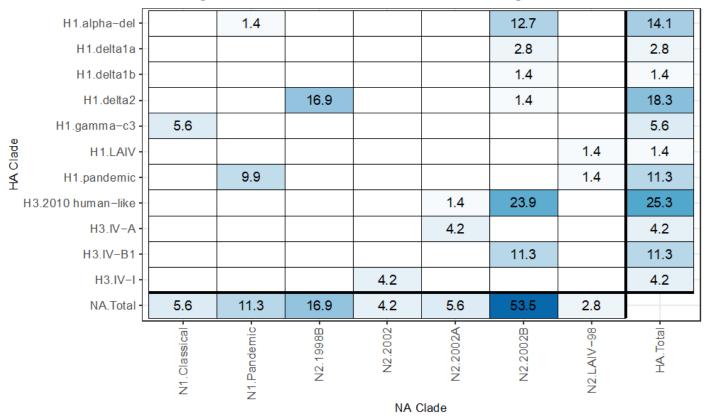


Percentage of HA and NA combinations - Oct 2022 to Sep 2023





Percentage of HA and NA combinations - Oct 2022 to A ug 2023





Percentage of HA and NA combinations - No v 2022 to Jul 2023

