

United States Department of Agriculture

Animal and Plant Health Inspection Service

Influenza A Virus in Swine Surveillance

Veterinary Services

Fiscal Year 2025 Quarterly Report

May 2025

Surveillance Summary for Second Quarter Fiscal Year 2025: January 1 to March 31, 2025

Report Summary

- This report covers the second quarter (Q2) of fiscal year (FY) 2025, from January 1 to March 31, 2025.
 - 925 samples were tested from 784 submitted accessions for a total of 2,163 samples from 1.854 accessions submitted in the first two quarters of 2025.
 - H1N2 was the predominant subtype reported in USDA data for the quarter.
 - Over the last 8 quarters, H1N1 was the predominant subtype in Regions 1 and 3; H1N2 was the predominant subtype in Regions 2 and 4; H3N2 was the most predominant in Region 5.
 - The Agricultural Research Service (ARS) characterized 200 isolates with published sequences in GenBank by phylogenetic analysis.
 - The National Veterinary Services Laboratory's (NVSL) Diagnostic Virology Laboratory (DVL) provided 19 isolates to one pharmaceutical and one government entity. NVSL received 207 isolates into the repository.

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 especially 5.
- Where relevant, this report includes previous years' data for historical perspective.

Introduction

This report, based on data received into the database as of May 20, 2025, 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 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

The Cycle threshold (Ct) maximum value differs depending on the sample type. 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.

In Q2 FY2025, 925 samples were tested from 784 submitted accessions for a total of 2,163 samples from 1,854 accessions submitted in the first two quarters of 2025 (Figure 1). Figure 2 shows the overall trends in number of accessions submitted, rRT-PCR and VI positive accessions and subtyped accessions with a notable increase in accessions submitted and positive accessions beginning in August 2022 and continuing through Q2 FY2025 with a large spike from January 2024 through May 2024. The positivity rate, calculated by the number of positive accessions divided by the total number of accessions submitted for each month, shows seasonal fluctuations but has otherwise remained relatively consistent since 2022.

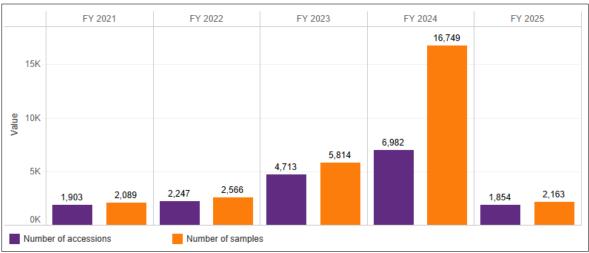


Figure 1. Number of IAV-S laboratory accessions and samples tested in swine FY2021 through Q2 FY2025

Figure 3 shows the number and distribution of subtype detections in Q2 FY2025. A total of 259 samples were subtyped, including H1N1 (n=85), H1N2 (n=101), H3N2 (n=61), H3N1 (n=1), and mixed subtype (n=11).

Figure 4 breaks down accessions by rRT-PCR subtype for FY2021 through Q2 FY2025. H1N2 was the predominant subtype detected in that time period. It is important to note that there is wide genetic diversity within each subtype.

Figure 5 displays the number of VI attempts in purple, successful VI attempts in orange, and the number of sequenced viral isolates submitted to GenBank in blue.

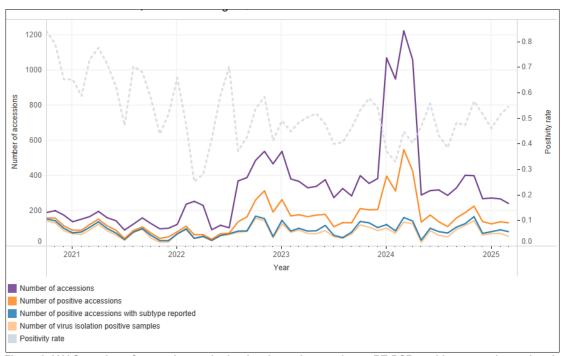


Figure 2. IAV-S number of accessions submitted, subtyped accessions, rRT-PCR positive accessions, virus isolation positive accessions, and positivity rate over time (monthly), FY2021 through Q2 FY2025

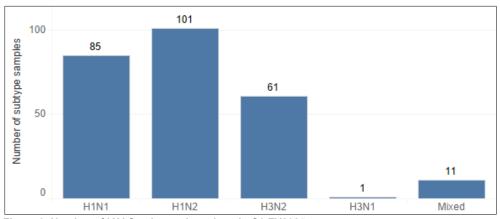


Figure 3. Number of IAV-S subtype detections in Q2 FY2025

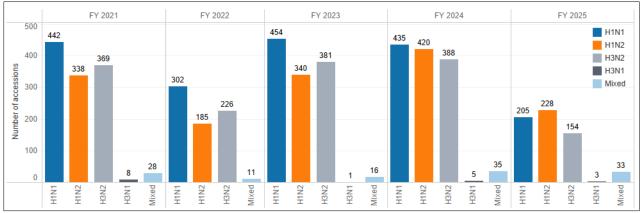


Figure 4. Breakdown of IAV-S accessions by subtype rRT-PCR from FY2021 through Q2 FY2025

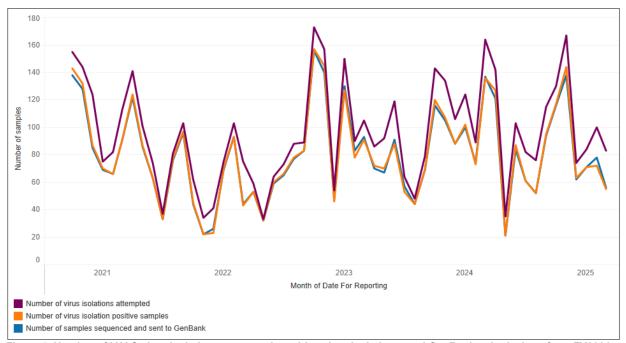


Figure 5. Number of IAV-S virus isolations attempted, positive virus isolations, and GenBank submissions from FY2021 through Q2 FY2025

Lab accessions were evaluated by age-class for the second quarter in FY2025. The most common subtype isolated in the suckling and nursing classes was H1N2. Subtype H1N1 was the most common subtype among the grow/finish class. H3N2 was the most common subtype among sows and boars, though please note the overall low 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 at least 51% or above in successful virus isolation attempts, peaking at 100% for 'Other Specimens' (n=2), with lung tissue providing a strong, more representative 84% success from 191 accessions.

Table 1. Number of positive accessions tested for IAV-S by age class and viral subtype, Q2 FY2025

Age Class (group)	Number of H1N1	Number of H1N2	Number of H3N1	Number of H3N2	Number of Mixed
Suckling	25	29	1	25	4
Nursery	30	43	0	19	4
Grow/Finish	16	15	0	9	3
Sow/Boar	1	1	0	2	0
Not Recorded/Unknown	12	12	0	6	0

Table 2. Number of positive accessions* tested for IAV-S by specimen type and by viral subtype. Q2 FY2025

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	191	84%	65	74	0	42	10	165
Nasal Swab or Wipe	51	51%	16	19	1	14	1	29
Oral Fluids	13	69%	2	7	0	4	0	9
Other Specimens	2	100%	1	0	0	1	0	2

^{*}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 April 2024 through March 2025, 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. Region 2 contains the most diversity of all the regions.

Figure 7 shows the distribution of rRT-PCR subtyped accessions across the five regions for Q2 FY2023 through Q2 FY2025. Over the last 8 quarters, H1N1 was the predominant subtype in Regions 1 and 3; H1N2 was the predominant subtype in Regions 2 and 4; H3N2 was the most predominant in Region 5, though please note the overall low accessions (n=20).

Table 3. Summary of predominant IAV-S HA/NA* phylo-types by US region for the 1-year window from April 2024 through March 2025

Region	Total Number	Predominant HA/NA subtypes
1	72	H1N2 (H1-1B.2.1 / N2-1998B) (n=13) H1N2 (H1-1B.2.1 / N2-2002B) (n=12) H1N1 (H1-1A.1.1.3 / N1-C.3.2) (n=10)
2+	538	H1N1 (H1-1A.3.3.3-c3 / N1-C.3.2) (n=109) H1N2 (H1-1B.2.1 / N2-1998B) (n=101) H3N2 (H3-2010.1 / N2-2002B) (n=98)
3	71	H1N2 (H1-1B.2.1/ N2-2002B) (n=17) H1N1 (H1-1A.1.1.3 / N1-C.2.1) (n=13) H1N1 (H1-1A.3.3.3-c3 / N1-C.3.2) (n=8)
4	44	H1N1 (H1-1A.3.33-c3 / N1-C.3.2) (n=9) H1N2 (H1-1B.2.1 / N2-1998B) (n=8) H1N1 (H1-1A.3.3.2 / N1-P) (n=4)
5++	2	H1N1 (H1-1A.3.3.2 / N1-P) (n=1) H3N2 (H3-1990.4.i / N2-2002) (n=1)
All	727	H1N1 (H1-1A.3.3.3-c3 / N1-C.3.2) (n=132) H1N2 (H1-1B.2.1 / N2-1998B) (n=124) H3N2 (H3-2010.1 / N2-2002B) (n=120)

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

^{*} Most diversity of all regions

^{**} Low participation

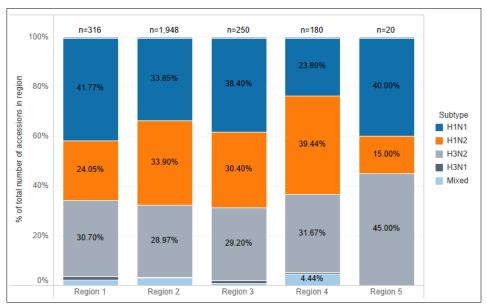


Figure 7. Distribution of IAV-S rRT-PCR subtyped accessions across five US regions for Q2 FY2023 through Q2 FY2025

Phylogenetic analysis of sequences from the IAV-S surveillance system

Phylogenetic analysis of gene sequences of IAV in swine is conducted by ARS 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 200 isolates with published sequences in GenBank was characterized by phylogenetic analysis for the Q2 FY2025 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.¹

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. Region 2 reported the most submissions with Regions 1, 3, and 4 all reporting a similar number of submissions. All were 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 region 5.

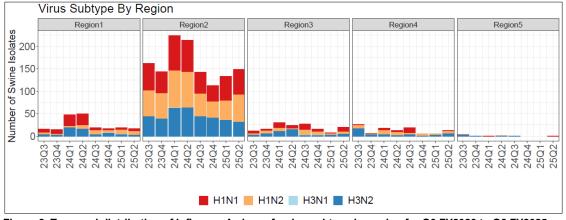


Figure 8. Temporal distribution of Influenza A virus of swine subtype by region for Q3 FY2023 to Q2 FY2025

¹ 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

Due to the US clade classification being too coarse and no longer informative, starting in Q1 of FY2024, the HA genes from H1 subtype viruses are no longer classified using the US phylogenetic clades alpha, beta, gamma, delta1, delta2, or pandemic H1N1 2009 (H1N1pdm09). Similarly, for H3 subtype viruses, US phylogenetic clades 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 are no longer used. Instead, they are classified using global clades as described in these two published nomenclature systems: A Phylogeny-Based Global Nomenclature System (2016) and Swine Influenza A Viruses and the Tangled Relationship with Humans (2021). In Q2 FY2025, there were 6 H1 and 6 H3 clades detected. The predominant H1 HA clades were H1-1B.2.1, H1-1A.3.3.3-c3, and H1-1A.1.1.3, representing 81.8% of all H1 detections (Figure 9). The predominant H3 HA clade was H3-2010.1, representing 78.3% of all H3 detections (Figure 10). There was an increase of: 2.0% in clade H1-1B.2.1, 1.7% in clade H1-1A.3.3.2, and 1.6% in clade H1-1A.3.3.3-c3 over the last year. There was a decrease of: 3.4% in clade H1-1A.3.3.3.-c3, 2.6% in clade H3-2010.1, and 1.3% in clade H3-1990.4.a over the last year.

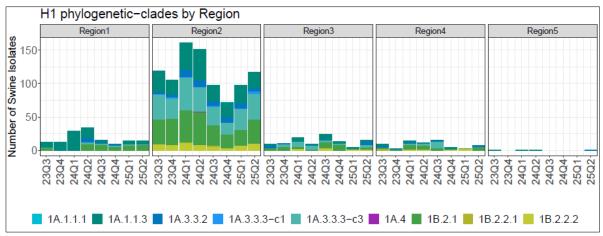


Figure 9. Temporal distribution of IAV-S H1 phylogenetic clades by region for Q3 FY2023 to Q2 FY2025

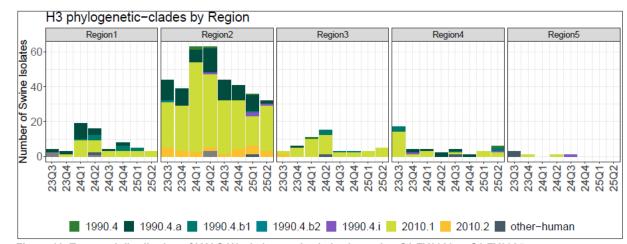


Figure 10. Temporal distribution of IAV-S H3 phylogenetic clades by region Q3 FY2023 to Q2 FY2025

National phylogenetic NA gene information

In Q2 FY2025, N1-C.3.2 and N1-P were the predominant N1 phylogenetic-clades, representing approximately 85.6% of the N1 collection (n=76). In Q2 FY2025, the predominant N2 phylogenetic-clades were N2-2002B and N2-1998B, representing approximately 90.3% of the N2 collection (n=124). Figures 11 and 12 show the temporal distribution of IAV-S N1 and N2 phylogenetic-clades by region for Q3 FY2023 through Q2 FY2025.

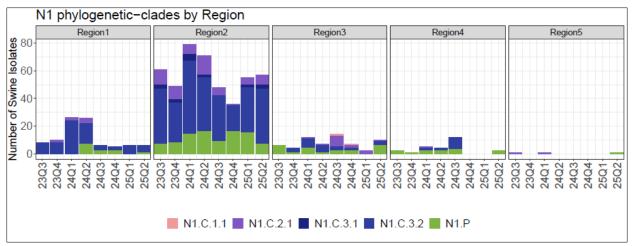


Figure 11. Temporal distribution of IAV-S N1 phylogenetic-clades by region for Q3 FY2023 to Q2 FY2025

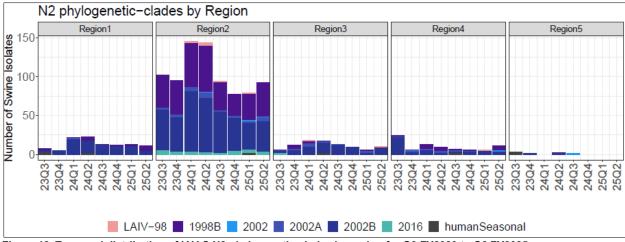


Figure 12. Temporal distribution of IAV-S N2 phylogenetic-clades by region for Q3 FY2023 to Q2 FY2025

Representative HA genes

Six months of IAV-S data, October 2024–March 2025, 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
A/swine/Minnesota/A02862941/2024	PQ584177	H1-1A.1.1.3
A/swine/Iowa/A02862962/2024	PQ584203	H1-1A.3.3.2
A/swine/Iowa/A02979311/2024	PQ851087	H1-1A.3.3.3-c3
A/swine/Iowa/A02863000/2024	PQ604811	H1-1B.2.1
A/swine/Iowa/A02863036/2024	PQ619883	H1-1B.2.2.2
A/swine/Minnesota/A02978922/2024	PQ589161	H3-1990.4.a
A/swine/Kansas/A02862984/2024	PQ604793	H3-2010.1
A/swine/Indiana/A02863053/2024	PQ619907	H3-2010.2

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

Representative NA genes

Six months of IAV-S data, October 2024–March 2025, 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
A/swine/Oklahoma/A02862967/2024	PQ584212	N1-C.2.1
A/swine/Iowa/A02979311/2024	PQ851088	N1-C.3.2
A/swine/Oklahoma/A02858367/2025	PV241391	N1-P
A/swine/Iowa/A02858421/2025	PV354497	N2-1998B
A/swine/Missouri/A02862939/2024	PQ537040	N2-2002A
A/swine/Missouri/A02862999/2024	PQ604810	N2-2002B
A/swine/Ohio/A02862954/2024	PQ584188	N2-2016

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

[•] October 2024 to March 2025 USDA HA data downloaded (n = 298 H1, n = 102 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 87% of observed diversity; 3 H3 selections cover 53% of observed diversity.

[•] Clades were required to have a detection rate of at least 2% to be considered for selection (n >= 8).

[•] Omitted H1-1A.3.3.3-c1 (n=5), H3-1990.4 (n=3), H3-1990.4.b1 (n=6), H3-1990.4.i (n=6) and H3-Other-Human-2020 (n=1)

[•] October 2024 to March 2025 USDA NA data downloaded (n = 155 N1, n = 245 N2) 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 3 N1 selections cover 78% of observed diversity; the 3 N2 selections cover 58% of observed diversity.

[•] Clades were required to have a detection rate of at least 2% to be considered for selection (n >= 8).

[•] Omitted N1-C.3.1 (n=5), N2-2002 (n=7), N2-Human-like (n=1), N2-LAIV-98 (n=3)

Gene constellations

Please note that complete WGS data and associated figures for Q2 FY2025 are not included in this report. This is due to a recent change in data processing timelines which impacted the availability of the most recent data needed for our comprehensive analysis. The comprehensive WGS analysis covering this time period will be provided in subsequent reports.

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 Q2 FY2025, the NVSL Diagnostic Virology Laboratory provided a total of 19 isolates to one pharmaceutical and one government entity. NVSL received 207isolates into the repository. Table 6 reports the total number of virus isolates received into the repository each year from FY2014 through Q2 of FY2025. 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
FY2025 Q2	6 <u>47</u>
FY2024	1,083
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	11,175

Table 7. Total number of subtyped IAV-S isolates collected from 2009- Q2 FY2025 and available

through the NVSL repository

Subtype	Number of Isolates Received in Q2 FY2025	Total Number of Isolates in Repository
H3N2	54	3,615
H3N1	2	31
H1N1	71	4,191
H1N2	80	4,066
Mixed	0	304
TOTAL	207	12,207

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 April 2024 through March 2025 for regions 1-4 and May 2024 through January 2025 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.

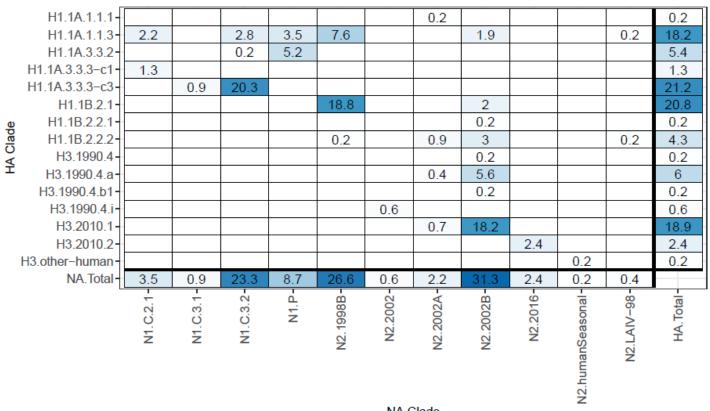


Percentage of HA and NA combinations - Apr 2024 to Mar 2025





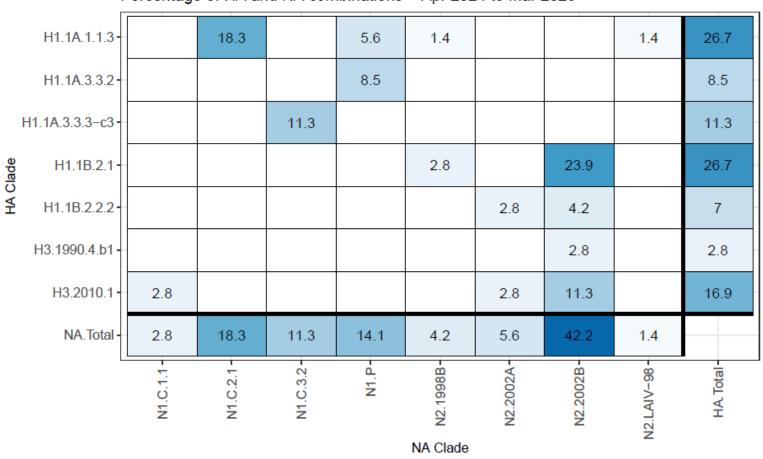
Percentage of HA and NA combinations - Apr 2024 to Mar 2025



NA Clade

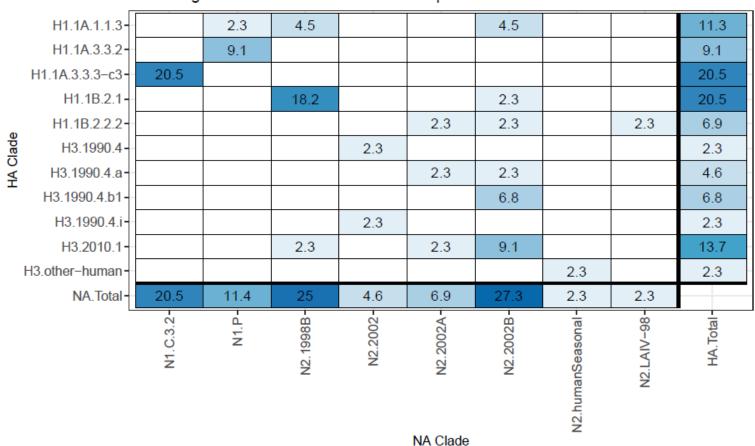


Percentage of HA and NA combinations - Apr 2024 to Mar 2025





Percentage of HA and NA combinations - Apr 2024 to Mar 2025





Percentage of HA and NA combinations - May 2024 to Jan 2025

