Influenza A Virus in Swine Surveillance



United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

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Fiscal Year 2024 Quarterly Report

Surveillance Summary for Third Quarter Fiscal Year 2024: April 1 to June 30, 2024

Report Summary

- This report covers the third quarter (Q3) of fiscal year (FY) 2024, from April 1 to June 30, 2024.
 - There were 4,089 samples submitted for influenza A virus (IAV) surveillance in swine from 1,657 accessions.
 - \circ $\,$ H1N1 was the predominant subtype reported in USDA data for the quarter.
 - Over the past 8 quarters, H1N1 was the predominant subtype in Regions 1-3, H1N2 was the predominant subtype in Region 4 and H3N2 was the predominant subtype in Region 5.
 - The Agricultural Research Service (ARS) characterized 206 isolates with published sequences in GenBank by phylogenetic analysis.
 - The National Veterinary Services Laboratory's (NVSL) Diagnostic Virology Laboratory (DVL) provided 18 isolates to one academic and two pharmaceutical 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 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 October 28, 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 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: <u>https://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/downloads/appendix_c_testing_g_uidelines.pdf</u>
- Instructions:
 <u>https://www-author.aphis.usda.gov/animal_health/animal_dis_spec/swine/downloads/iav-s-algorithm-instructions.pdf</u>

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

 Isolate request: <u>https://www.aphis.usda.gov/animal_health/lab_info_services/downloads/OrderingIAV-</u> 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

In FY2018, 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 entering into the program; however, they also yielded a higher percentage of accessions resulting in a virus isolate that could be sequenced and analyzed.

In Q3 FY2024, 4,089 samples were tested from 1,657 accessions for a total of 14,383 samples from 6,001 accessions collected through Q3 FY2024 (Figure 1). Figure 2 shows the overall trends in rRT-PCR and VI positive accessions and subtyped accessions.

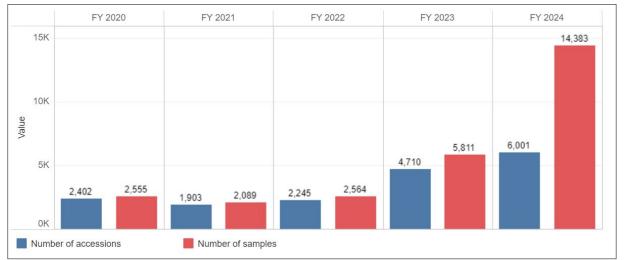


Figure 1. Number of IAV-S laboratory accessions and samples tested in swine FY2020 through Q3 FY2024

Figure 3 shows the number and distribution of subtype detections in Q3 FY2024. A total of 276 samples were subtyped, including H1N1 (n=103), H1N2 (n=81), H3N2 (n=77), H3N1 (n=2), and mixed subtype (n=13).

Figure 4 breaks down accessions by rRT-PCR subtype for FY2020 through Q3 FY2024. H1N1 has been 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 blue, successful VI attempts in purple, and the number of sequenced viral isolates submitted to <u>GenBank</u> in green.

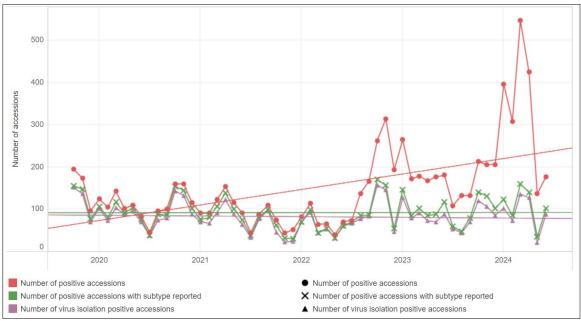
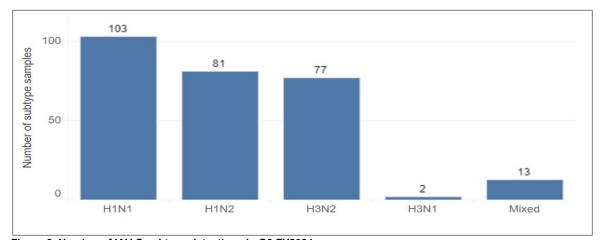
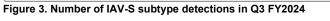


Figure 2. IAV-S subtyped accessions, rRT-PCR positive accessions, and virus isolation positive accessions over time (monthly) with trend lines, FY2020 through Q3 FY2024





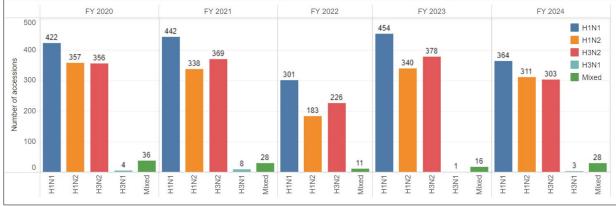


Figure 4. Breakdown of IAV-S accessions by subtype rRT-PCR from FY2020 through Q3 FY2024

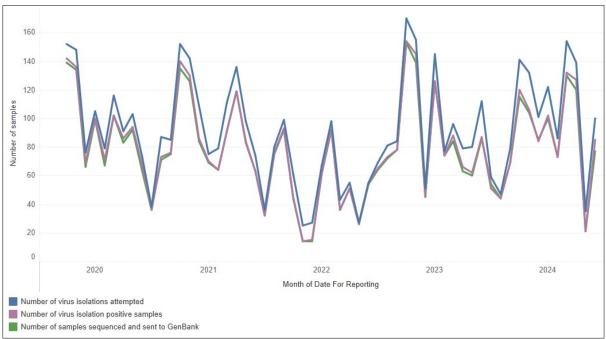


Figure 5. Number of IAV-S virus isolations attempted, positive virus isolations, and GenBank submissions from FY2020 through Q3 FY2024

Laboratory accessions were evaluated by age-class for the third quarter in FY2024. The most common subtype isolated among the nursery class was H1N2. Subtype H1N1 was the most common subtype among the suckling, grow/finish, and unknown classes. There were no positive sow/boar accessions (Table 1). Table 2 displays the number of IAV-S positive accessions by specimen and viral subtype. All sample types yielded above 70% in successful virus isolation attempts, with lung tissue yielding the most success with 89%.

Table 1. Number of positive accessions tested for IAV-S by age class and viral subtype, Q3 FY2024

Age Class (group)	Number of H1N1	Number of H1N2	Number of H3N1	Number of H3N2	Number of Mixed
Suckling	30	25	1	18	4
Nursery	30	34	1	27	3
Grow/Finish	23	13	0	14	3
Sow/Boar	0	0	0	0	0
Not Recorded/Unknown	19	9	0	18	3

Table 2. Number of positive accessions* tested for IAV-S by specimen type and by viral subtype, Q3 FY2024

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	201	89%	82	63	1	47	8	172
Nasal or Nasal Swab	57	77%	17	17	1	21	1	41
Oral Fluids	10	70%	0	1	0	6	3	3
Other Specimens	7	71%	3	0	0	3	1	6

*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 July 2023 through June 2024, 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 Q4 FY2022 through Q3 FY2024. Over the last 8 quarters, H1N1 was the predominant subtype in Regions 1, 2, and 3, H1N2 was the predominant subtype in Region 4 and where the region was unknown, and H3N2 was the predominant subtype in Region 5.

Region	Total Number	Predominant HA/NA subtypes
1	131	H1N1 (H1-1A.1.1.3 / N1-C.3.2) (n=46) H3N2 (H3-2010.1 / N2-2002B) (n=18) H3N2 (H3-1990.4.a / N2-2002B) (n=16)
2+	721	H1N2 (H1-1B.2.1 / N2-1998B) (n=153) H3N2 (H3-2010.1 / N2-2002B) (n=139) H1N1 (H1-1A.3.3.3-c3 / N1-C.3.2) (n=131)
3	97	H3N2 (H3-2010.1 / N2-2002B) (n=20) H1N1 (H1-1A.3.3.3-c3 / N1-C.3.2) (n=18) H1N1 (H1-1A.1.1.3 / N1-C.2.1) (n=10)
4	57	H1N2 (H1-1B.2.1 / N2-1998B) (n=14) H1N1 (H1-1A.3.33-c3 / N1-C.3.2) (n=13) H1N1 (H1-3.3.2 / N1-P) (n=6)
5**	5	H3N2 (H3-2010.1 / N2-2002B) (n=2) H1N1 (H1-1A.1.1.3 / N1-C.2.1) (n=1) H1N2 (H3-1A.1.1.3 / N2-1998B) (n=1)
All	1,011	H1N2 (H1-1B.2.1 / N2-1998B) (n=182) H3N2 (H3-2010.1 / N2-2002B) (n=182) H1N1 (H1-1A.3.3.3-c3 / N1-C.3.2) (n=167)

Table 3. Summary of predominant IAV-S HA/NA* phylo-types by US region for the 1-year window from July 2023 through June 2024

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

* Most diversity of all regions

++ Low participation

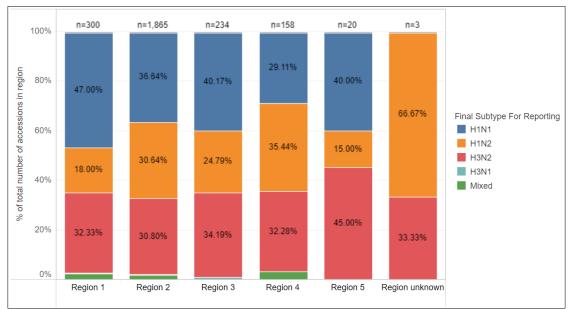


Figure 7. Distribution of IAV-S rRT-PCR subtyped accessions across five US regions for Q4 FY2022 through Q3 FY2024

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 206 isolates with published sequences in GenBank was characterized by phylogenetic analysis for the Q3 FY2024 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 reported 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.

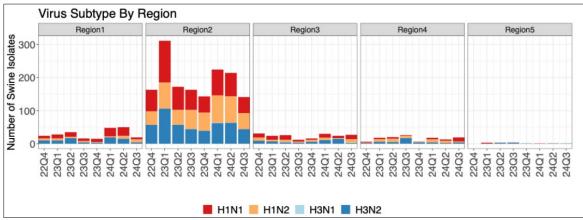


Figure 8. Temporal distribution of Influenza A virus of swine subtype by region for Q4 FY2022 to Q3 FY2024

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 longer be 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 be used. Instead, they are classified using global clades as described in these two published nomenclature systems: <u>A Phylogeny-Based Global Nomenclature</u> System (2016) and Swine Influenza A Viruses and the Tangled Relationship with Humans (2021). In Q3 FY2024, there were 7 H1 and 6 H3 clades detected. The predominant H1 HA genes were H1-1B.2.1, H1-1A.3.3.3-c3, and H1-1A.1.1.3, representing 61.7% of all HA detections (Figure 9). The predominant H3 HA genes were H3-2010.1 and the H3-1990.4.a, representing 24.8% of all HA detections (Figure 10). There was an increase of: 5.3% in clade H1-1A.1.1.3, 3.9% in clade H1-1B.2.1, and 1.9% in clade H3-2010.1 over the last year. There was a decrease of: 4% in clade H1-1A.3.3.3-c3 and 3.9% in clade H3-1990.4.a over the last year.

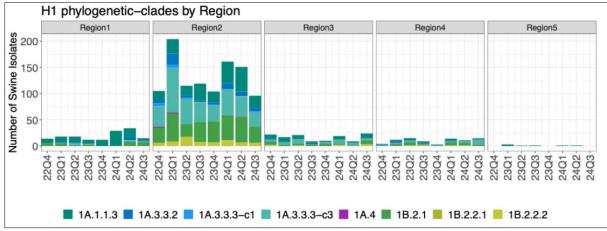


Figure 9. Temporal distribution of IAV-S H1 phylogenetic clades by region for Q4 FY2022 to Q3 FY2024

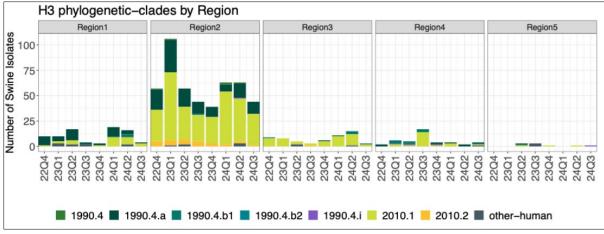


Figure 10. Temporal distribution of IAV-S H3 phylogenetic clades by region Q4 FY2022 to Q3 FY2024

National phylogenetic NA gene information

In Q3 FY2024, N1-C.3.2 was the predominant N1 phylogenetic-clade (Figure 11), representing approximately 61.3% of the Q2 FY2024 N1 collection (n=80). In Q3 FY2024, the predominant N2 phylogenetic-clade was N2-2002B (Figure 12), representing approximately 61.9% of the Q3 FY2024 N2 collection (n=126).

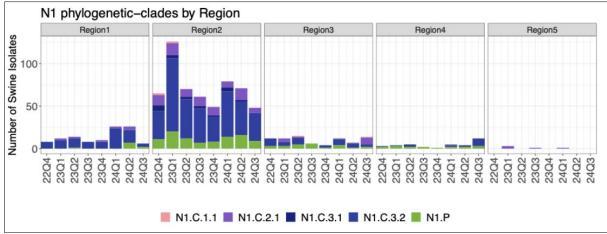


Figure 11. Temporal distribution of IAV-S N1 phylogenetic-clades by region for Q4 FY2022 to Q3 FY2024

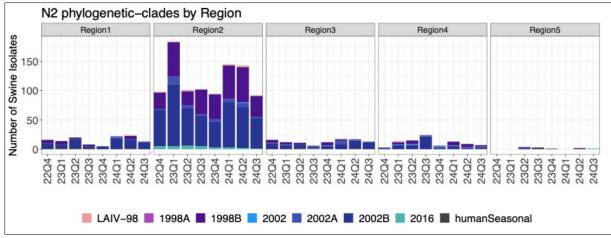


Figure 12. Temporal distribution of IAV-S N2 phylogenetic-clades by region for Q4 FY2022 to Q3 FY2024

Representative HA genes

Six months of IAV-S data, January 2024–June 2024, 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/Iowa/A02862338/2024	PP525113	H1-1A.1.1.3
A/swine/Indiana/A02862346/2024	PP525127	H1-1A.3.3.2
A/swine/South_Dakota/A02862203/2024	PP203192	H1-1A.3.3.3-c3
A/swine/Iowa/A02862379/2024	PP620663	H1-1B.2.1
A/swine/Indiana/A02862262/2024	PP379601	H1-1B.2.2.2
A/swine/Iowa/A02862437/2024	PP657398	H3-1990.4.a
A/swine/Iowa/A02862299/2024	PP467088	H3-2010.1

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

January 2024 to June 2024 USDA HA data downloaded (n = 358 H1, n = 154 H3) and a phylogenetic tree was inferred. For each HA clade, an objective representative selection was made using PARNAS (<u>https://github.com/flu-crew/parnas</u>)

• The 5 H1 selections cover 88% of observed diversity; 2 H3 selections cover 60% of observed diversity.

• Clades were required to have a detection rate of at least 2% to be considered for selection (n >= 11).

Omitted H1-1A.3.3.3-c1 (n=3), H1-1A.4 (n=1), H1-1B.2.2.1 (n=1), H3-1990.4 (n=1), H3-1990.4.b1 (n=8), H3-1990.4.i (n=2), H3-2010.2 (n=3), H3-Other-Human-2020 (n=7)

Representative NA genes

Six months of IAV-S data, January 2024–June 2024, 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/A02862679/2024	PQ083505	N1-C.2.1
A/swine/Iowa/A02862202/2024	PP346622	N1-C.3.2
A/swine/North_Carolina/A02978811/2024	PP580013	N1-P
A/swine/Iowa/A02862662/2024	PQ083483	N2-1998B
A/swine/Iowa/A02862265/2024	PP379606	N2-2002A
A/swine/Iowa/A02862294/2024	PP467081	N2-2002B

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

January 2024 to June 2024 USDA NA data downloaded (n = 189 N1, n = 322 N2) and a phylogenetic tree was inferred. For each HA clade, an objective representative selection was made using PARNAS (<u>https://github.com/flu-crew/parnas</u>).

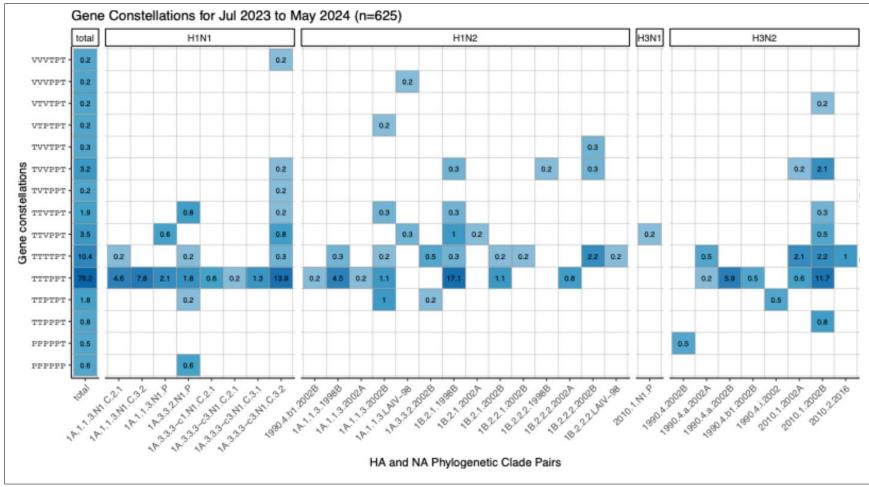
• The 3 N1 selections cover 80% of observed diversity; the 3 N2 selections cover ~65% of observed diversity.

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

• Omitted N1-C.1.1 (n=1), N1-C.3.1 (n=2), N2-2002 (n=2), N2-2016 (n=3), N2-Human-like (n=3), N2-LAIV-98 (n=4)

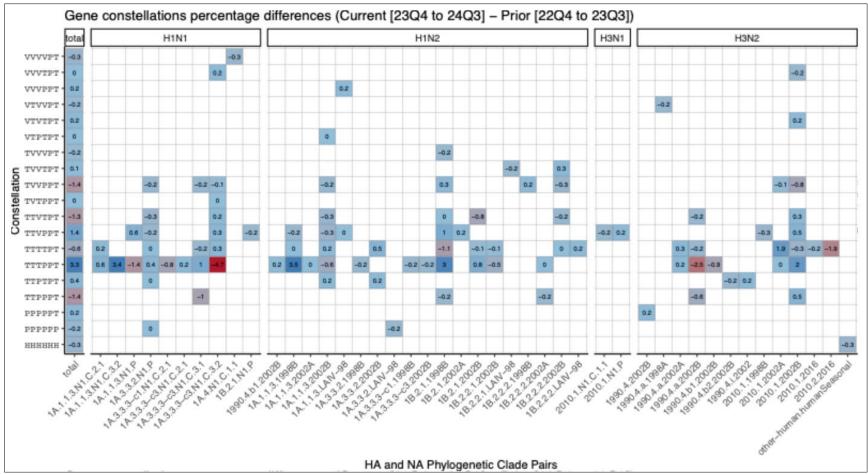
Gene constellations

The most dominant internal gene constellations for July 2023 to May 2024 were TTTPPT (76.2%), TTTTPT (10.4%), and TTVPPT (3.5%). Out of 625 strains with completed whole genome sequencing that were analyzed, 36.6% were H1N1, 33.6% were H1N2, 29.6% were H3N2, and 0.2% were H3N1with 15 unique gene constellations and 31 unique HA/NA pairs (Figure 13). Of observed constellations, 9.8% 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, Q4 FY2023–Q3 FY2024, against the prior timeframe of interest, Q4 FY2022–Q3 FY2023.



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 July 2023 to May 2024



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 [Q4 FY2023 to Q3 FY2024] - Prior [Q4 FY2022 to Q3 FY2023])

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 Q3 FY2024, the NVSL Diagnostic Virology Laboratory provided a total of 18 isolates to two pharmaceutical and one academic entity. NVSL received 201 isolates into the repository in Q3 FY2024. Table 6 reports the total number of virus isolates received into the repository each year from FY2014 through Q3 of FY2024. 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: <u>https://www.aphis.usda.gov/animal_health/lab_info_services/downloads/OrderingIAV-</u> <u>SRepositoryIsolates.pdf</u>

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

Fiscal Year	Number of
	Isolates
FY2024 (Q3)	767
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	10,212

Table 7. Total number of subtyped IAV-S isolates collected from 2009- Q3 FY2024 and available through the NVSL repository

Subtype	Number of Isolates
H3N2	3,397
H3N1	27
H1N1	4,306
H1N2	3,799
Mixed	305
TOTAL	11,834

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 <u>GenBank</u> 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.

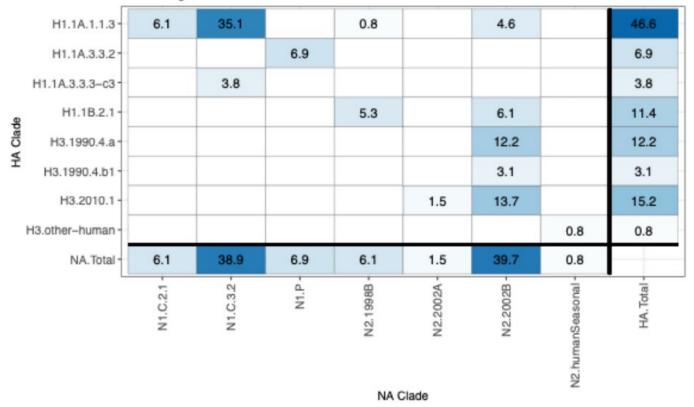
Appendix 1. Regional Charts of IAV-S HA and NA Combinations by Percentage

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 July 2023 through June 2024 for regions 1 through 4 and July 2023 through May 2024 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.



Region 1

Percentage of HA and NA combinations - Jul 2023 to Jun 2024

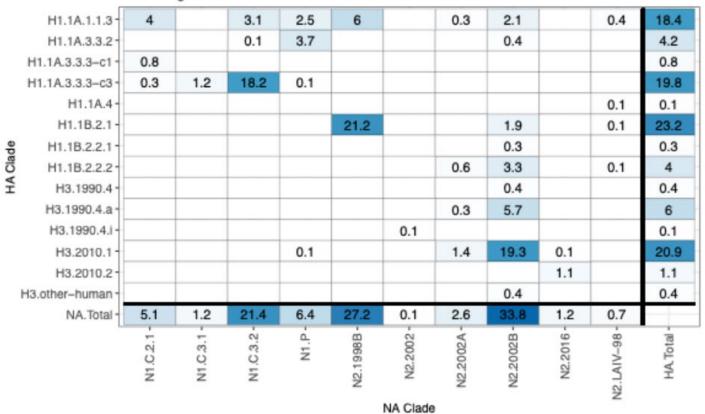


Total HA & NA combinations – 131

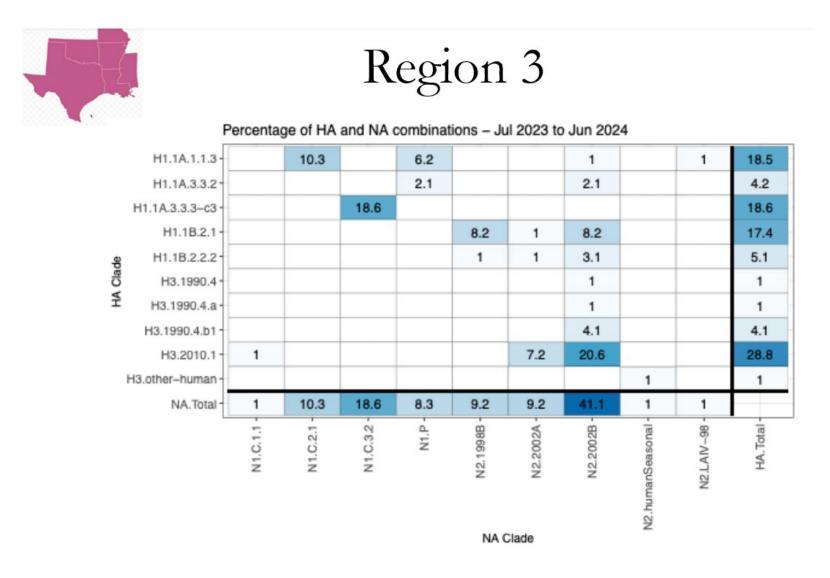


Region 2

Percentage of HA and NA combinations - Jul 2023 to Jun 2024



Total HA & NA combinations - 721

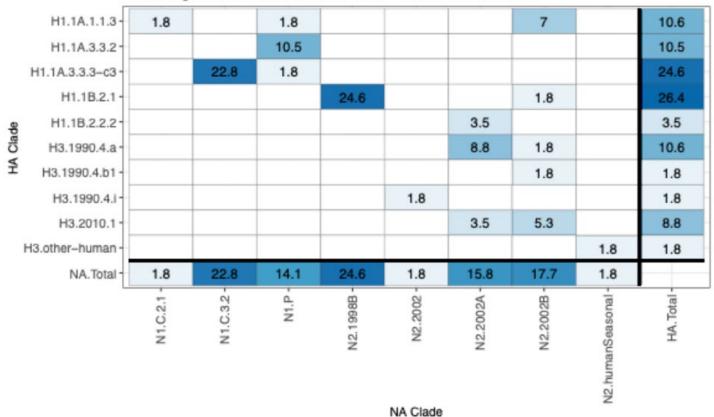


Total HA & NA combinations – 97

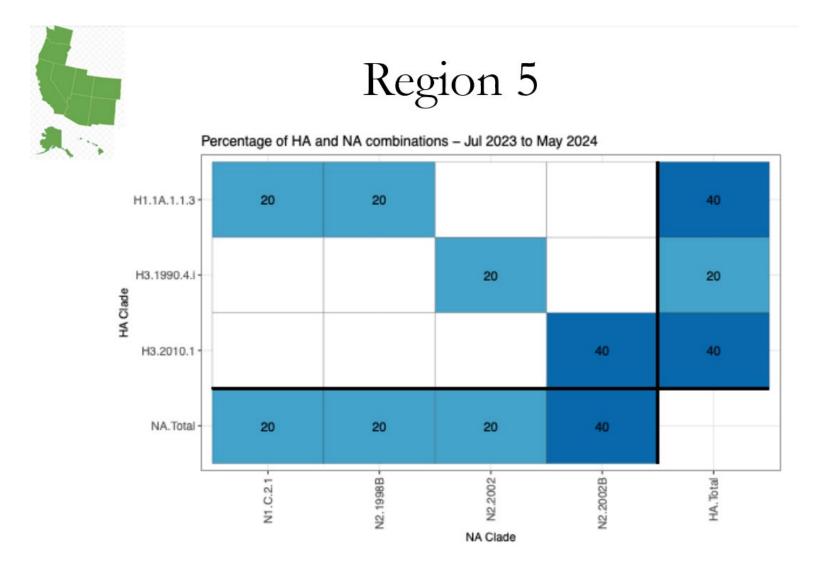


Region 4

Percentage of HA and NA combinations - Jul 2023 to Jun 2024



Total HA & NA combinations - 57



Total HA & NA combinations - 5