Influenza A Virus in Swine Surveillance Quarterly Report for Fiscal Year 2023, Quarter 3



United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

December 2023 Influenza A Virus in Swine Surveillance

Fiscal Year 2023 Quarterly Report

Surveillance Summary for Third Quarter Fiscal Year 2023: April 1 to June 30, 2023

Report Summary¹

- This report covers the third quarter (Q3) of fiscal year (FY) 2023, from April 1 to June 30, 2023.
- In Q3 of FY2023, there were 3,886 samples submitted for influenza A virus (IAV) surveillance in swine from 3,045 accessions.
- H1N1 was the predominant subtype reported in USDA data in Q3 FY2023.
- Over the past 8 quarters, H1N1 was the predominant subtype in all regions.
- The Agricultural Research Service (ARS) characterized 219 isolates with published sequences in GenBank by phylogenetic analysis for the Q3 FY2023 report.
- In Q3 FY2023, the National Veterinary Services Laboratory's (NVSL) Diagnostic Virology Laboratory (DVL) provided 11 total isolates to one governmental and one pharmaceutical entity.

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 November 28, 2023, 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

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.

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 <u>GenBank</u>, 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 <u>Genbank</u>. 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 <u>GenBank</u>.

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_testin_ g_guidelines.pdf
- 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-SRepositoryIsolates.pdf</u>

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, through the end of Q3, a total of 4,740 samples were tested from 3,828 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 FY2019 through Q3 FY2023

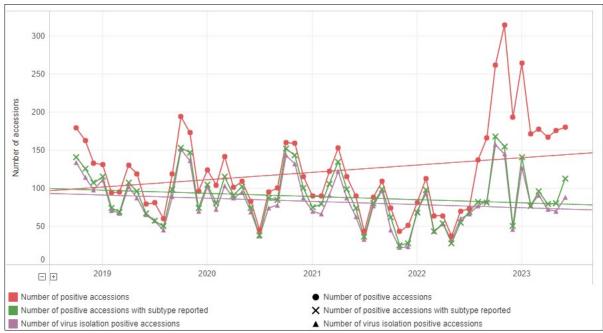


Figure 2. IAV-S subtyped accessions, rRT-PCR positive accessions, and virus isolation positive accessions over time with trend lines, FY2019 through Q3 FY2023

Figure 3 shows the number and distribution of subtype detections in Q3 FY2023. A total of 206 samples were subtyped, including H1N1 (n=105), H1N2 (n=79), H3N2 (n=73), H3N1 (n=0), and mixed subtype (n=9).

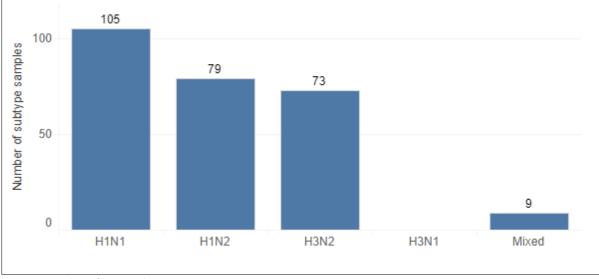


Figure 3. Number of IAV-S subtype detections in Q3 FY2023

Figure 4 breaks down accessions by rRT-PCR subtype for FY2019 through Q3 FY2023. H1N1 has been the predominant subtype detected from 2020 through Q3 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.

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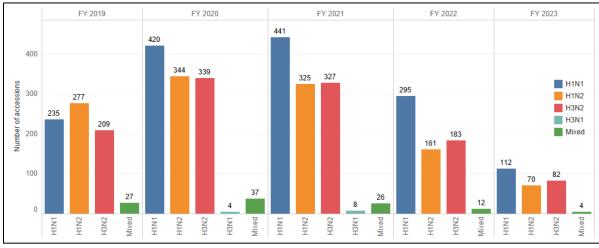


Figure 4. Breakdown of IAV-S accessions by subtype rRT-PCR from FY2019 through Q3 FY2023

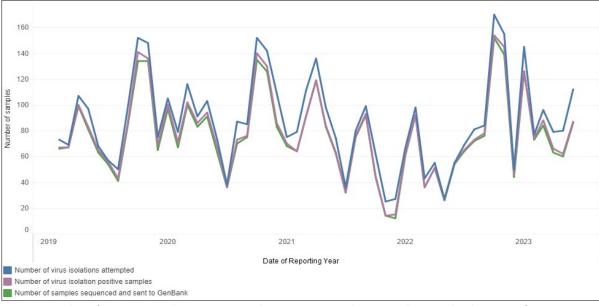


Figure 5. Number of IAV-S virus isolations attempted, positive virus isolations, and GenBank submissions from FY2019 through Q3 FY2023

Laboratory accessions were evaluated by age-class for the third quarter in FY2023. The most common subtype isolated among the suckling, nursery and grow/finish classes was H1N1. Subtype H3N2 was the most common subtype among the sow/boar and the unknown group (Table 1). Table 2 displays the number of IAV-S positive accessions by specimen and viral subtype. All sample types yielded above 75% successful virus isolation and submission to GenBank.

Table 1. Number of positive accessions tested for IAV-S by age class and viral subtype, Q3 FY2023

	Number Numbe		Number of	Number	Number	
Age Class (group)	of H1N1	of H1N2	H3N1	of H3N2	of Mixed	
Suckling	27	22	0	23	1	
Nursery	43	32	0	20	4	
Grow/Finish	30	20	0	15	1	
Sow/Boar	0	0	0	1	0	
Not Recorded/Unknown	5	5	0	14	0	

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	241	79%	98	75	0	62	6	185
Nasal or Nasal Swab	19	78%	5	2	0	8	3	13
Oral Fluids	6	83%	2	2	0	2	0	5
Other Specimens	1	100%	0	0	0	1	0	1

Table 2. Number of positive accessions* tested for IAV-S by specimen type and by viral subtype, Q3 FY2023

*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.

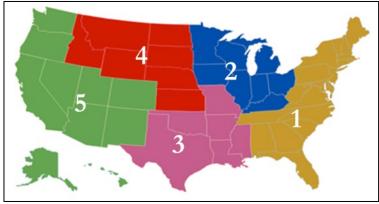


Figure 6. A map of the regions for national IAV-S surveillance

Summary of Regional Data from ARS

Table 3 lists the predominant HA/NA phylo-type pairs by region from July 2022 through June 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.

Region	Total number	Predominant HA/NA subtypes
1	100	H1N1 (H1-Alpha-del / N1-Classical) (n=37) H3N2 (H3-Cluster IV-A / N2-2002B) (n=27) H1N2 (H1-Delta2 / N2-1998B) (n=15)
2+	803	H1N1 (H1-Gamma-c3 / N1-Classical) (n=208) H3N2 (H3-2010.1 / N2-2002B) (n=147) H1N2 (H1-Delta2 / N2-1998B) (n=137)
3	88	H1N1 (H1-Gamma-c3 / N1-Classical) (n=17) H3N2 (H3-2010.1 / N2-2002B) (n=11) H1N1 (H1-Alpha-del / N1-Pandemic) (n=11)
4	68	H3N2 (H3-2010.1 / N2-2002B) (n=15) H1N2 (H1-Delta2 / N2-1998B) (n=11) H1N2 (H1-Alpha-del / N2-2002B) (n=9)
5++	11	H1N1 (H1-Alpha-del / N1-Classical) (n=4) H3N2 (H3-Other-Human / N2-Human-like) (n=3) H3N2 (H3-Cluster IV-A / N2-1998A) (n=1)
All	1,070	H1N1 (H1-Gamma-c3 / N1-Classical) (n=233) H3N2 (H3-2010.1 / N2-2002B (n=181) H1N2 (H1-Delta2 / N2-1998B) (n=173)

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

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

* Most diversity of all regions

** Low participation

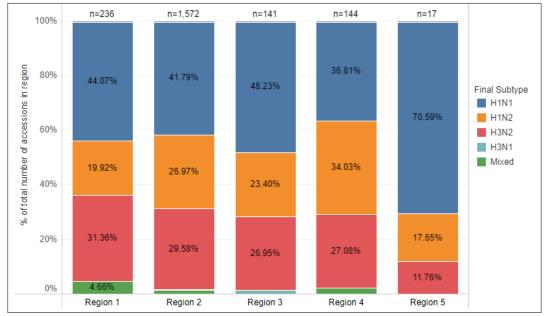


Figure 7. Distribution of rRT-PCR subtyped accessions across the five regions for Q3 FY2021 through Q3 FY2023

Figure 7 shows the distribution of rRT-PCR subtyped accessions across the five regions for Q4 FY2021 through Q3 FY2023. Over the last 8 quarters, H1N1 was the predominant subtype in all regions.

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 219 isolates with published sequences in GenBank was characterized by phylogenetic analysis for the Q3 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 the 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.

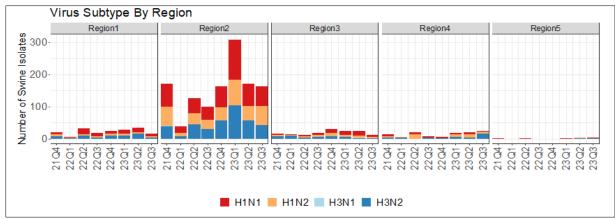


Figure 8. Temporal distribution of Influenza A virus of swine subtype by region for Q4 FY2021 to Q3 FY2023

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 Q3 FY2023, the predominant H1 HA genes were gamma-c3, delta2, and alpha-del (Figure 9), and 2010.1 and IV-A were the predominant H3 HA genes (Figure 10).

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.

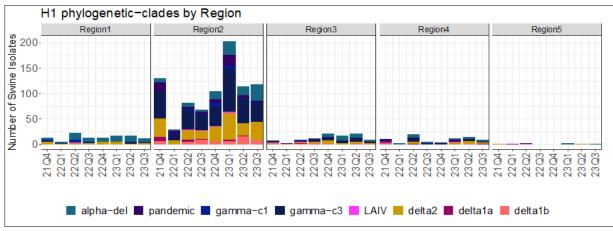


Figure 9. Temporal distribution of IAV-S H1 phylogenetic clades by region for Q4 FY2021 to Q3 FY2023

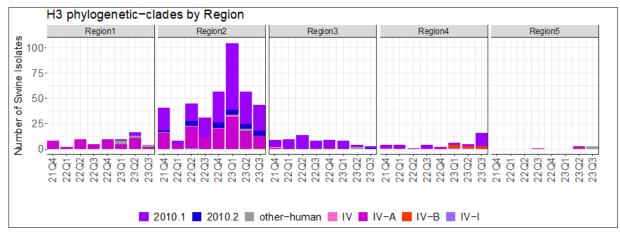


Figure 10. Temporal distribution of IAV-S H3 phylogenetic clades by region for Q4 FY2021 to Q3 FY202

National phylogenetic NA gene information

In Q3 FY2023, N1-Classical was the predominant N1 phylogenetic-clade (Figure 11), representing approximately 80.8% of the Q3 FY2023 N1 collection (n=78).

In Q3 FY2023, the predominant N2 phylogenetic-clade was 2002B-lineage (Figure 12), representing approximately 56.0% of the Q3 FY2023 N2 collection (n=141).

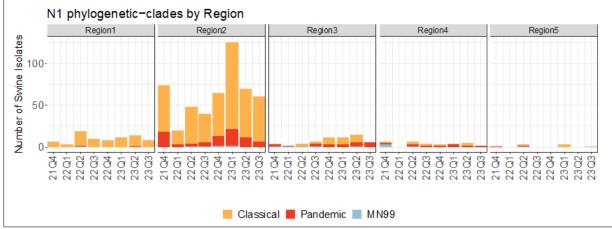


Figure 11. Temporal distribution of IAV-S N1 phylogenetic-clades by region for Q4 FY2021 to Q3 FY2023

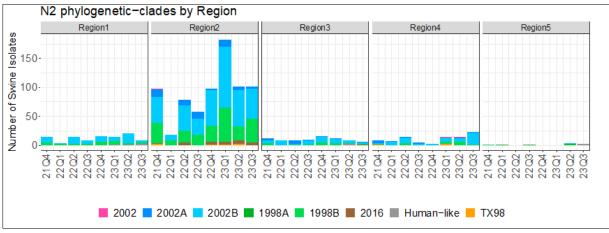


Figure 12. Temporal distribution of IAV-S N2 phylogenetic-clades by region for Q4 FY2021 to Q3 FY2023

Representative HA genes

Six months of IAV-S data, January 2023 - June 2023, were used by the NADC to identify circulating HA clades. For each circulating HA clade, an amino acid alignment for the HA1 was used to generate a majority consensus sequence. NADC used genetic distance to the clade consensus to identify the best-matched field strain housed in the USDA IAV-S virus repository (Table 4).

Strain	HA GenBank	Global Clade	US Clade	
A/swine/Iowa/A02751457/2023	OQ575459	1A.1.1.3	alpha-del	
A/swine/Pennsylvania/A02861308/2023	OQ870345	1A.3.3.2	pandemic	
A/swine/Minnesota/A02861318/2023	OQ859575	1A.3.3.3-c3	gamma-c3	
A/swine/Iowa/A02861395/2023	OQ996081	1B.2.1	delta2	
A/swine/Ohio/A02751535/2023	OQ698870	1B.2.2.2	delta1b	
A/swine/Iowa/A02861264/2023	OQ836500	1990.4.a	IV-A	
A/swine/Ohio/A02861299/2023	OQ836528	2010.1	2010.1	
A/swine/Ohio/A02685047/2023	OR075062	2010.2	2010.2	
A/swine/Missouri/A02524408/2023	OQ469428	Other-Human- 2020	Other-Human	

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

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

January 2023 to June 2023 USDA HA data downloaded (n = 321 H1, n = 158 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; the 4 H3 selections cover ~76% of observed diversity.

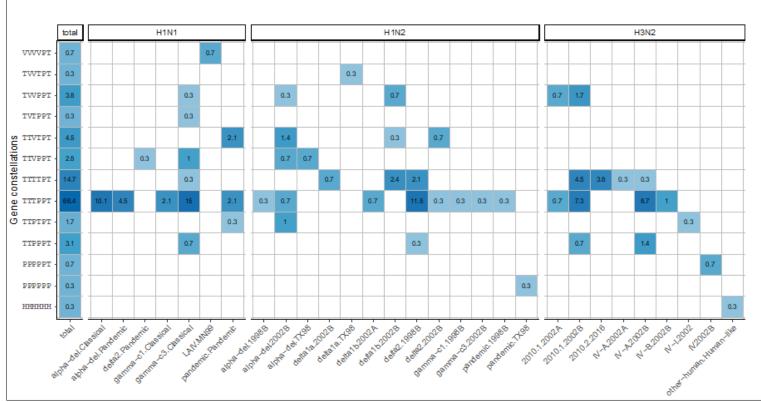
Clades were required to have a detection rate of at least 2% to be considered for selection (n >= 10).

Omitted 1B.2.2.1 (n=4), 1A.3.3.3-c1 (n=4), 1990.4.i (n=1), 1990.4.b1 (n=7), 1990.4.b2 (n=1)

· Other-human represents novel human-to-swine spillovers from the 2022-23 human seasonal H3 flu season.

Gene constellations

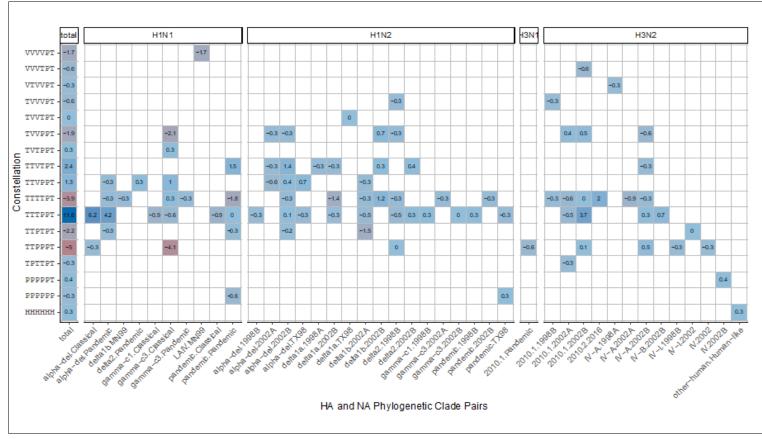
The most dominant internal gene constellations for July 2022 to April 2023 were TTTPPT (66.6%), TTTTPT (14.7%) and TVVPPT (4.6%). Out of 286 strains with completed whole genome sequencing that were analyzed, 40.2% were H1N1, 26.9% were H1N2, and 32.9% were H3N2, with 13 unique gene constellations and 30 unique HA/NA pairs (Figure 13). Of observed constellations, 12.6% had at least one vaccine gene. Figure 14 displays the gene constellation percentage differences from the current timeframe of interest, Q4 FY2022- Q3FY2023, against the prior timeframe of interest, Q4 FY2021 – Q3FY2022.



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 2022 to April 2023



*Incomplete data from June 2022 to March 2023 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 FY2022 to Q3 FY2023] – Prior [Q4 FY2021 to Q3 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 Q3 FY2023, the NVSL Diagnostic Virology Laboratory provided a total of 11 to one governmental and one pharmaceutical entity. NVSL received 323 isolates into the repository in Q3 FY2023. Table 5 reports the total number of virus isolates received into the repository each year from FY2014 through Q3 of FY2023. Table 6 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 5. IAV-S isolates received in NVSL					
repository by fiscal year					

Fiscal Year	Number of isolates
FY2023 (Q1-Q3)	839
FY2022	641
FY2021	1,108
FY2020	1,074
FY2019	1,055
FY2018	994
FY2017	844
FY2016	1,046
FY2015	883
FY2014	765
TOTAL	9,249

Table 6. Total number of subtyped IAV-S isolates collected from 2009- Q3 FY2023 and available through the NVSL repository

Subtype	Number of Isolates
H3N2	3,105
H3N1	25
H1N1	3,953
H1N2	3,485
Mixed	303
TOTAL	10,871

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.

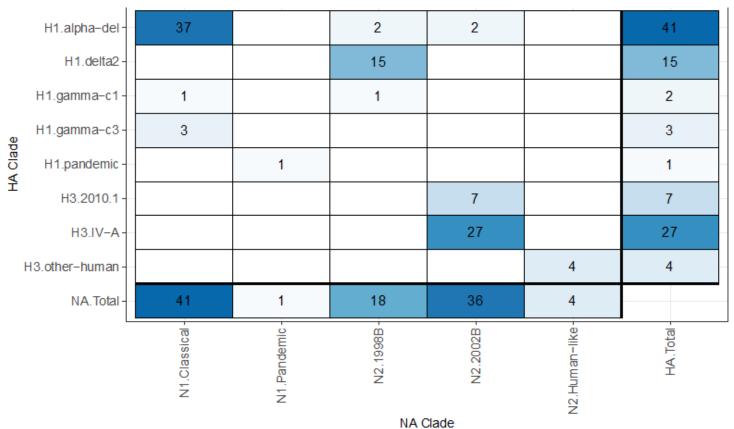
Appendix 1. Regional Charts of IAV-S HA and NA Combinations by Percentage

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 2022 through June 2023 for regions 1 through 4, and November 2022 through May 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.



Region 1

Percentage of HA and NA combinations - Jul 2022 to Jun 2023





Region 2

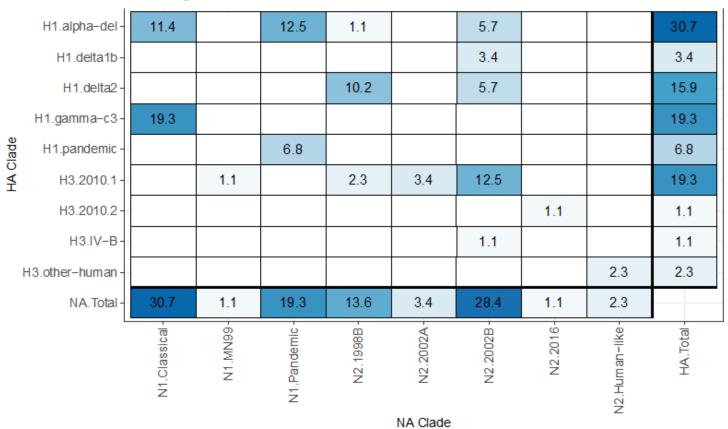
Percentage of HA and NA combinations – Jul 2022 to Jun 2023

	1									
I	H1.alpha-del-	5.2		2.1	1.5	0.4	2.1		0.4	11.7
	H1.delta1a -						0.4		0.1	0.5
	H1.delta1b -					1.2	3.4			4.6
	H1.delta2 -	0.1		0.1	17.1		0.5			17.8
Н	1.gamma-c1 -	1.9								1.9
Н	1.gamma-c3-	25.9					0.1			26
de	H1.LAIV		0.5							0.5
HA Clade	H1.pandemic -	0.1		3.9	0.4		0.1			4.5
¥	H3.2010.1 -					0.9	18.3	0.1		19.3
	H3.2010.2 -							2.5		2.5
	H3.IV -						0.2			0.2
	H3.IV-A-					0.4	9.8			10.2
	H3.IV-B-						0.1			0.1
H3.	other-human -						0.1			0.1
	NA.Total -	33.2	0.5	6.1	19	2.9	35.1	2.6	0.5	
	L	N1.Classical -	N1.MN99-	N1.Pandemic-	N2.1998B-	N2.2002A-	N2.2002B -	N2.2016-	N2.TX98 -	HA. Total -
		NA Clade								



Region 3

Percentage of HA and NA combinations - Jul 2022 to Jun 2023





Region 4

Percentage of HA and NA combinations - Jul 2022 to Jun 2023





Region 5

Percentage of HA and NA combinations - Nov 2022 to May 2023

