USDA
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Veterinary Services

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Influenza A Virus in Swine Surveillance

Fiscal Year 2023 Quarterly Report

Surveillance Summary for Second Quarter Fiscal Year 2023: January 1 to March 31, 2023

Report Summary[1]

- This report covers the second quarter (Q2) of fiscal year (FY) 2023 from January 1 through March 31, 2023.
- In Q2 of FY2023, there were 486 samples submitted for influenza A virus (IAV) surveillance in swine from 442 accessions.
- H1N1 was the predominant subtype reported in USDA data in Q2 FY2023.
- Over the past 8 quarters, H1N1 was the predominant subtype in all regions.
- The Agricultural Research Service (ARS) characterized 203 isolates with published sequences in GenBank by phylogenetic analysis for the Q2 FY2023 report.
- In Q2 FY2023, the NVSL Diagnostic Virology Laboratory (NVSL) provided 75 total isolates to one government entity, three pharmaceutical entities, and two academic institutions, noting that 20 of the 75 isolates were a reshipment due to package thawing.

Key Points

- Where relevant, the report also includes previous years' data for historical perspective.
- 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.
- 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.

Introduction

This report, based on data received into the database as of July 7, 2023, provides a brief update on the status of national surveillance for IAV in swine 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. Reporting months are based on the month the sample was

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[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.

collected. The IAV-S surveillance program is voluntary and, as a result, the accessions and samples submitted represent a subset of the swine population. Submitted samples should only be collected from animals displaying influenza-like illness. When the submitter does not report relevant information, data are recorded as "unknown." Due to its voluntary nature, this 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.

A laboratory accession generally represents a set of samples collected at a single premises on a single day and 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 genomic 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, the public sequence database.

Program Updates

Information on IAV-S and the IAV-S surveillance program, as well as previous IAV-S quarterly reports, are 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_guidelines.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

USDA's National Surveillance Plan for Swine Influenza Virus in Pigs (July 2010) 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. Changes initiated in the program in FY2016 resulted in decreased laboratory accessions and samples, however yielded higher percentage of accessions resulting in a virus isolate that could be sequenced and analyzed. 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.

In FY2023, through the end of Q2, a total of 854 samples were tested from 783 accessions (Figure 1). Figure 2 shows the overall trends in rRT-PCR and VI positive accessions and subtyped accessions.
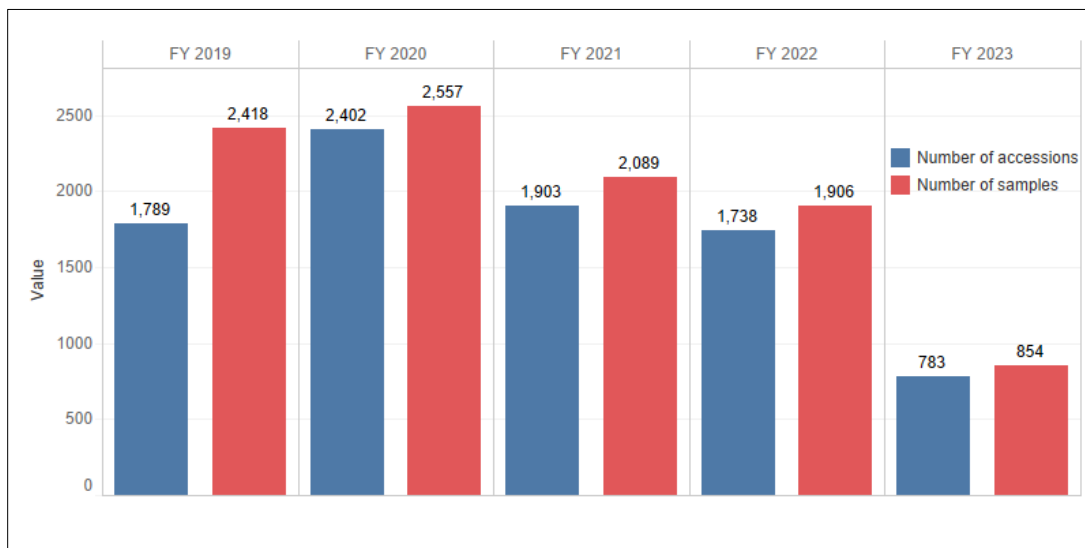

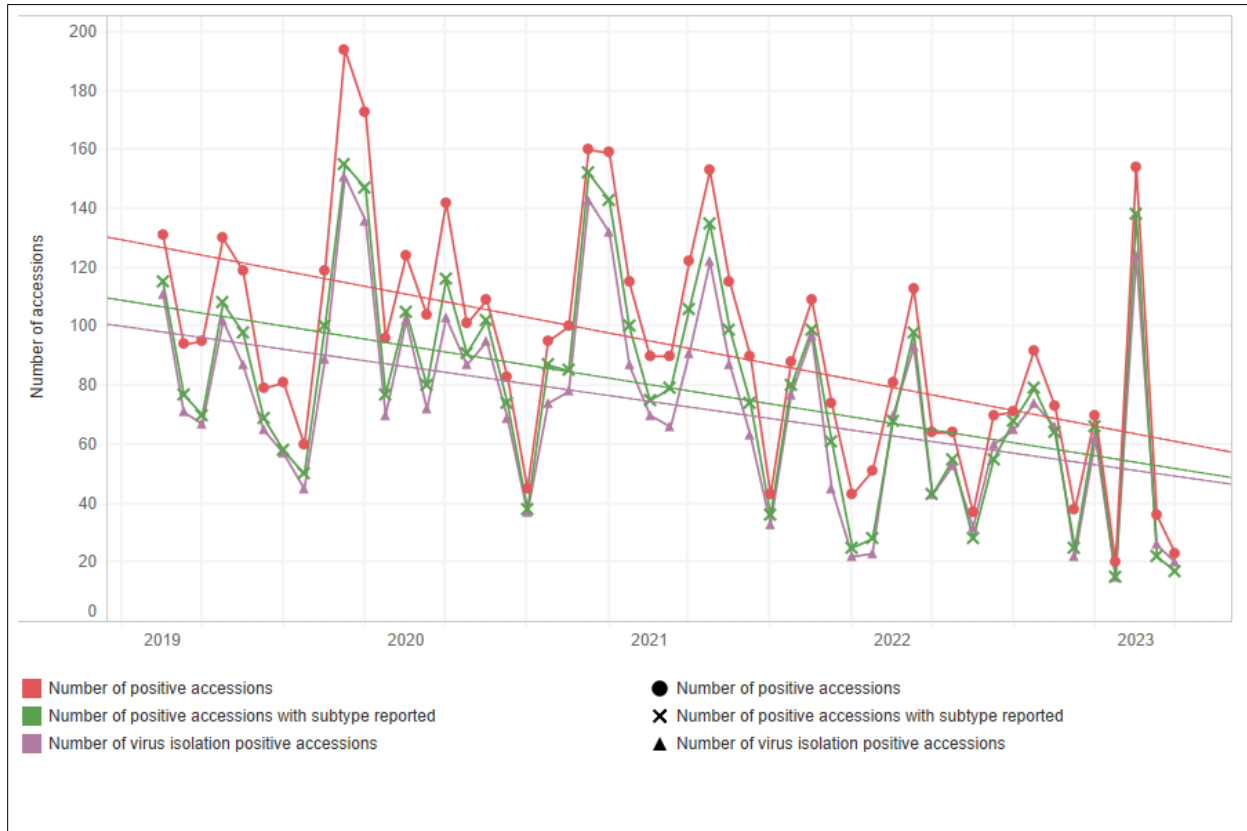**Figure 1. Number of IAV laboratory accessions and samples tested in swine FY2019 through Q2 FY2023**

Figure 2. Subtyped accessions, rRT-PCR positive accessions, and virus isolation positive accessions over time with trend lines for IAV-S, FY2019 through Q2 FY2023

Figure 3 shows the number and distribution of subtype detections in Q2 FY2023. A total of 174 samples were subtyped, including H1N1 (n=68), H1N2 (n=44), H3N2 (n=59), H3N1 (n=1), and mixed subtype (n=2).
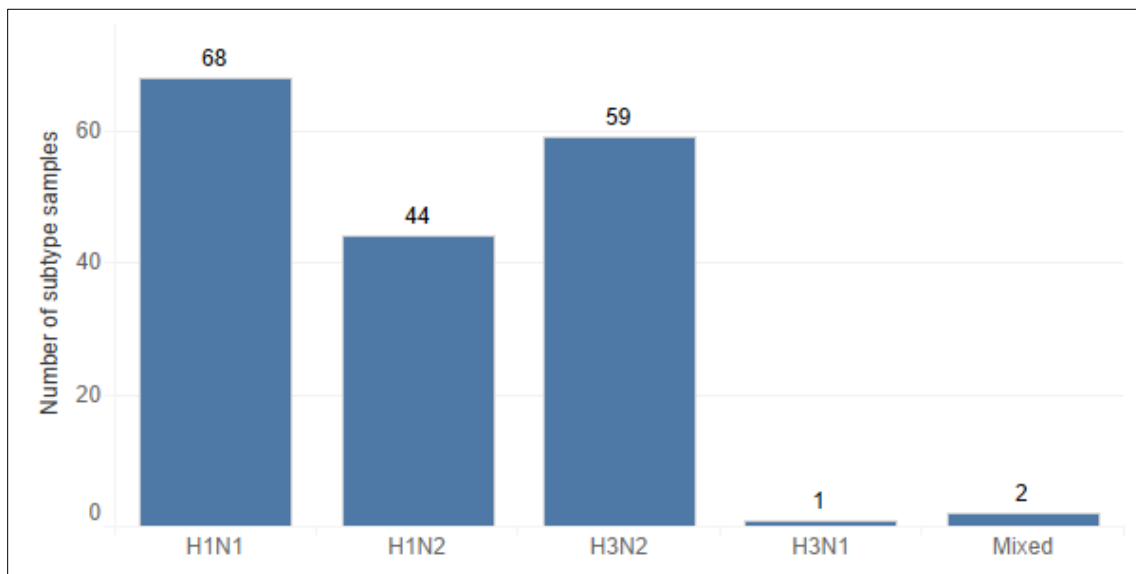

Figure 3. Number of IAV-S subtype detections in Q2 FY2023

Figure 4 breaks down accessions by rRT-PCR subtype for FY2019 through Q2 FY2023. H1N1 was the predominant subtype detected in 2020, 2021 and through Q2 FY2023. H1N2 was detected most often in 2019. It is important to note that there is wide genetic diversity within each subtype.
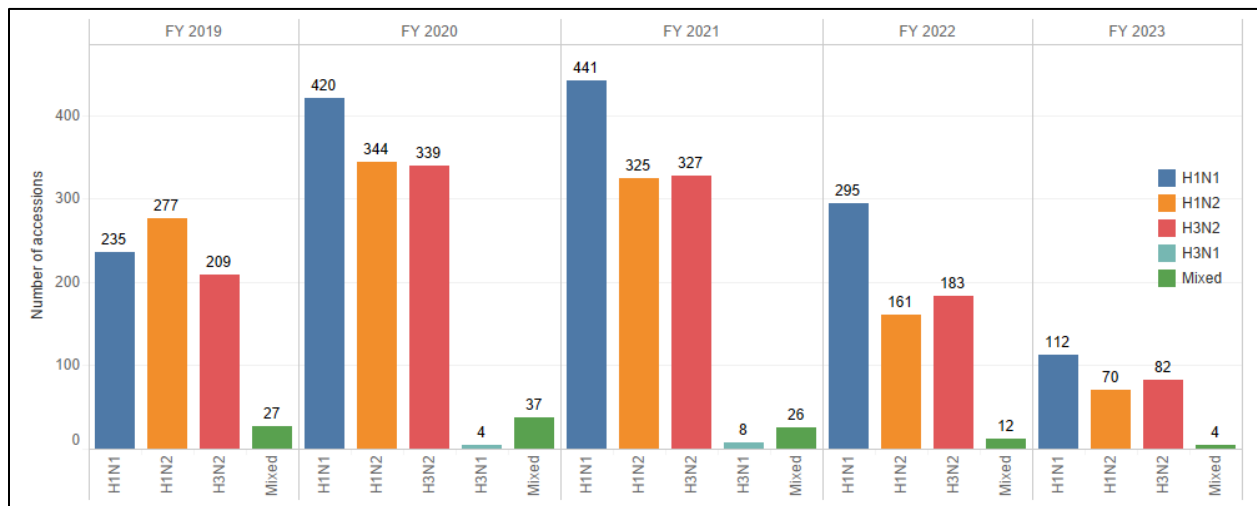
Figure 4. Breakdown of accessions by subtype rRT-PCR from FY2019 through Q2 FY2023

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 GenBank in green. Since the implementation of the June 2016 program modifications, almost all VIs attempted now yield a virus with the sequences submitted to Genbank for analysis.
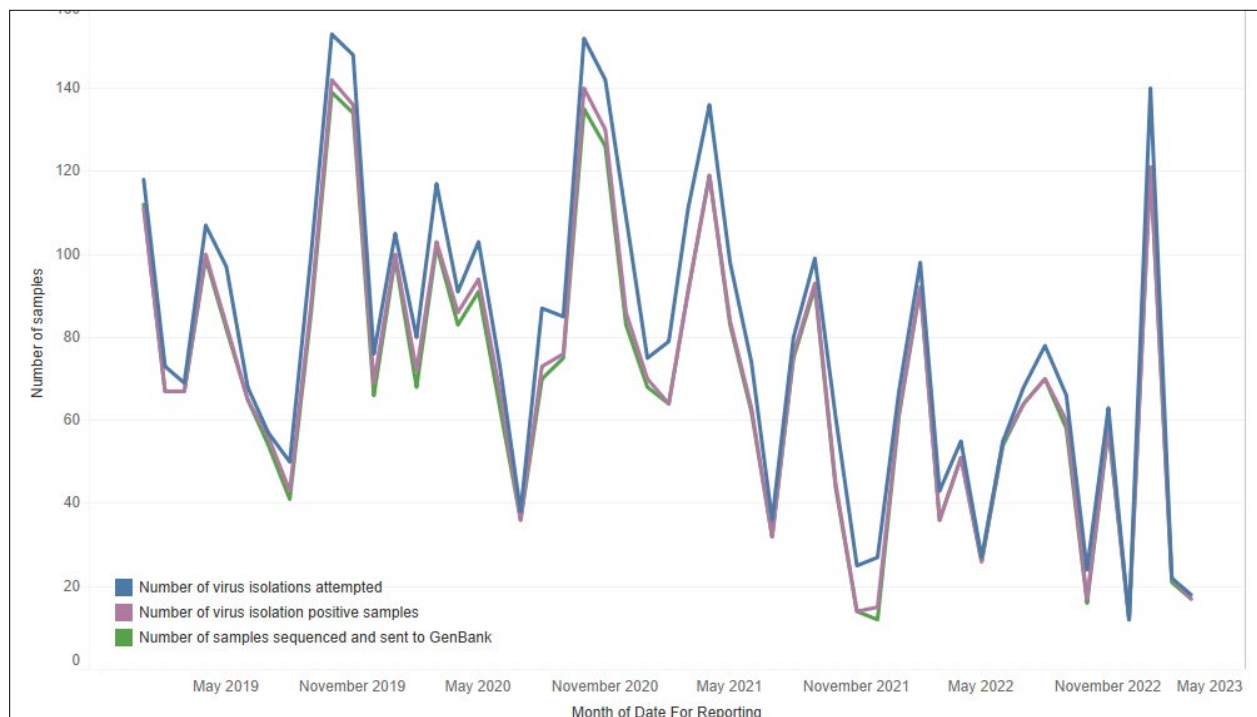

Figure 5. Number of virus isolations attempted, positive virus isolations, and GenBank submissions from FY2019 through Q2 FY2023

Laboratory accessions were evaluated by age-class for the second quarter in FY2023. The most common subtype isolated among the suckling and grow/finish classes was H1N1 and subtype H3N2 was most common among the nursery class and the unknown group (Table 1). After excluding specimen types that comprised less than 10 percent of total sample submissions, samples taken from lung tissue were the most successful at providing positive virus isolation and submission to GenBank (Table 2).

Table 1. Number of positive accessions tested for IAV-S by age class and viral subtype, Q2 FY2023

Age Class (group)	Number of H1N1	Number of H1N2	Number of H3N1	Number of H3N2	Number of Mixed
Suckling	21	13	0	14	1
Nursery	24	17	1	25	0
Grow/Finish	15	10	0	7	0
Sow/Boar	0	0	0	0	0
Not Recorded/Unknown	8	4	0	13	1

Table 2. Number of positive accessions* tested for IAV-S by specimen type and by viral subtype, Q2 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	151	94%	60	40	1	50	0	139
Nasal or Nasal Swab	17	65%	5	3	0	7	2	11
Oral Fluids	6	100%	3	1	0	2	0	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 data across five different regions (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.
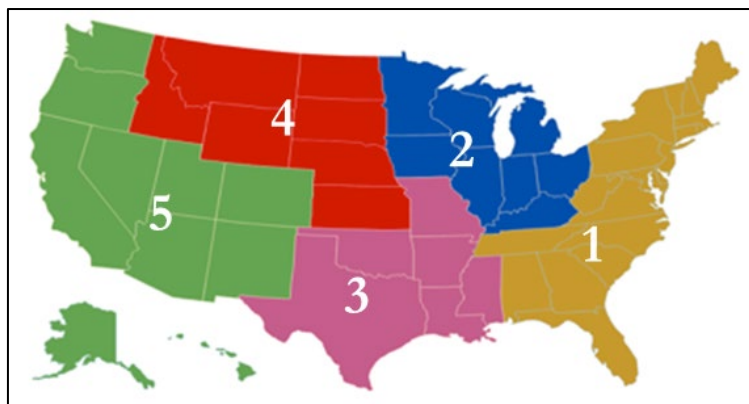

Figure 6. A map of the regions for national IAV-S surveillance

Summary of Regional Data from ARS

Table 3 lists the most predominant HA/NA phylo-type pairs by region from April 2022 - March 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.

Table 3. Summary of predominant HA/NA* phylo-types by region in a 1-year window from April 2022 through March 2023

Region	Total number	Predominant HA/NA subtypes
1	96	H1N1 (H1-Alpha-del / N1-Classical) (n=36)
		H3N2 (H3-Cluster IV-A / N2-2002B) (n=28)
		H1N2 (H1-Delta2 / N2-1998B) (n=14)
2+	700	H1N1 (H1-Gamma-c3 / N1-Classical) (n=191)
		H3N2 (H3-2010.1 / N2-2002B) (n=129)
		H1N2 (H1-Delta2 / N2-1998B) (n=117)
3	83	H1N1 (H1-Gamma-c3 / N1-Classical) (n=18)
		H3N2 (H3-2010.1 / N2-2002B) (n=13)
		H1N2 (H1-Delta2 / N2-1998B) (n=10)
4	49	H1N2 (H1-Delta2 / N2-1998B) (n=9)
		H1N1 (H1-Pandemic / N1-Pandemic) (n=7)
		H1N2 (H1-Alpha-del / N2-2002B) (n=7)
5++	5	H1N1 (H1-Alpha-del / N1-Classical) (n=3)
		H3N2 (H3-Cluster IV-A / N2-1998A) (n=1)
		H3N2 (H3-2010.1 / N2-2002B) (n=1)
All	933	H1N1 (H1-Gamma-c3 / N1-Classical) (n=216)
		H3N2 (H3-2010.1 / N2-2002B (n=155)
		H1N2 (H1-Delta2 / N2-1998B) (n=150)

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

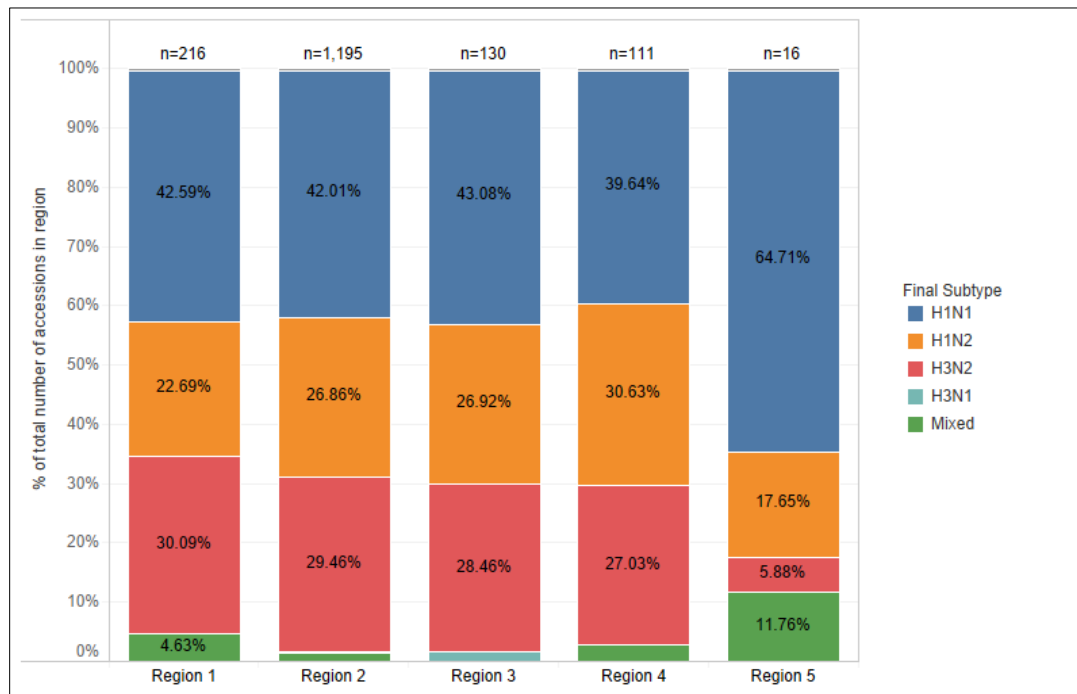* Most diversity of all regions

** Low participation

Figure 7. Distribution of rRT-PCR subtyped accessions across the five regions for Q2 FY2021 through Q2 FY2023

Figure 7 shows the distribution of rRT-PCR subtyped accessions across the five regions for Q2 FY2021 through Q2 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, NA, and Matrix (M) genes of this rapidly changing virus. Through collaboration

with ARS, a dataset[2,3] of 203 isolates with published sequences in GenBank was characterized by phylogenetic analysis for the Q2 FY2023 report. This analysis provides information on the genetic diversity and evolutionary patterns of influenza in swine and allows for inferences about population and/or vaccine immunity.

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 and 2 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 3, 4, and 5.
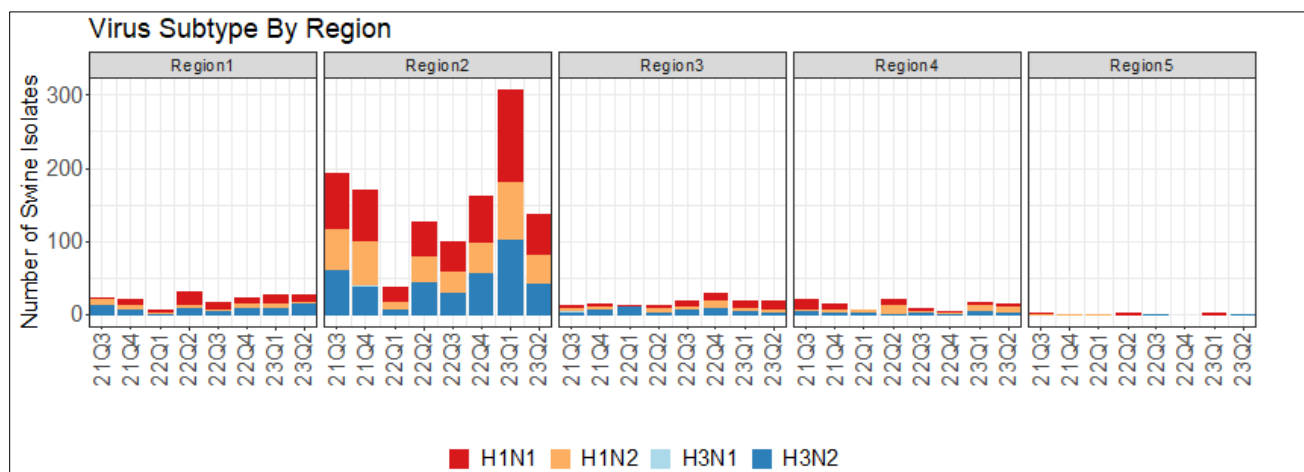

Figure 8. Temporal distribution of Influenza A virus subtype by region for Q3 FY2021 to Q2 FY2023

National phylogenetic HA gene information

HA genes from H1 subtype viruses are classified as alpha, beta, gamma, delta-1, delta-2, or pandemic H1N1 2009 (H1N1pdm09) phylogenetic clades based on a previously published nomenclature system. 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, or human-like. In Q2 FY2023, the predominant H1 HA genes were alpha-del, pandemic, gamma-c3, and delta2 (Figure 9) and 2010.1 and IV-A were the predominant H3 HA genes (Figure 10).

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[2] Participating NAHLN labs included M gene sequencing in their testing until July 2016 because the 2009 H1N1 M gene was the predominant circulating gene.

[3] The ARS dataset is comprised of IAV-S surveillance isolate sequences that were posted in 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.
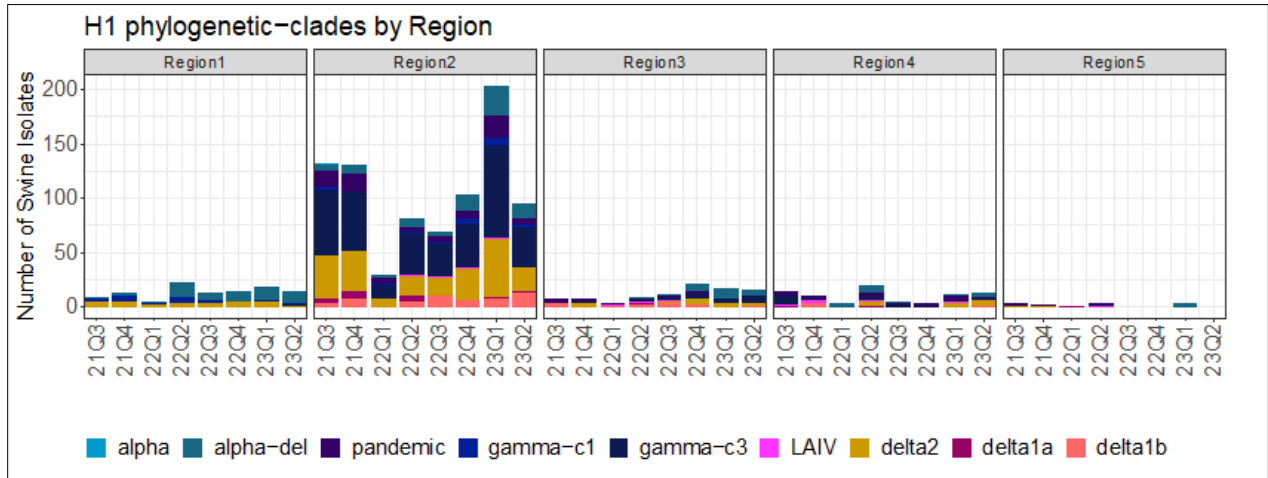
Figure 9. Temporal distribution of H1 phylogenetic clades by region for Q3 FY2021 to Q2 FY2023
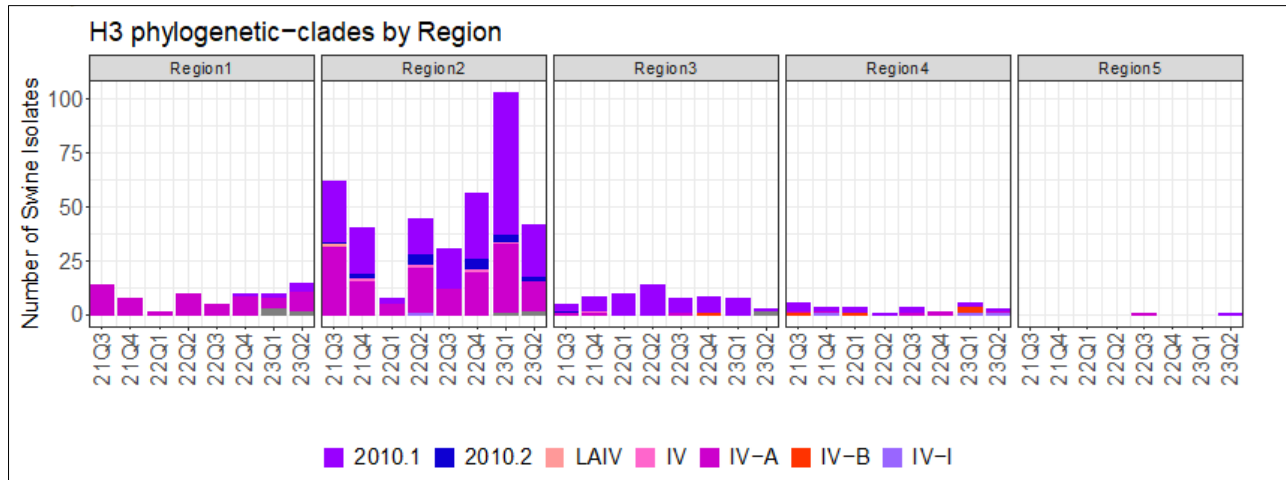

Figure 10. Temporal distribution of H3 phylogenetic clades by region for Q3 FY2021 to Q2 FY2023

National phylogenetic NA gene information

In Q2 FY2023, N1-Classical was the most predominant N1 phylogenetic-clade (Figure 11) and represented approximately 80.5% of the Q2 FY2023 N1 collection total of 82.

In Q2 FY2023, the most predominant N2 phylogenetic-clade was 2002B-lineage (Figure 12) and represented approximately 62.2% of the Q2 FY2023 N2 collection total of 119.
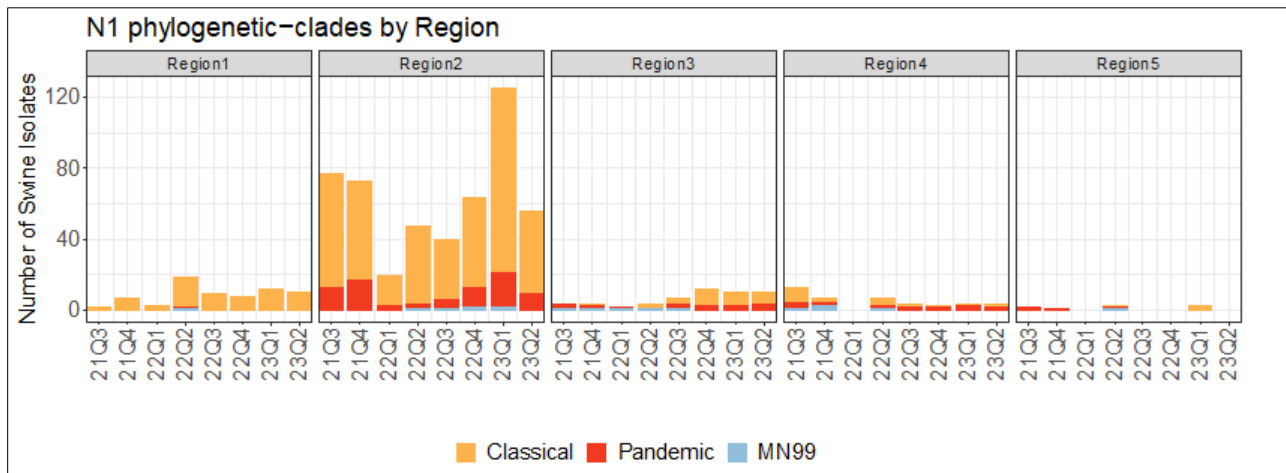

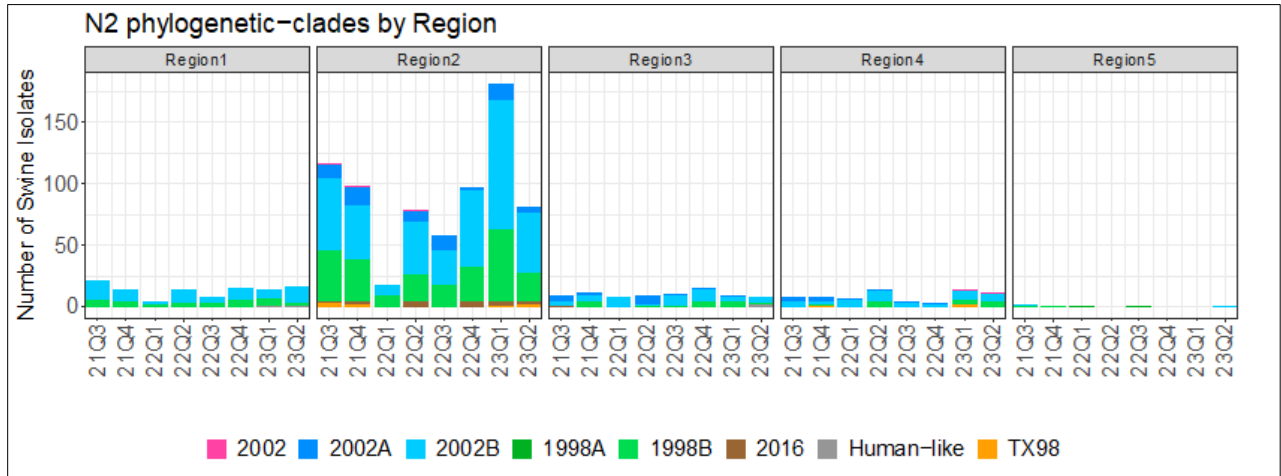**Figure 11. Temporal distribution of N1 phylogenetic-clades by region for Q3 FY2021 to Q2 FY2023**

Figure 12. Temporal distribution of N2 phylogenetic-clades by region for Q3 FY2021 to Q2 FY2023

Representative HA genes

Six months of IAV-S data, October 2022-March 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).

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

Strain	HA GenBank	Global Clade	US Clade
A/swine/Ohio/A02750994/2022	OP903621	1A.1.1.3	alpha-del
A/swine/Iowa/A02751240/2022	OQ079206	1A.3.3.2	pandemic
A/swine/South Dakota/A02751313/2022	OQ151554	1A.3.3.3-c3	gamma-c3
A/swine/Iowa/A02751429/2022	OQ508802	1B.2.1	delta2
A/swine/Ohio/A02751535/2023	OQ698870	1B.2.2.2	delta1b
A/swine/Iowa/A02751181/2022	OP967181	1990.4.a	IV-A
A/swine/Iowa/A02751052/2022	OP951003	2010.1	2010.1

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

- October 2022 to March 2023 USDA HA data downloaded (n = 393 H1, n = 189 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 2 H3 selections cover 71% of observed diversity.
- Clades were required to have a detection rate of at least 2% to be considered for selection (n >= 12).
- Omitted 1B.2.2.1 (n=3), 1A.4 (n=3), 1A.3.3.2-vaccine (n=2), 1A.3.3.3-c1 (n=10), 1990.4.i (n=2), 1990.4.b1 (n=3), 2010.2 (n=5), Other-Human-2020 (n=10), 1990.4 (n=1)

Gene constellations information

The most dominant internal gene constellations for April 2022 to January 2023 year were TTTPPT (62.9%), TTTTPT (18.0%) and TVVPPT (4.5%). Out of 90 strains with completed whole genomic sequencing that were analyzed, 43% were H1N1, 26% were H1N2, and 31% were H3N2, with 11 unique gene constellations and 22 unique HA/NA pairs (Figure 13). Thirteen percent of observed constellations had at least one vaccine gene.
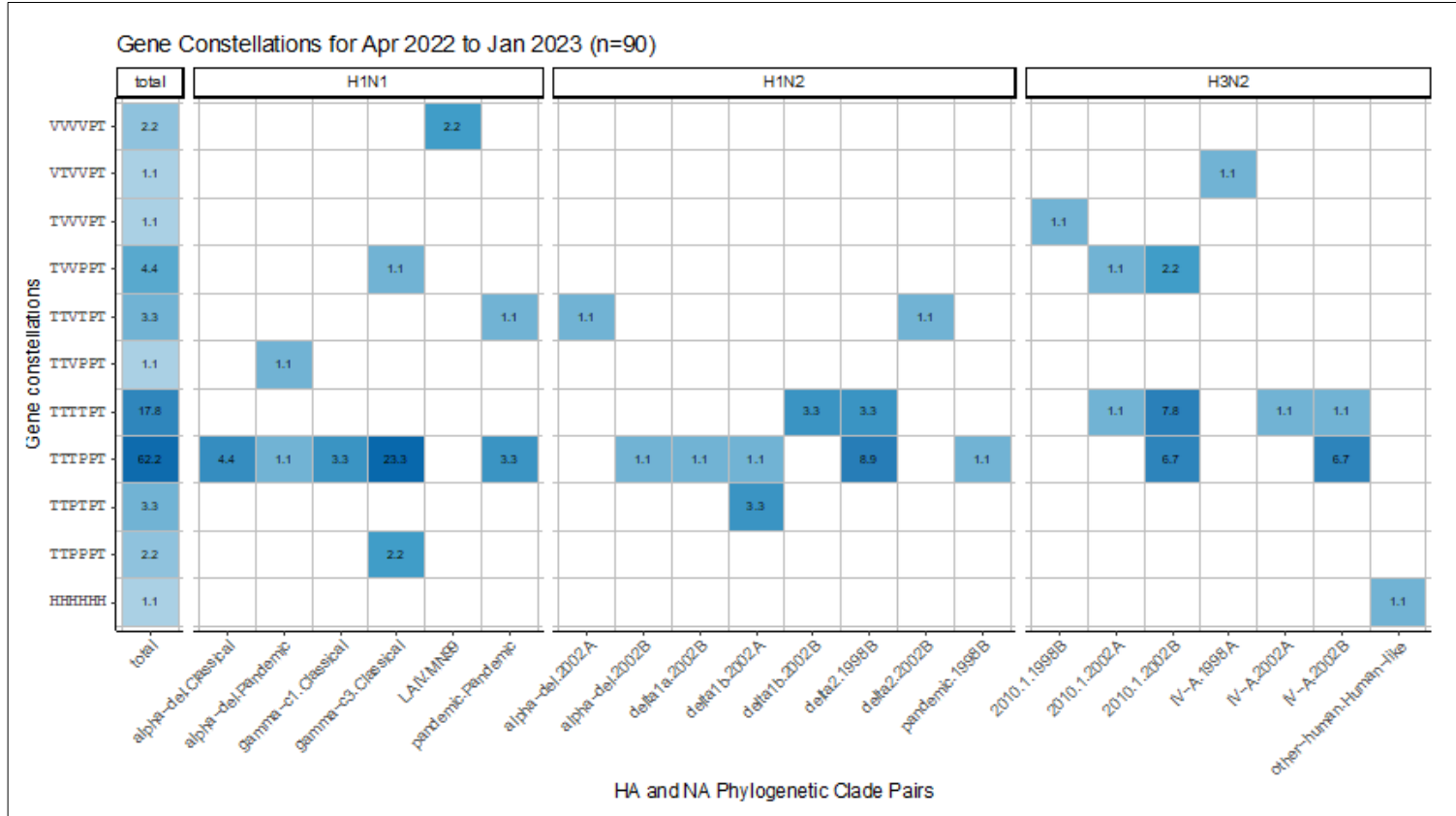
*Incomplete data from June 2022 to March 2023

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 13. Gene constellations by HA and NA phylogenetic clade pairs for April 2022 to January 2023*

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 FY2023, the NVSL Diagnostic Virology Laboratory provided a total of 75 isolates (20 of the 75 were due to a reshipment due to package thawing) to one government entity, three pharmaceutical entities, and two academic institutions. NVSL received 138 isolates into the repository in Q2 FY2023. Table 5 reports the total number of virus isolates received into the repository each year from FY2014 through Q2 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: https://www.aphis.usda.gov/animal_health/lab_info_services/downloads/OrderingIAV-SRepositoryIsolates.pdf

Table 5. Virus isolates received in NVSL repository by year

Fiscal Year	Number of isolates
FY2023	516*
FY2022	641
FY2021	1,108
FY2020	1,074
FY2019	1,055
FY2018	994
FY2017	844
FY2016	1,046
FY2015	883
FY2014	765
TOTAL FY14-Q2FY23	8,926

*Through Q2 FY23

Table 6. Total number of subtyped isolates collected from 2009- Q2 FY2023 and available through the NVSL repository

Subtype	Number of Isolates
H3N2	3,002
H3N1	25
H1N1	3,824
H1N2	3,394
Mixed	303
TOTAL	10,548

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 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 HA and NA Combinations by Percentage

The following charts present the percentages of combinations of HA and NA by region based on ARS-NADC phylogenetic analyses. The results are reported from April 2022 through March 2023 for regions 1 through 4, and June 2022 through March 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 − Apr 2022 to Mar 2023

HA Clade	N1.Classical	N2..1998B	N2.2002B	N2.Human−like	HA.Total
H1.alpha−del	37.5	2.1	1		40.6
H1.delta2		14.6			14.6
H1.gamma−c1	4.2	1			5.2
H1.gamma−c3	1				1
H3.2010.1			7.3		7.3
H3.IV−A			29.2		29.2
H3.other−human				2.1	2.1
NA.Total	42.7	17.7	37.5	2.1	

NA Clade

Total HA & NA combinations − 96

Region 2

Percentage of HA and NA combinations − Apr 2022 to Mar 2023

HA Clade	N1.Classical	N1.MN99	N1.Pandemic	N2.1998B	N2.2002A	N2.2002B	N2.2016	N2.TX98	HA.Total
H1.alpha−del	3.7		1.6	0.9	0.4	1.7		0.3	8.6
H1.delta1a						0.4		0.1	0.5
H1.delta1b					2.4	2.7			5.1
H1.delta2			0.1	16.7		0.4			17.2
H1.gamma−c1	2.1								2.1
H1.gamma−c3	27.3					0.1			27.4
H1.LAIV		0.7							0.7
H1.pandemic	0.1		4.7	0.4		0.1			5.3
H3.2010.1				0.1	1	18.4	0.1		19.6
H3.2010.2							1.4		1.4
H3.IV						0.3			0.3
H3.IV−A				0.1	0.6	10.4			11.1
H3.other−human						0.1			0.1
NA.Total	33.2	0.7	6.4	18.2	4.4	34.6	1.5	0.4	

NA Clade

Total HA & NA combinations − 700

Region 3

Percentage of HA and NA combinations – Apr 2022 to Mar 2023

HA Clade	N1.Classical	N1.MN99	N1.Pandemic	N2.1998B	N2.2002A	N2.2002B	N2.Human-like	HA.Total
H1.alpha-del	10.8		9.6			3.6		24
H1.delta1b					1.2	6		7.2
H1.delta2				12		3.6		15.6
H1.gamma-c3	21.7							21.7
H1.LAIV		1.2						1.2
H1.pandemic			6					6
H3.2010.1				1.2	2.4	15.7		19.3
H3.IV-A						1.2		1.2
H3.IV-B						1.2		1.2
H3.other-human							2.4	2.4
NA.Total	32.5	1.2	15.6	13.2	3.6	31.3	2.4	

NA Clade

Total HA & NA combinations – 83

Region 4

Percentage of HA and NA combinations – Apr 2022 to Mar 2023
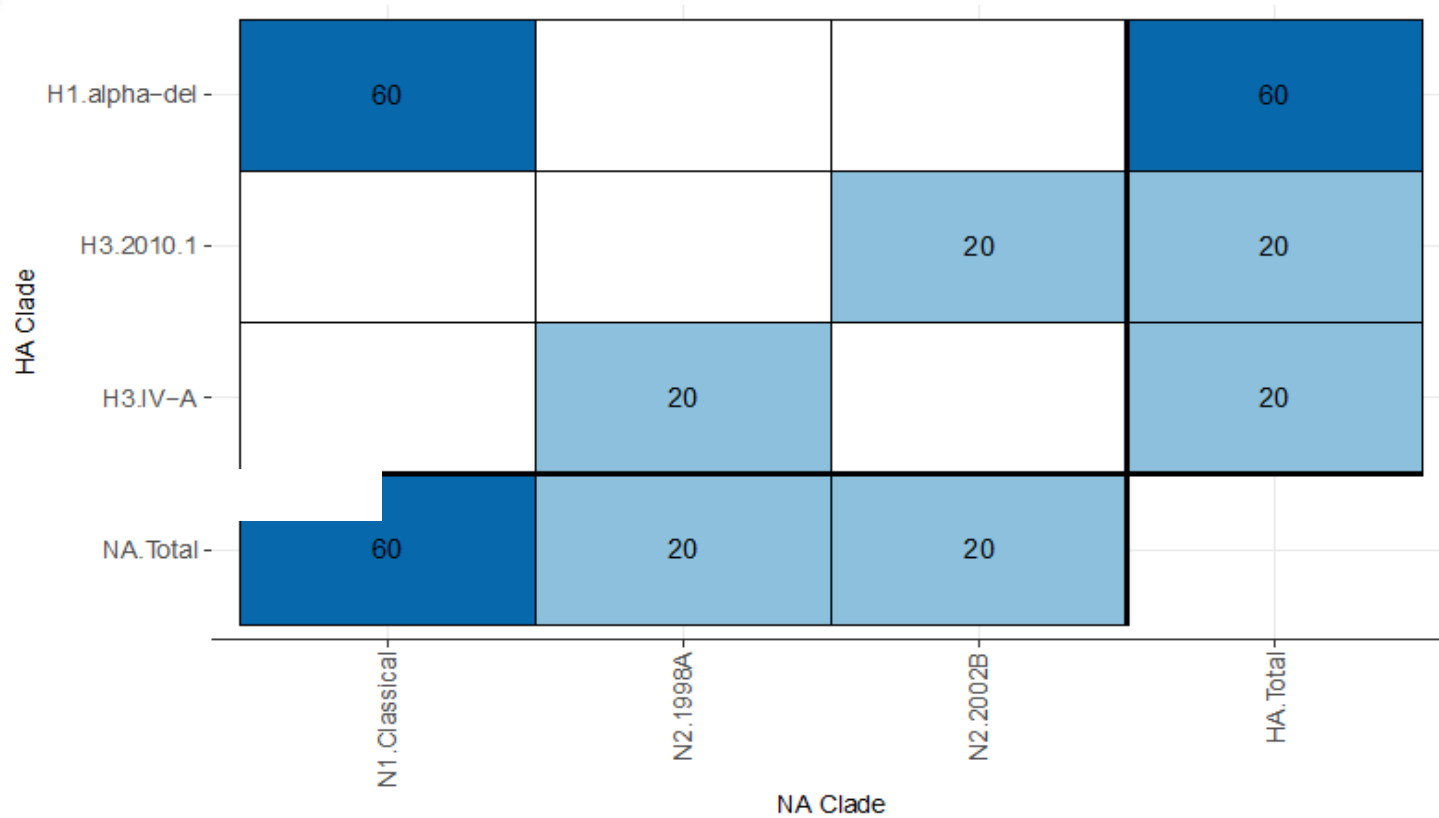
HA Clade	N1.Classical	N1.Pandemic	N2.1998B	N2.2002	N2.2002A	N2.2002B	N2.TX98	HA.Total
H1.alpha–del		4.1				14.3		18.4
H1.delta1b						2		2
H1.delta2			18.4					18.4
H1.gamma–c3	12.2							12.2
H1.LAIV							2	2
H1.pandemic		14.3					2	16.3
H3.2010.1					2	10.2		12.2
H3.IV–A					6.1	2		8.1
H3.IV–B						6.1		6.1
H3.IV–I				4.1				4.1
NA.Total	12.2	18.4	18.4	4.1	8.1	34.6	4	

NA Clade

Total HA & NA combinations – 49

Region 5

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

HA Clade	N1.Classical	N2.1998A	N2.2002B	HA.Total
H1.alpha-del	60			60
H3.2010.1			20	20
H3.IV-A		20		20
NA.Total	60	20	20	

NA Clade

Total HA & NA combinations – 5