United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

February 2023

Influenza A Virus in Swine Surveillance

Fiscal Year 2022 Quarterly Report

Surveillance Summary for Fourth Quarter Fiscal Year 2022: July 1 to September 30, 2022

Report Summary[1]

- This report covers the fourth quarter (Q4) of fiscal year (FY) 2022 from July 1 through September 30, 2022.
- Through Q4 of FY2022, there were 1,905 samples submitted for influenza A virus (IAV) surveillance in swine from 1,737 accessions.
- H1N1 was the predominant subtype reported in USDA data in Q4 FY2022.
- Over the past 8 quarters, H1N1 was the predominant subtype in all regions.
- The Agricultural Research Service (ARS) characterized 218 isolates with published sequences in GenBank by phylogenetic analysis for the Q4 FY2022 report
- In Q4 FY2022, the NVSL Diagnostic Virology Laboratory (NVSL) provided 36 isolates to one academic institution and two government entities. NVSL received 297 isolates into the repository in Q4 FY2022.

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 February 15, 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

[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.

addition of data from participating laboratories. Reporting months are based on the month the sample was 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.

Through Q4 of FY2022, a total of 1,905 samples were tested from 1,737 accessions in FY2022 (Figure 1). Figure 2 shows the overall trends in rRT-PCR and VI positive accessions and subtyped accessions.

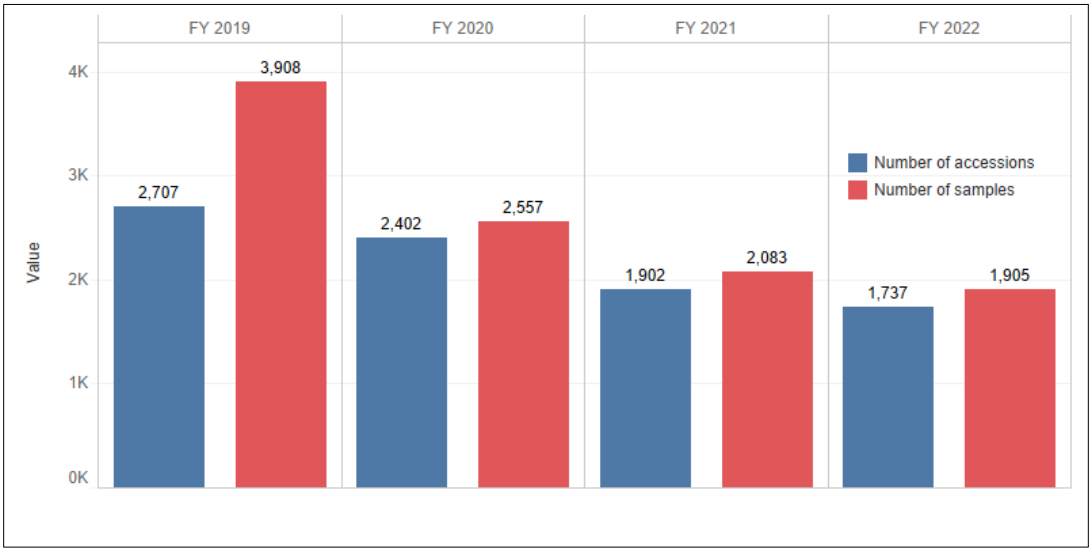


Figure 1. Number of IAV laboratory accessions and samples tested in swine FY2019 through Q4 FY2022

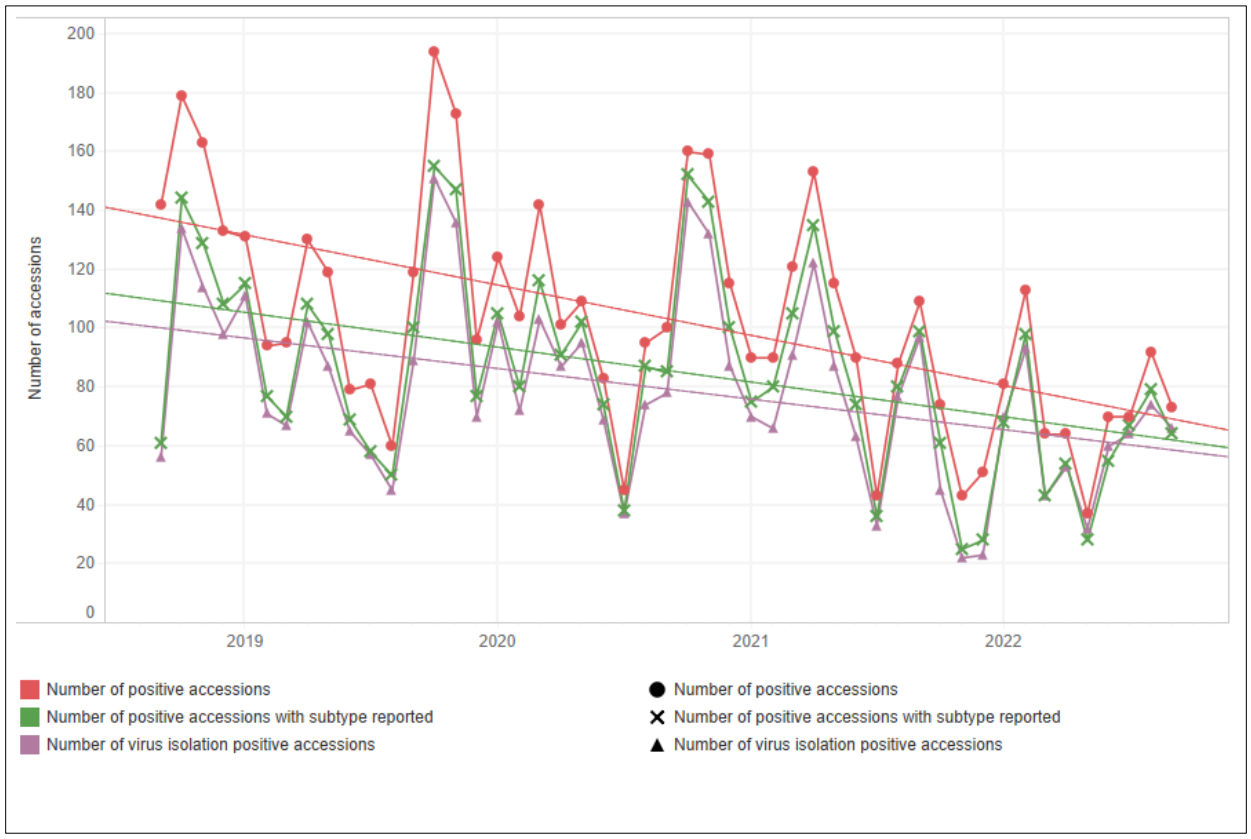


Figure 2. Subtyped accessions, rRT-PCR positive accessions, and virus isolation positive accessions over time with trend lines for IAV-S, FY2018 through Q4 FY2022

Figure 3 shows the number and distirbution of subtype detections in Q4 FY2022. A total of 205 samples were subtyped, including H1N1 (n=84), H1N2 (n=52), H3N2 (n=65), mixed subtype (n=4), and there were no H3N1 subtypes.

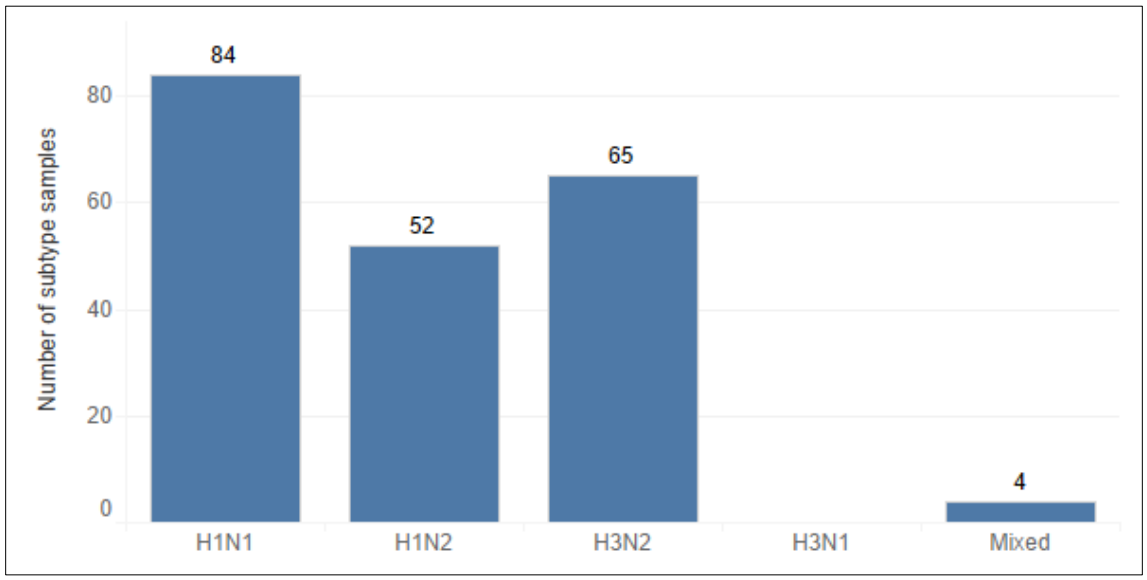


Figure 3. Number of IAV-S subtype detections in Q4 FY2022

Figure 4 breaks down accessions by rRT-PCR subtype for FY2018 through Q4 FY2022. H1N1 was the predominant subtype detected in 2018, 2020, 2021 and through Q4 FY2022. H1N2 was detected most often in 2019. It is important to note that there is wide genetic diversity within each subtype.

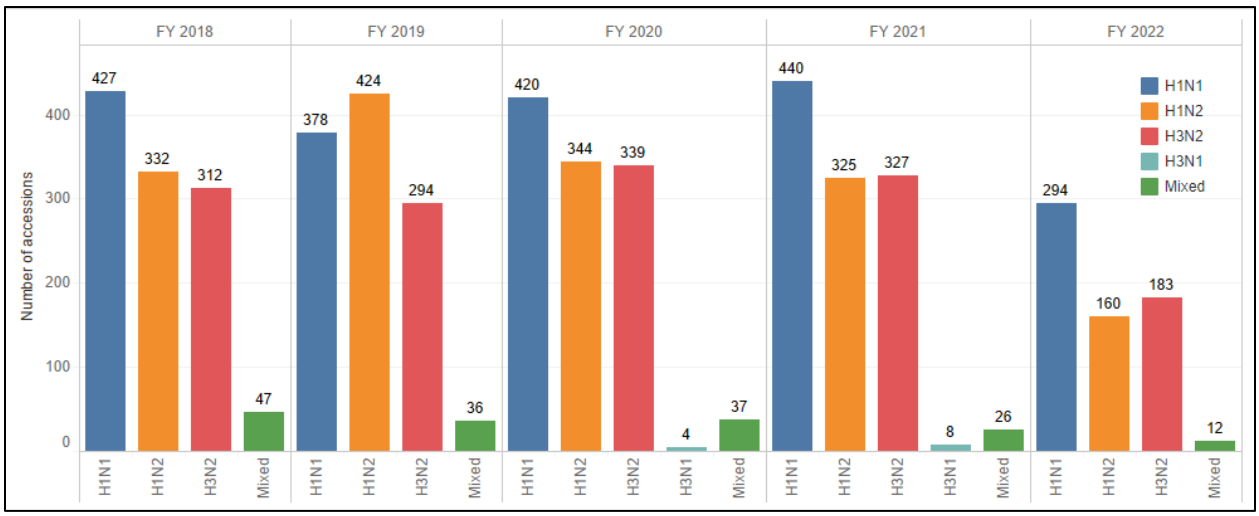


Figure 4. Breakdown of accessions by subtype rRT-PCR from FY2018 through Q4 FY2022

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.

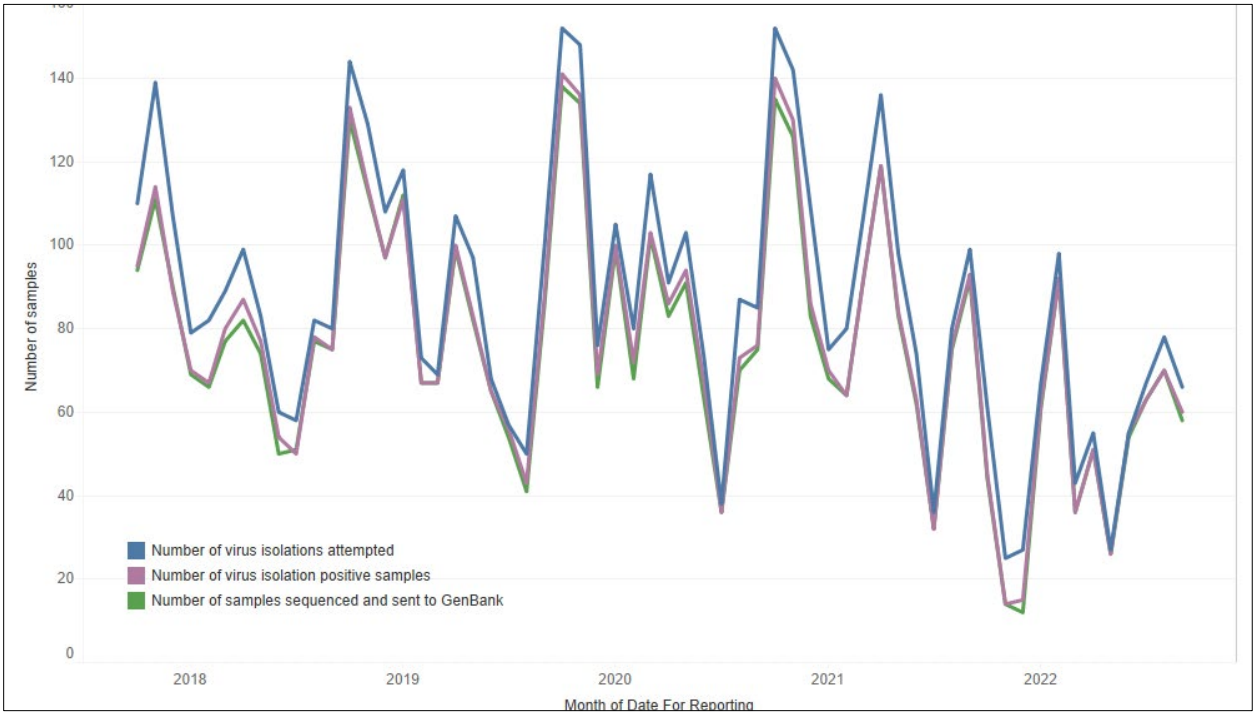


Figure 5. Number of virus isolations attempted, positive virus isolations, and GenBank submissions from FY2018 through Q4 FY2022

Laboratory accessions were evaluated by age-class for the fourth quarter. The most common subtype isolated among all classes was H1N1 (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, Q4 FY2022

Age Class (group)	Number of H1N1	Number of H1N2	Number of H3N1	Number of H3N2	Number of Mixed
Suckling	18	10	0	12	0
Nursery	35	27	0	30	3
Grow/Finish	21	13	0	18	1
Sow/Boar	2	0	0	0	0
Not Recorded/Unknown	7	2	0	5	0

Table 2. Number of positive accessions* tested for IAV-S by specimen type and by viral subtype, Q4 FY2022

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	93%	78	49	0	61	3	176
Nasal or Nasal Swab	13	85%	5	3	0	4	0	11
Oral Fluids	0	NA	0	0	0	0	0	0

*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.

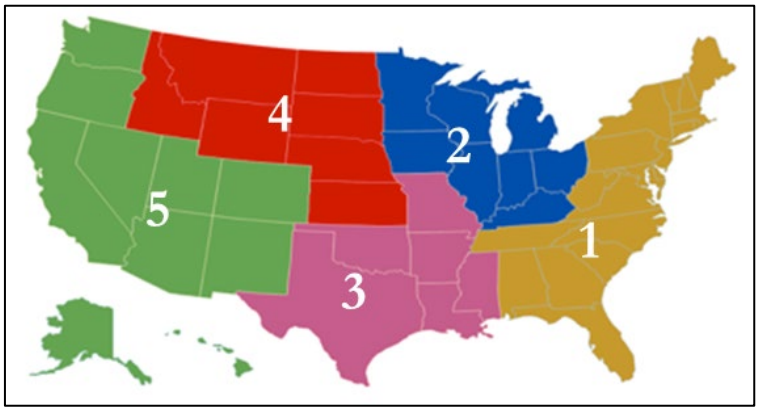


Figure 6. A map of the regions for national IAV-S surveillance

Summary of Regional Data from ARS

Table 3 lists the most predominant HA/NA phylo-type pairs by region from October 2021-September 2022, 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 October 2021 through September 2022

Region	Total number	Predominant HA/NA subtypes
1	81	H1N1 (H1-Alpha-del / N1-Classical) (n=29) H3N2 (H3-Cluster IV-A / N2-2002B) (n=26) H1N2 (H1-Delta2 / N2-1998B) (n=13)
2[+]	417	H1N1 (H1-Gamma / N1-Classical) (n=131) H1N2 (H1-Delta2 / N2-1998B) (n=68) H3N2 (H3-2010.1 / N2-2002B) (n=60)
3	58	H1N1 (H1-Gamma-c3 / N1-Classical) (n=10) H3N2 (H3-2010.1 / N2-2002B) (n=10) H1N2 (H1-Delta2 / N2-1998B) (n=7)
4	43	H1N2 (H1-Alpha-del / N2-2002B) (n=10) H1N1 (H1-Gamma-c3 / N1-Classical) (n=7) H3N2 (H3-2010.1 / N2-2002B (n=6)
5[++]	5	H1N1 (H1-Gamma / N1-Classical) (n=1) H1N1 (H1-Gamma2-beta-like / N1-MN99) (n=1) H1N1 (H1-Pandemic / N1-Pandemic) (n=1)
All	604	H1N1 (H1-Gamma / N1-Classical) (n=158) H1N2 (H1-Delta2 / N2-1998B) (n=92) H3N2 (H3-Cluster IV-A / N2-2002B (n=81)

*HA/NA pairs included if they compromise over 10% from a region
[+] Most diversity of all regions
[++] Low participation

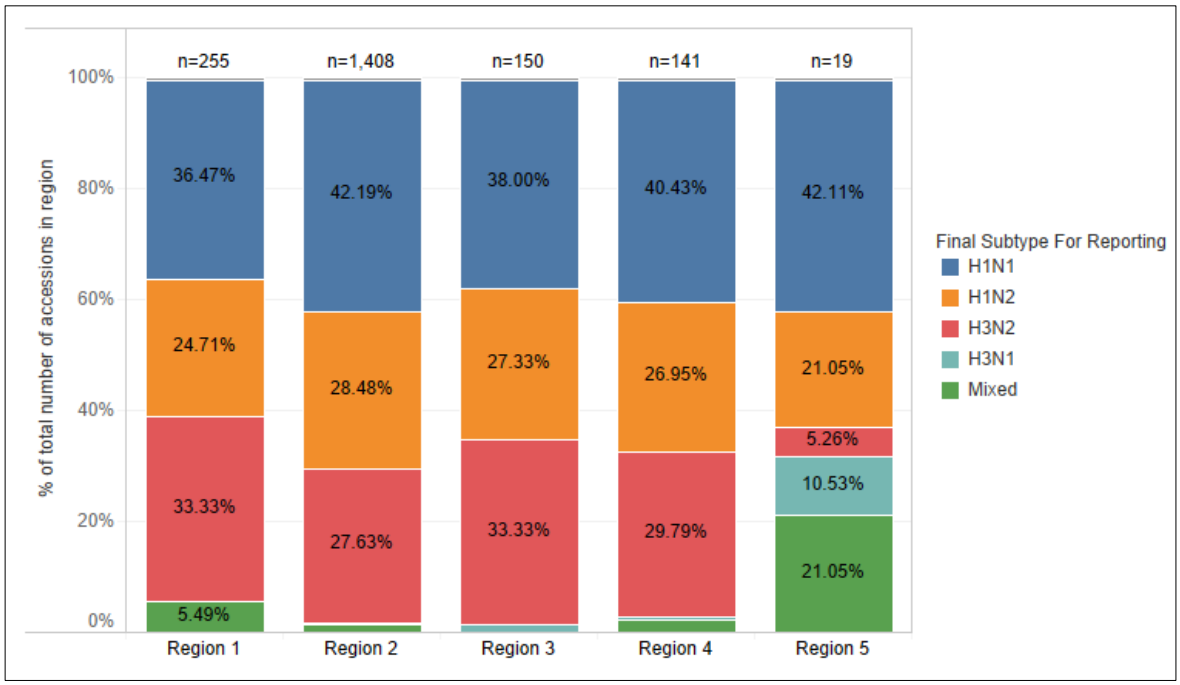


Figure 7. Distribution of rRT-PCR subtyped accessions across the five regions for Q4 FY2020 through Q4 FY2022

Figure 7 shows the distribution of rRT-PCR subtyped accessions across the five regions for Q4 FY2020 through Q4 FY2022. 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 218 isolates with published sequences in GenBank was characterized by phylogenetic analysis for the Q4 FY2022 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.

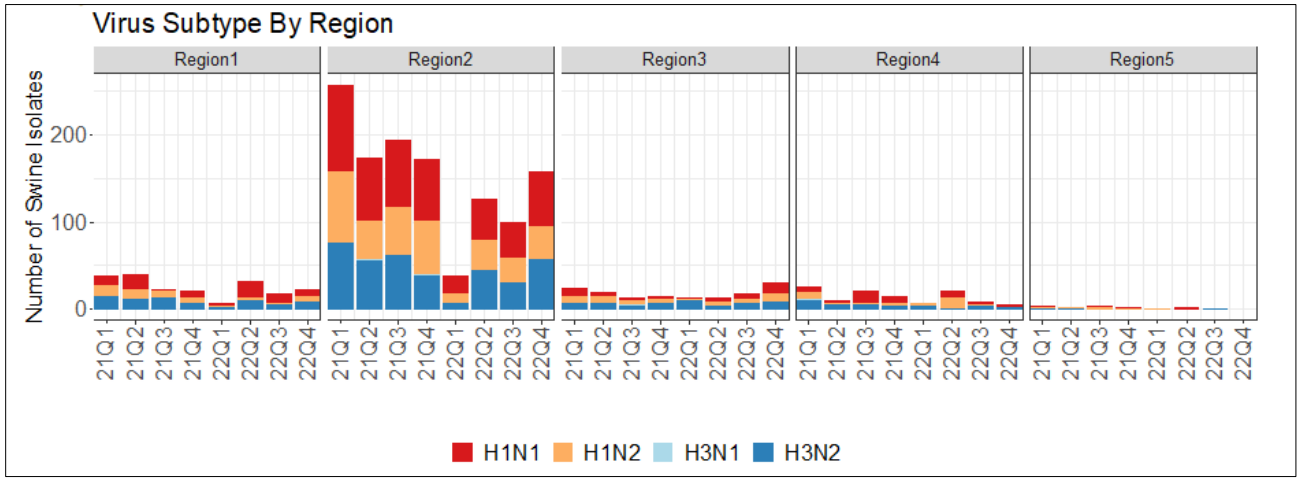


Figure 8. Temporal distribution of Influenza A virus subtype by region for Q4 FY2020 to Q4 FY2022

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. From Q4 FY2020 through Q4 FY2022, H1-Gamma remained the predominant H1 HA gene (Figure 9) and H3-Cluster IV-A and 2010.1 were the predominant H3 HA gene (Figure 10).

[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.

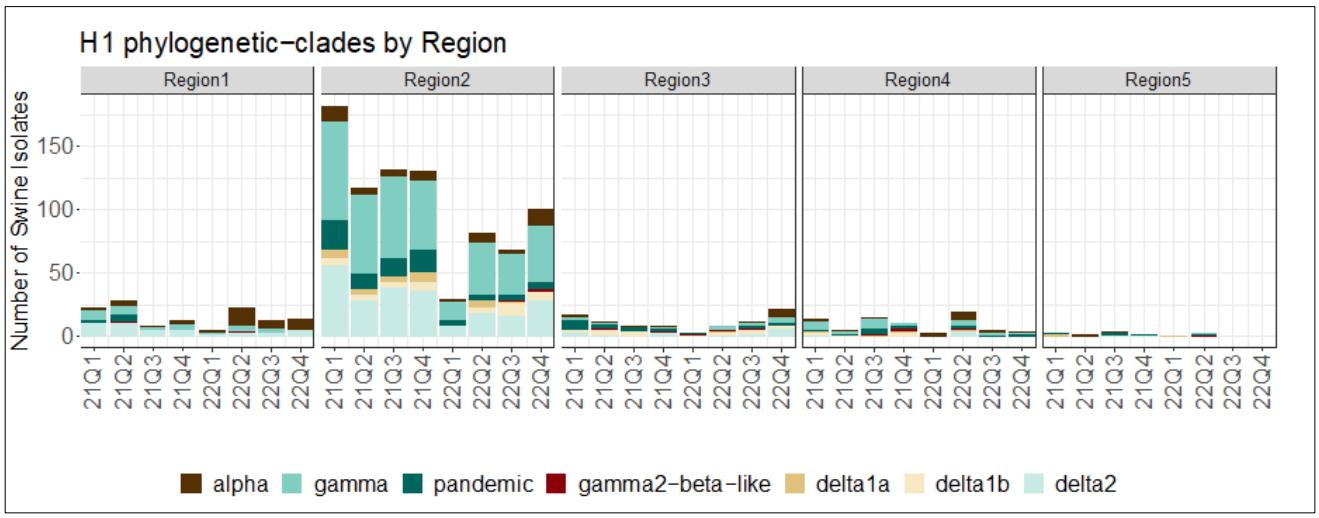


Figure 10. Temporal distribution of H1 phylogenetic clades by region for Q4 FY2020 to Q4 FY2022

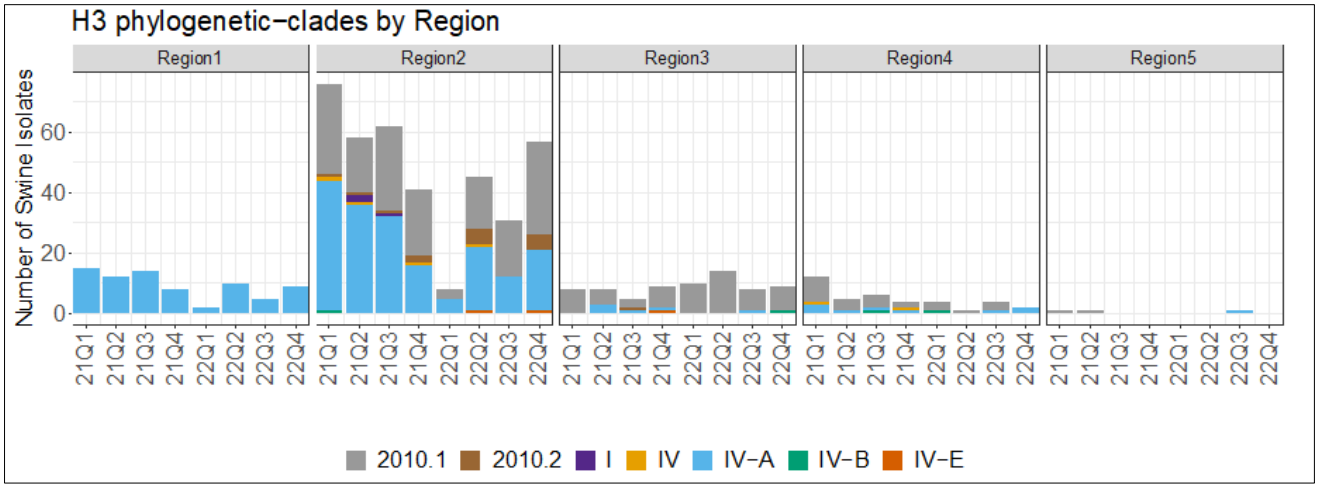


Figure 9. Temporal distribution of H3 phylogenetic clades by region for Q4 FY2020 to Q4 FY2022

National phylogenetic NA gene information

In Q4 FY2022, N1-Classical was the most predominant N1 phylogenetic-clade (Figure 11) and represented approximately 79.1% of the Q4 FY2022 N1 collection.

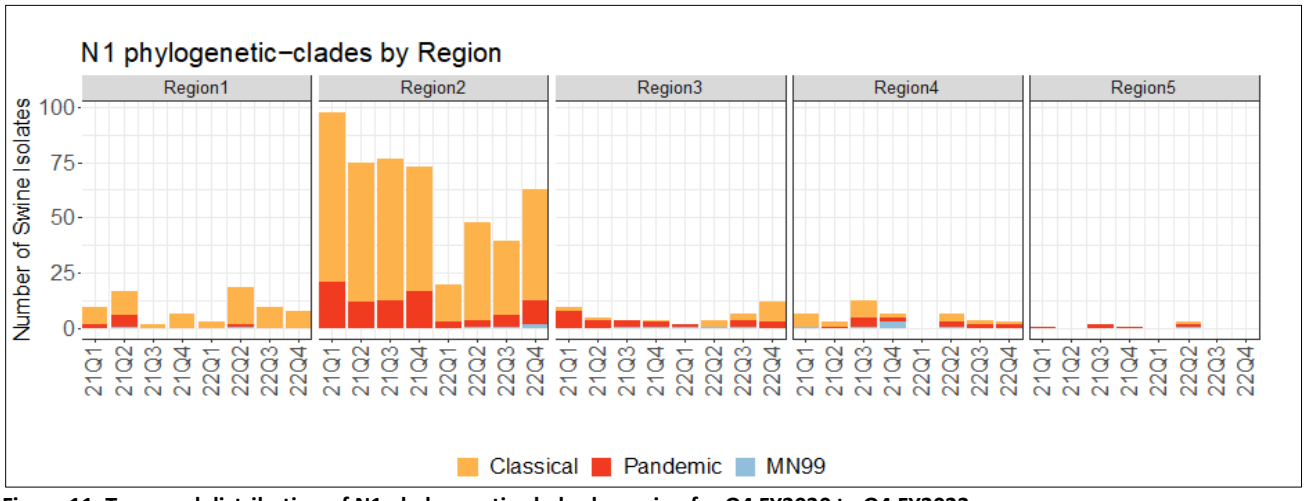


Figure 11. Temporal distribution of N1 phylogenetic-clades by region for Q4 FY2020 to Q4 FY2022

In Q4 FY2022, the most predominant N2 phylogenetic-clade was 2002B-lineage (Figure 12) and represented approximately 63.3% of the Q4 FY2022 N2 collection.

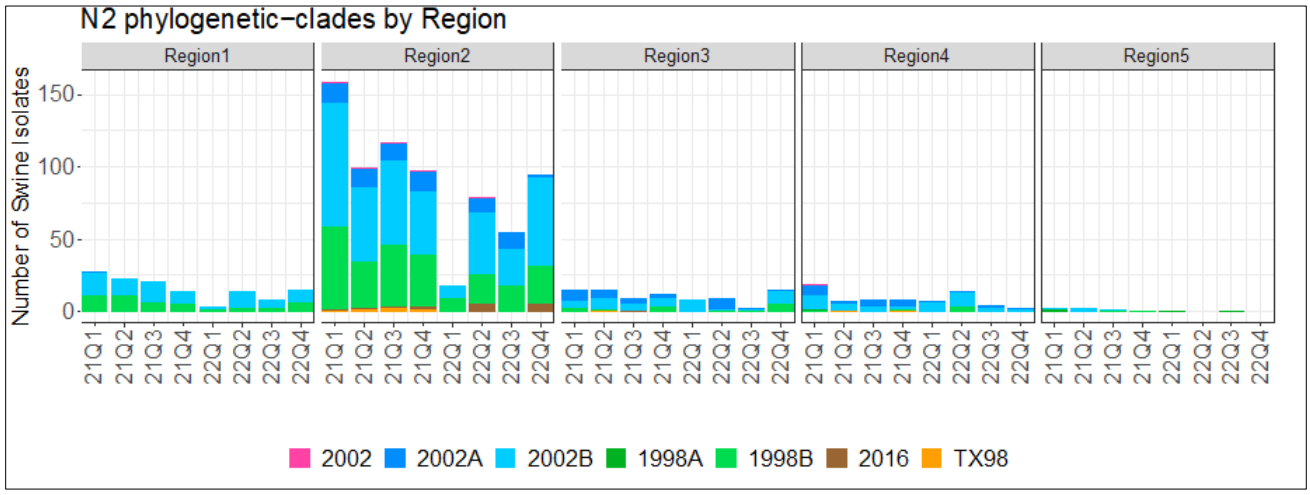


Figure 12. Temporal distribution of N2 phylogenetic-clades by region for Q4 FY2020 to Q4 FY2022

Representative HA genes

Six months of IAV-S data, April 2022-September 2022, 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*

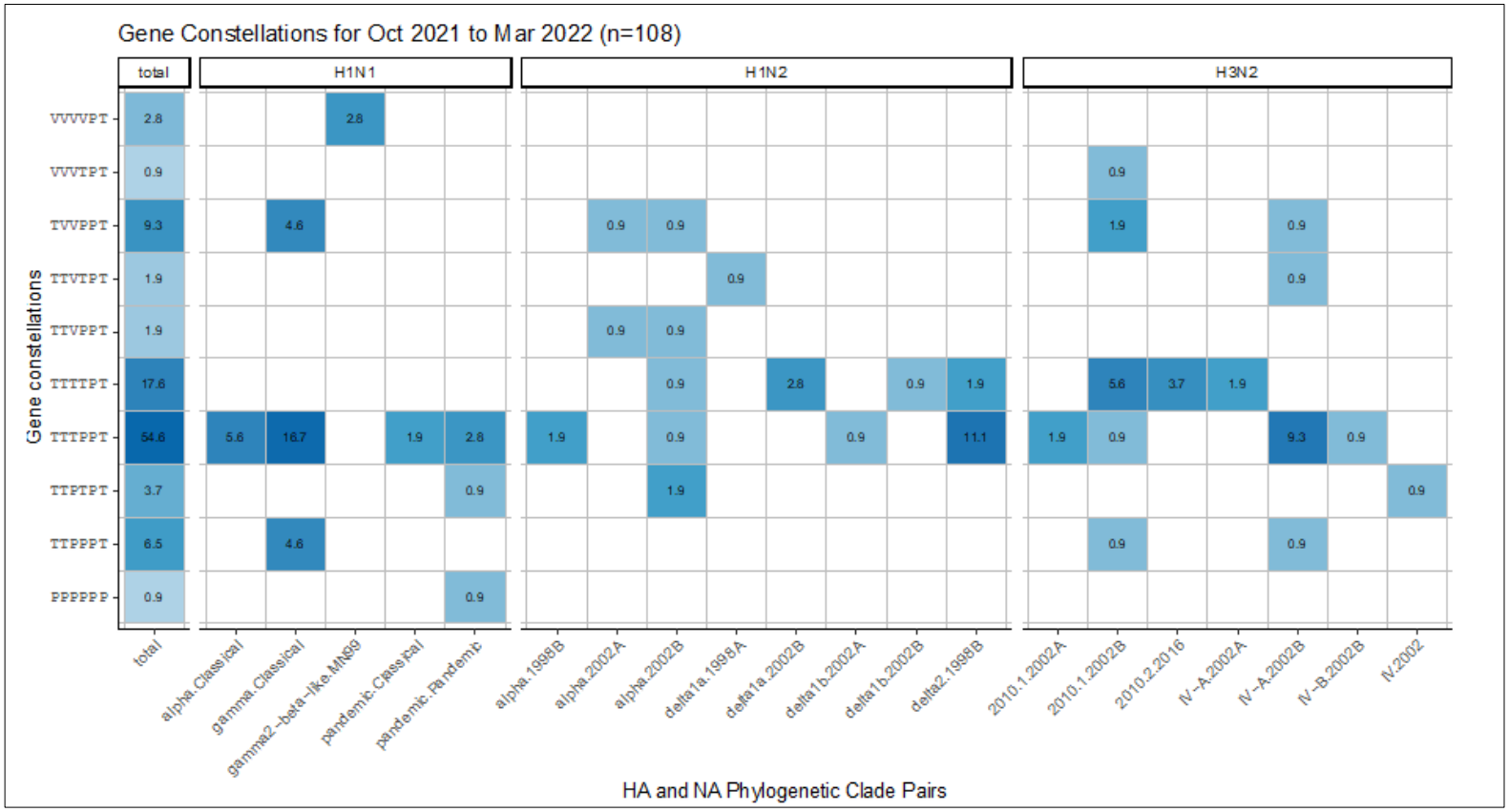
Strain	HA GenBank	Global Clade	US Clade
A/swine/Iowa/A02750864/2022	OP596618	1A.1.1.3	alpha-del
A/swine/Nebraska/A02711851/2022	OP292923	1A.3.3.2	pandemic
A/swine/North_Carolina/A02636642/2022	ON426971	1A.3.3.3-c1	gamma-c1
A/swine/Iowa/A02711806/2022	ON427112	1A.3.3.3-c3	gamma-c3
A/swine/Iowa/A02750859/2022	OP622342	1B.2.1	delta2
A/swine/Oklahoma/A02710705/2022	OP292920	1B.2.2.2	delta1b
A/swine/Iowa/A02750897/2022	OP622360	1990.4.a	IV-A
A/swine/Iowa/A02750686/2022	OP099959	2010.1	2010.1

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

- *April 2022 to September 2022 USDA HA data downloaded (n = 239 H1, n = 126 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 6 H1 selections cover 88% of observed diversity; the 2 H3 selections cover 67% of observed diversity.*
- *Clades were required to have a detection rate of at least 2% to be considered for selection (n >= 8).*
    - *Omitted 1A.4 (n = 4), 1B.2.2.1 (n = 1), 2010.2 (n = 5), 1990.4.b1 (n = 2), and 1990.4 (n = 1)*

Gene constellations information

The most dominant internal gene constellations for FY2022 year were TTTPPT (52.7%), TTTTPT (20.5%) and TTPPPT (8.9%). From October 2021 to March 2022, out of 108 strains with completed whole genomic sequencing that were analyzed, 38% were H1N1, 34% were H1N2, 1% H3N1, and 26% were H3N2, with 10 unique gene constellations and 21 unique HA/NA pairs (Figure 13). Seventeen percent of observed constellations had at least one vaccine gene.



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. Temporal distribution of N2 phylogenetic-clades by region for October 2021 – March 2022

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

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

In Q4 FY2022, the NVSL Diagnostic Virology Laboratory provided 36 isolates to one academic institution and two government entities. NVSL received 297 isolates into the repository in Q4 FY2022. Table 5 reports the total number of virus isolates received into the repository each year from FY2014 through Q4 of FY2022. 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
FY2022	641
FY2021	1,108
FY2020	1,074
FY2019	1,055
FY2018	994
FY2017	844
FY2016	1,046
FY2015	883
FY2014	765
TOTAL FY14-FY22	**8,410**

Table 6. Total number of subtyped isolates collected from 2009-2022 and available through the NVSL repository

Subtype	Number of Isolates
H3N2	2,832
H3N1	25
H1N1	3,615
H1N2	3,257
Mixed	303
TOTAL	10,032

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.

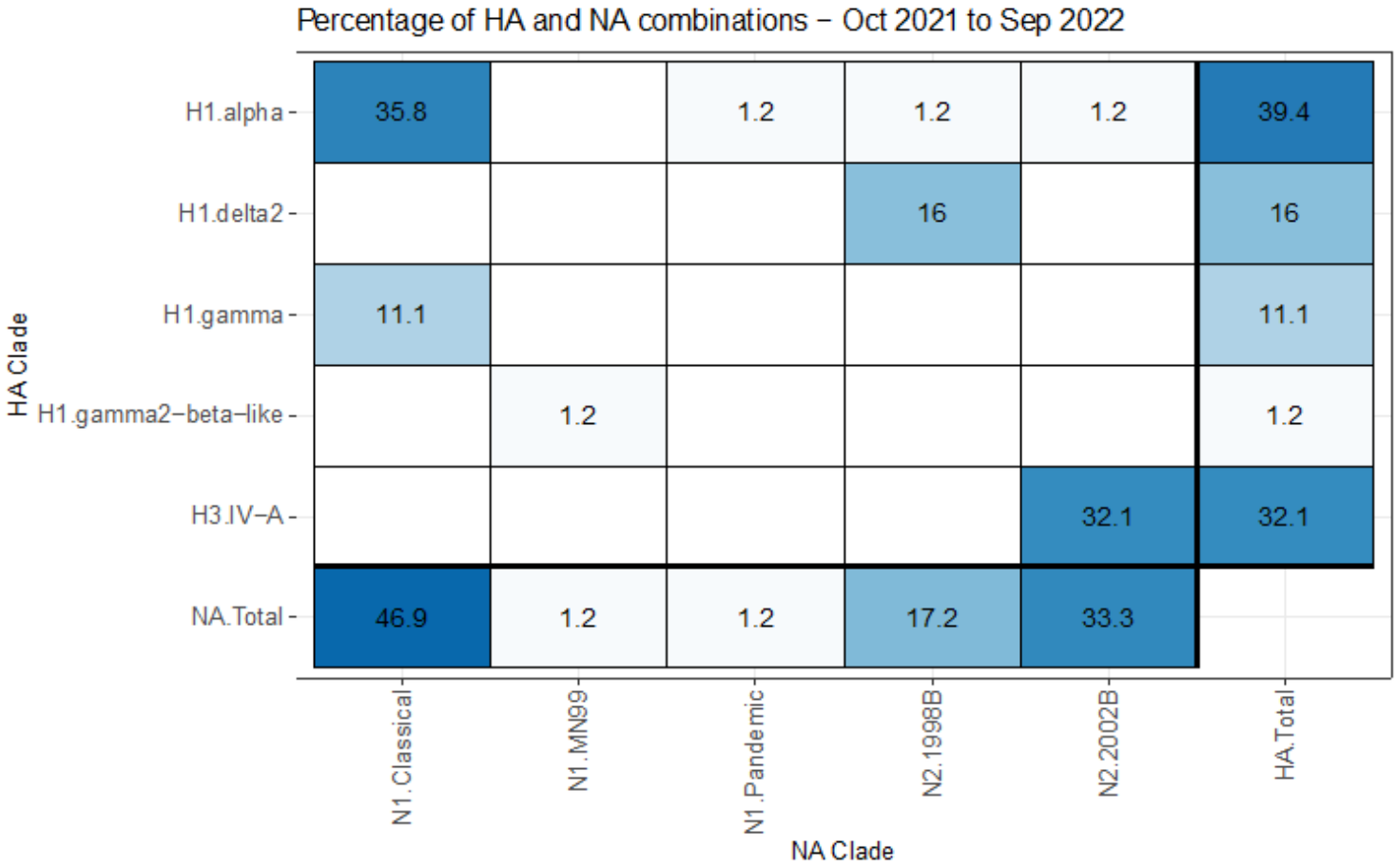
Conclusion

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 October 2021 through September 2022 for regions 1-4, and November 2021 through June 2022 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



Total HA & NA combinations – 81

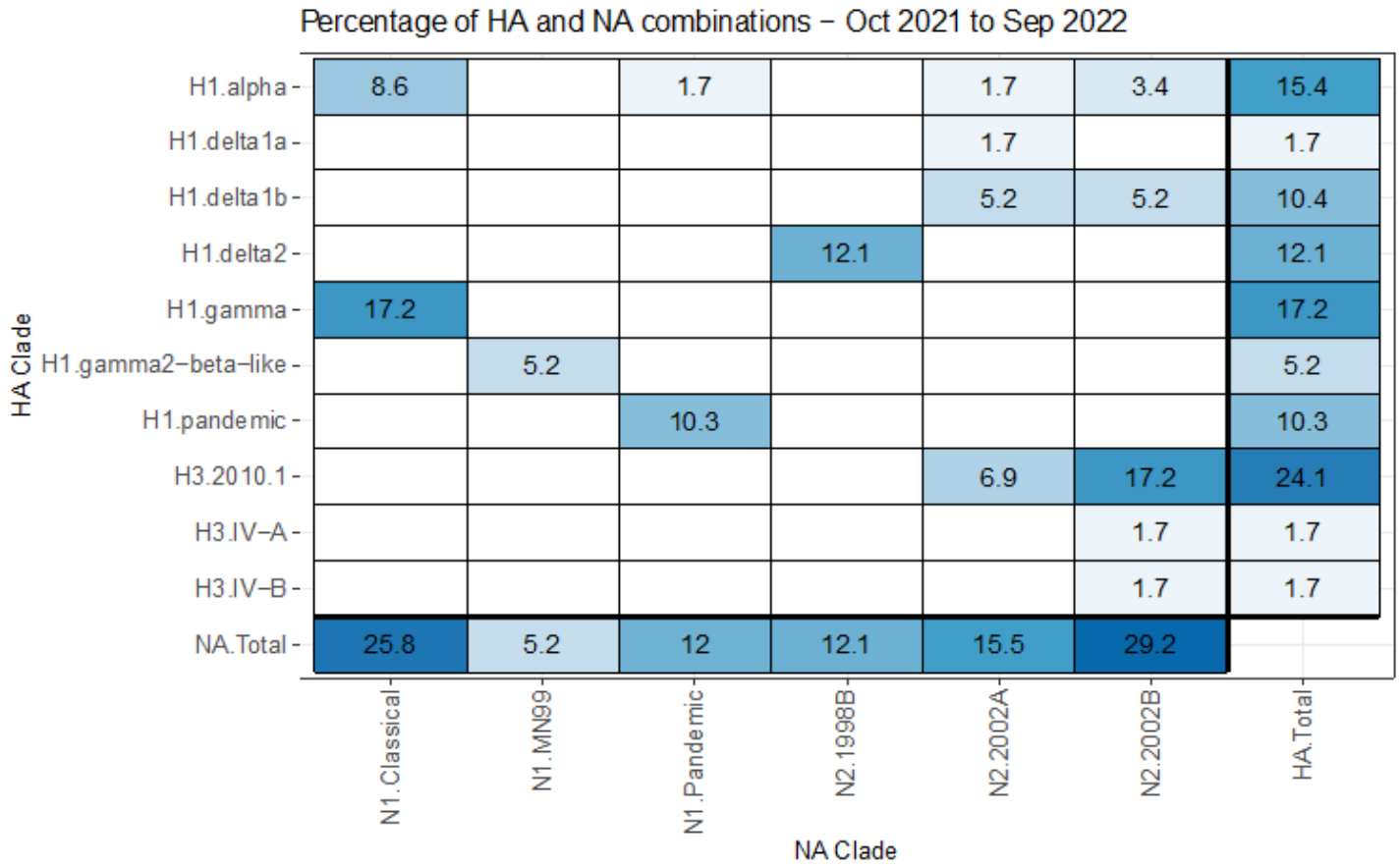
Region 2

Percentage of HA and NA combinations – Oct 2021 to Sep 2022

HA \ NA Clade	N1.Classical	N1.MN99	N1.Pandemic	N2.1998B	N2.2002	N2.2002A	N2.2002B	N2.2016	HA.Total
H1.alpha	2.6		1	1		0.2	1.9		6.7
H1.delta1a							1.4		1.4
H1.delta1b						3.4	1.7		5.1
H1.delta2			0.2	16.3			0.5		17
H1.gamma	31.4								31.4
H1.gamma2–beta–like		1							1
H1.pandemic	0.7		3.8	0.2					4.7
H3.2010.1				0.2		1	14.4		15.6
H3.2010.2								2.4	2.4
H3.IV					0.2				0.2
H3.IV–A				0.2		1	12.7		13.9
H3.IV–E							0.5		0.5
NA.Total	34.7	1	5	17.9	0.2	5.6	33.1	2.4	

Total HA & NA combinations – 417

Region 3



Total HA & NA combinations – 58

Region 4

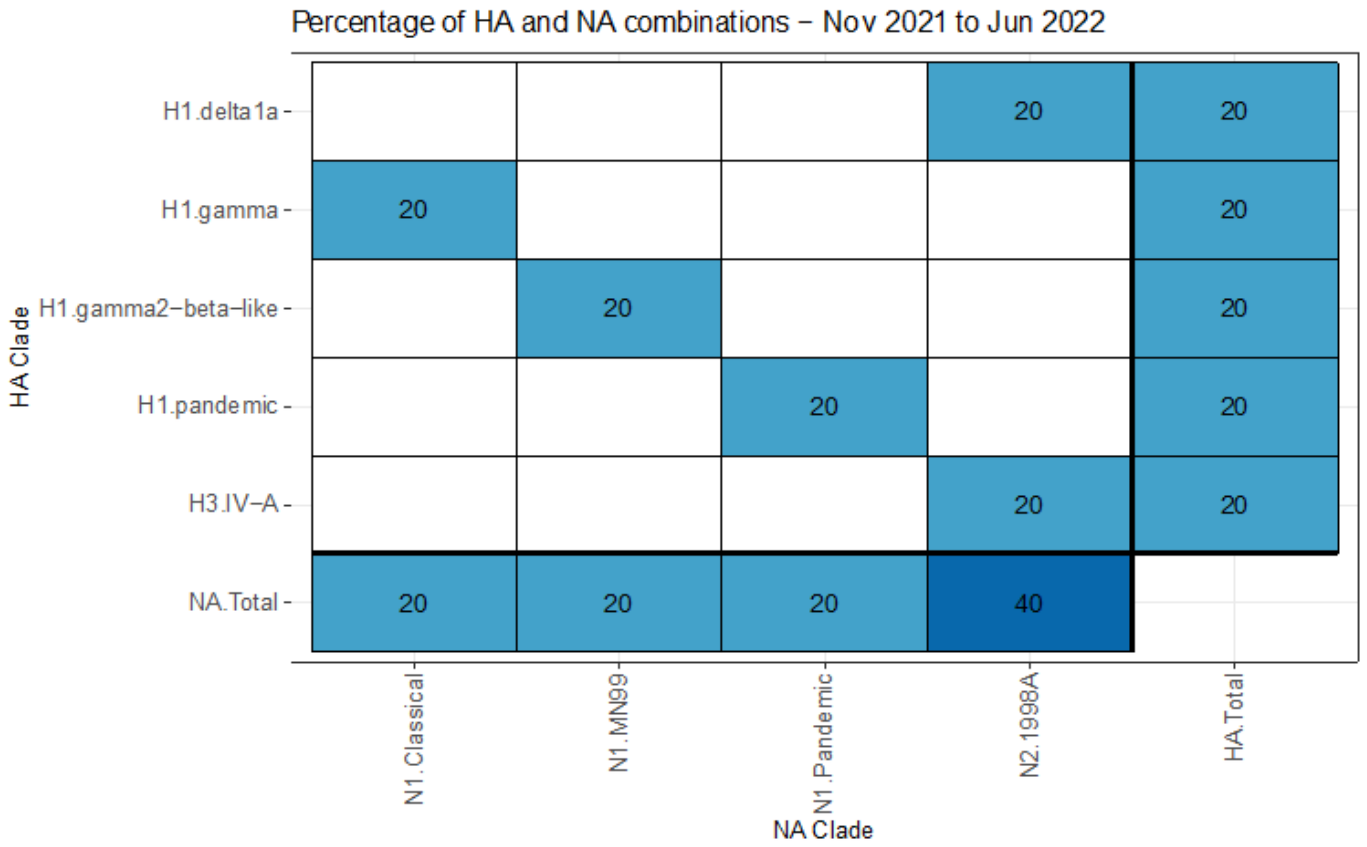
Percentage of HA and NA combinations – Oct 2021 to Sep 2022

HA Clade	N1.Classical	N1.MN99	N1.Pandemic	N2.1998B	N2.2002A	N2.2002B	HA.Total
H1.alpha			2.3		4.7	23.3	30.3
H1.delta1a						2.3	2.3
H1.delta2				9.3			9.3
H1.gamma	16.3					2.3	18.6
H1.gamma2–beta–like		2.3					2.3
H1.pandemic			11.6				11.6
H3.2010.1					2.3	14	16.3
H3.IV–A					4.7	2.3	7
H3.IV–B						2.3	2.3
NA.Total	16.3	2.3	13.9	9.3	11.7	46.5	

NA Clade

Total HA & NA combinations – 43

Region 5



Total HA & NA combinations – 5