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Influenza A Virus in Swine Surveillance

Fiscal Year 2021 Quarterly Report

Surveillance Summary for Fourth Quarter Fiscal Year 2021: July 1 to September 30, 2021

Report Summary[1]

- This report covers the fourth quarter (Q4) of fiscal year (FY) 2021 from July 1 through September 30, 2021.
- In Q4 of FY2021, there were 409 samples submitted for influenza A virus (IAV) surveillance in swine from 376 accessions.
- H1N1was the predominant subtype reported in USDA data in Q4 FY2021.
- Over the past 8 quarters, H1N2 was the predominant subtype in region 1. In regions 2, 4, and 5 H1N1 was the most predominant. H3N2 was the predominant subtype for region 3.
- The Agricultural Research Service (ARS) characterized 201 isolates with published sequences in GenBank by phylogenetic analysis for the Q4 FY2021 report
- In Q4 FY2021, the NVSL Diagnostic Virology Laboratory provided 69 isolates to five academic institutions, three government entities, and three pharmaceutical requestors. NVSL received 324 isolates into the repository in Q4 FY2021.

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 January 20, 2022, provides a brief update on the status of national surveillance for IAV in swine for producers, swine practitioners, diagnosticians and

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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.

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 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 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 ARS 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 National Animal Health Laboratory Network (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 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 National Veterinary Services Laboratories (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 causing high mortality, but the samples have higher than the established maximum Ct values, they will still enter the surveillance stream. In FY 2021, through the end of Q4, a total of 2,078 samples were tested from 1,898 accessions (Figure 1), with 409 samples tested from 376 accessions in Q4. Figure 2 shows the overall trends in rRT-PCR and VI positive accessions and subtyped accessions.
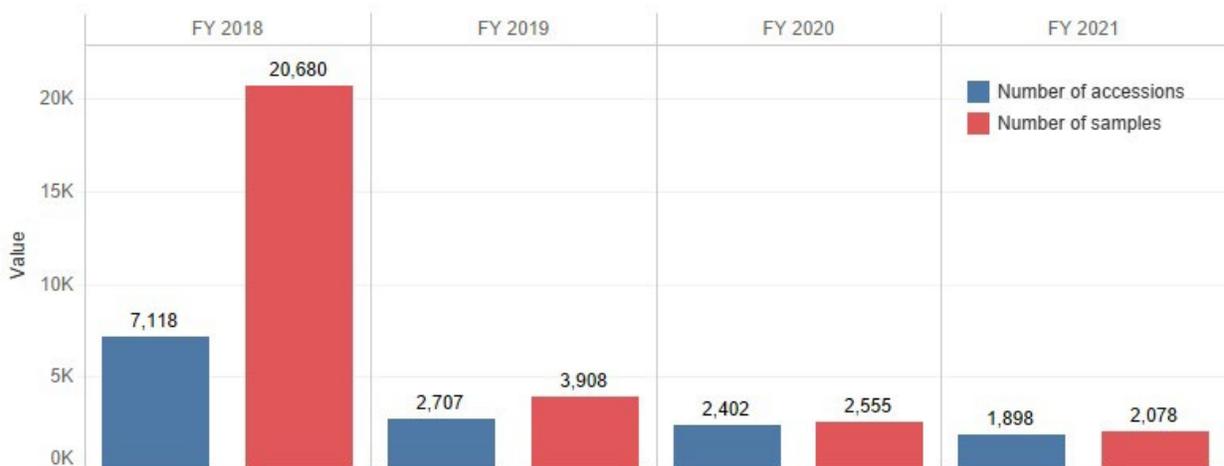

Figure 1. Number of IAV laboratory accessions and samples tested in swine FY2018 through Q4 FY2021
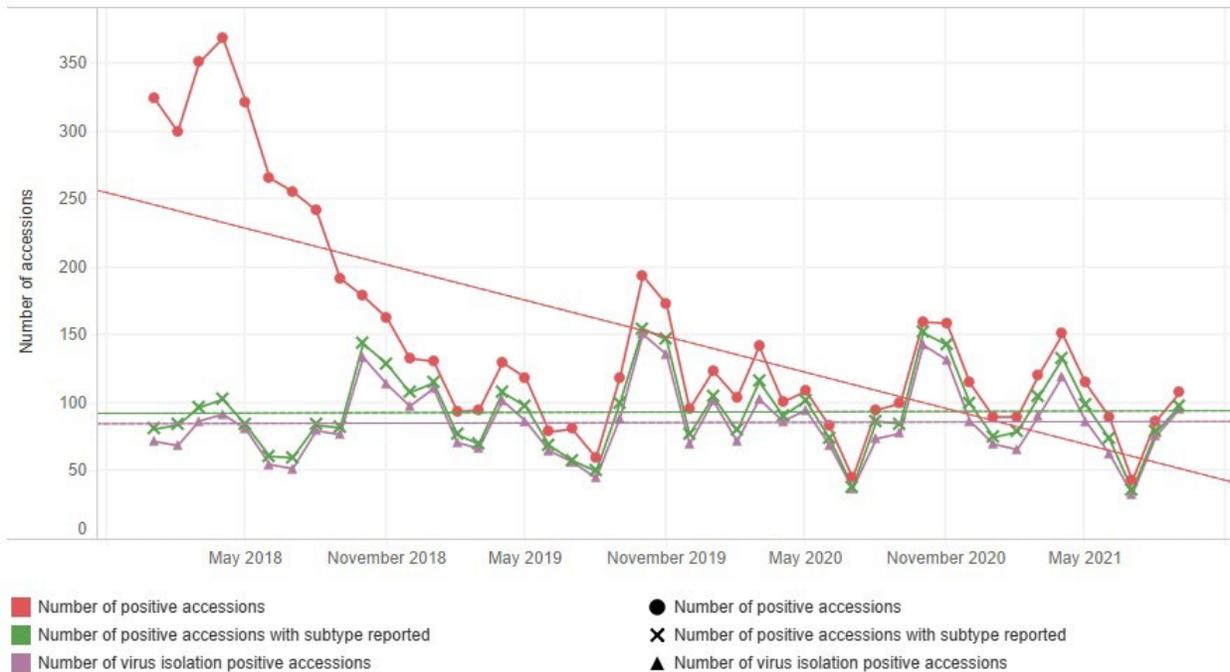
Figure 2. Subtyped accessions, rRT-PCR positive accessions, and virus isolation positive accessions over time with trend lines for IAV-S, FY2017 through Q4 FY2021

Figure 3 shows the number and distirbution of subtype detections in Q4 FY2021. A total of 208 samples were subtyped, including H1N1 (n=80), H1N2 (n=71), H3N2 (n=52), H3N1 (n=2), and mixed (n=3).
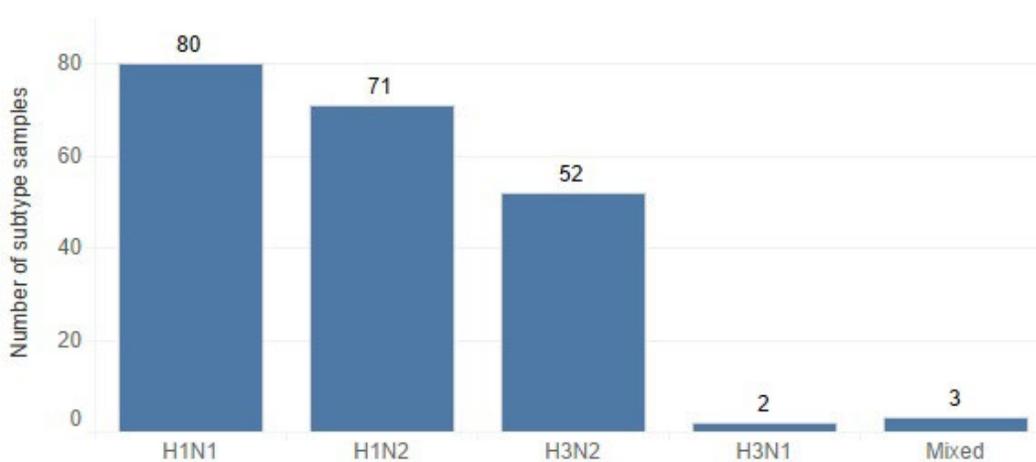
Figure 3. Number of IAV-S subtype detections in Q4 FY2021

Figure 4 breaks down accessions by rRT-PCR subtype for FY2017 through Q4 FY2021. H1N1 was the predominant subtype detected in 2018, 2020 and through Q4 FY2021. H1N2 was detected most often in 2017 and 2019. It is important to note that there is wide genetic diversity within each subtype.
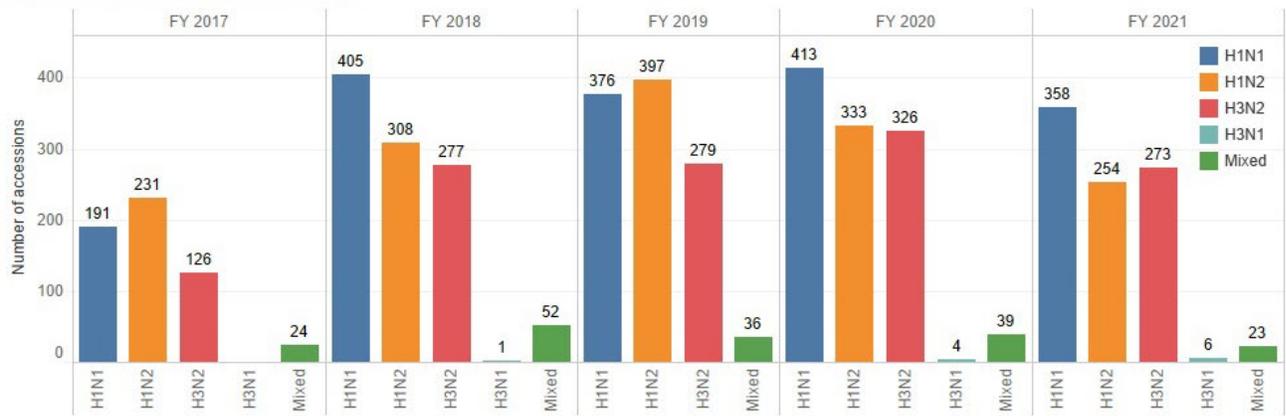
Figure 4. Breakdown of accessions by subtype rRT-PCR from FY2017 through Q4 FY2021

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.
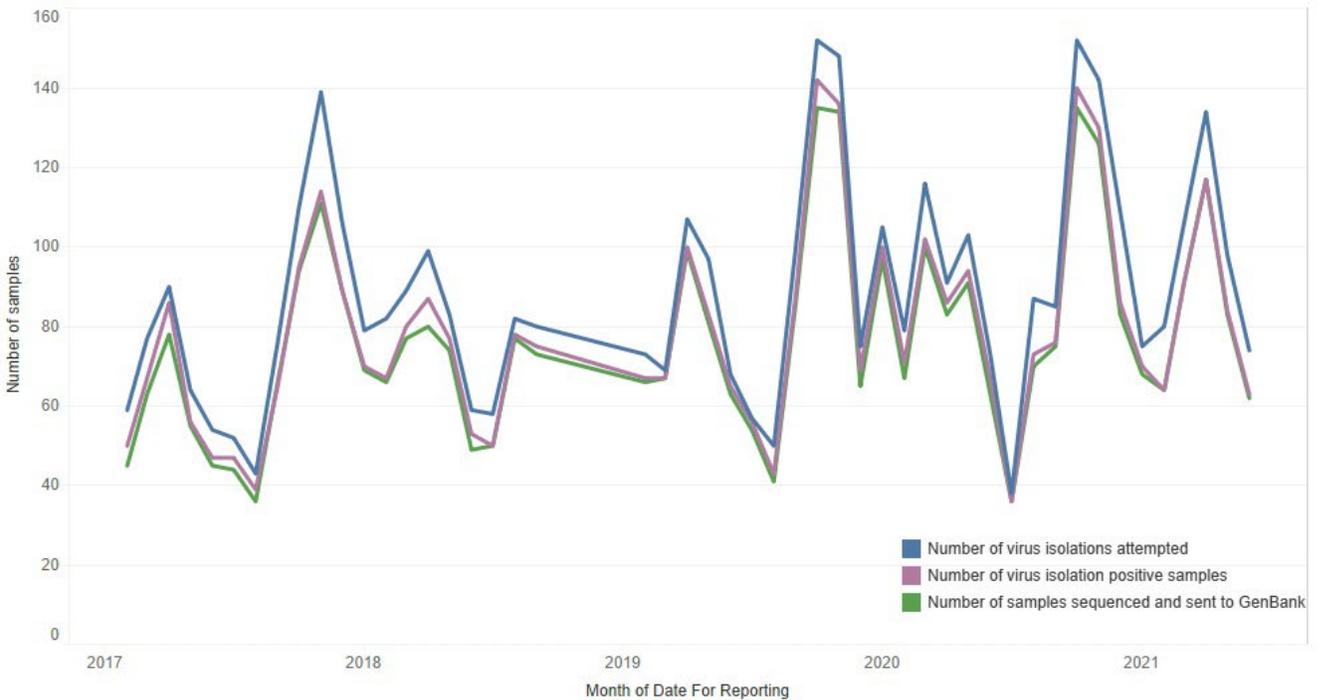
Figure 5. Number of virus isolations attempted, positive virus isolations, and GenBank submissions from FY2017 through Q4 FY2021

Laboratory accessions were evaluated by age-class for the fourth quarter. The most common subtype isolated among the nursery and grow/finish classes was H1N1. Among the suckling age class, H3N2 was the predominant isolated subtype. The sow/boar class had limited testing, with one isolate of H1N2 reported. Among isolates for which the age class was unknown or not recorded, H1N1, H1N2, and H3N2 were all equally subtyped (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 FY2021

Age Class (group)	Number of H1N1	Number of H1N2	Number of H3N1	Number of H3N2	Number of Mixed
Suckling	14	14	1	16	1
Nursery	31	27	1	12	0
Grow/Finish	29	23	0	18	2
Sow/Boar	0	1	0	0	0
Not Recorded/Unknown	6	6	0	6	0

Table 2. Number of positive accessions* tested for IAV-S by specimen type and by viral subtype, Q4 FY2021

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	199	94%	119	72	1	73	6	187
Nasal or Nasal Swab	9	100%	3	2	0	3	0	7
Oral Fluids	0	NA	0	0	0	0	0	0
Other Specimens	1	0%	0	0	0	1	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.
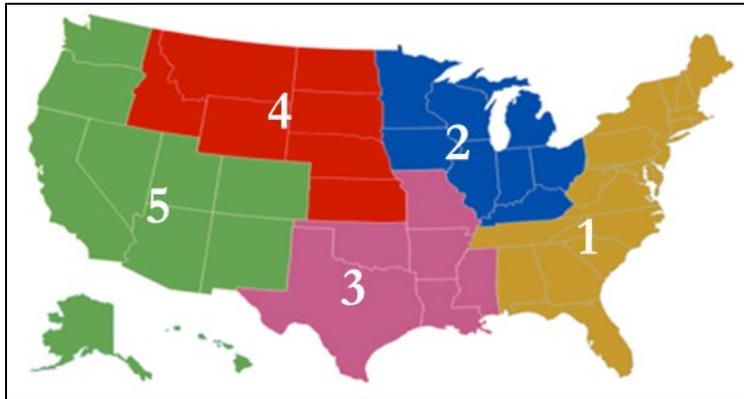
Figure 6. A map of the regions for national IAV-S surveillance

Table 3. Summary of predominant HA/NA* phylo-types by region in a 1-year window from October 2020 through September 2021.

Region	Total number	Predominant HA/NA subtypes
1	117	H3N2 (H3-Cluster IV-A / N2-2002B) (n=48) H1N2 (H1-Delta2 / N2-1998B) (n=32) H1N1 (H1-Gamma / N1-Classical) (n=20)
2[+]	773	H1N1 (H1-Gamma / N1-Classical) (n=241) H1N2 (H1-Delta2 / N2-1998B) (n=155) H3N2 (H3-Cluster IV-A / N2-2002B) (n=115)
3	70	H1N1 (H1-Pandemic / N1-Pandemic) (n=16) H3N2 (H3-2010.1 / N2-2002A) (n=11) H3N2 (H3-2010.1 / N2-2002B) (n=9) H1N1(H1-Delta1B / N2-2002A) (n=7)
4	70	H1N1 (H1-Gamma / N1-Classical) (n=18) H3N2 (H3-2010.1 / N2-2002B) (n=10) H1N1 (H1-Pandemic / N1-Pandemic) (n=7) H3N2 (H3-2010.1 / N2-2002A) (n=7)
5[++]	13	H1N1 (H1-Pandemic / N1-Pandemic) (n=4) H1N2 (H1-Alpha / N2-2002B) (n=3) H1N2 (H1-Delta1A / N2-1998A) (n=2) H1N2 (H1-Delta2 / N2-1998B) (n=2) H3N2 (H3-2010.1 / N2-2002B) (n=2)
All	1043	H1N1 (H1-Gamma / N1-Classical) (n=283) H1N2 (H1-Delta2 / N2-1998B) (n=195) H3N2 (H3-Cluster IV-A / N2-2002B) (n=170)

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

[+] Most diversity of all regions

[++] Low participation

Summary of Regional Data from ARS

Table 3 lists the most predominant HA/NA phylo-type pairs by region from October 2020-September 2021, with predominant being defined as comprising at least 10% of a region's HA/NA pairs. The total number column displays the number of isolates that were phylo-typed for each region from October 2020-September 2021.

Figure 7 shows the distribution of rRT-PCR subtyped accessions across the five regions for Q4 FY2019 through Q4 FY2021. Over the last 8 quarters, H1N2 was the predominant subtype in region 1. In regions 2, 4, and 5 H1N1 was the most predominant. H3N2 was the predominant subtype for region 3.
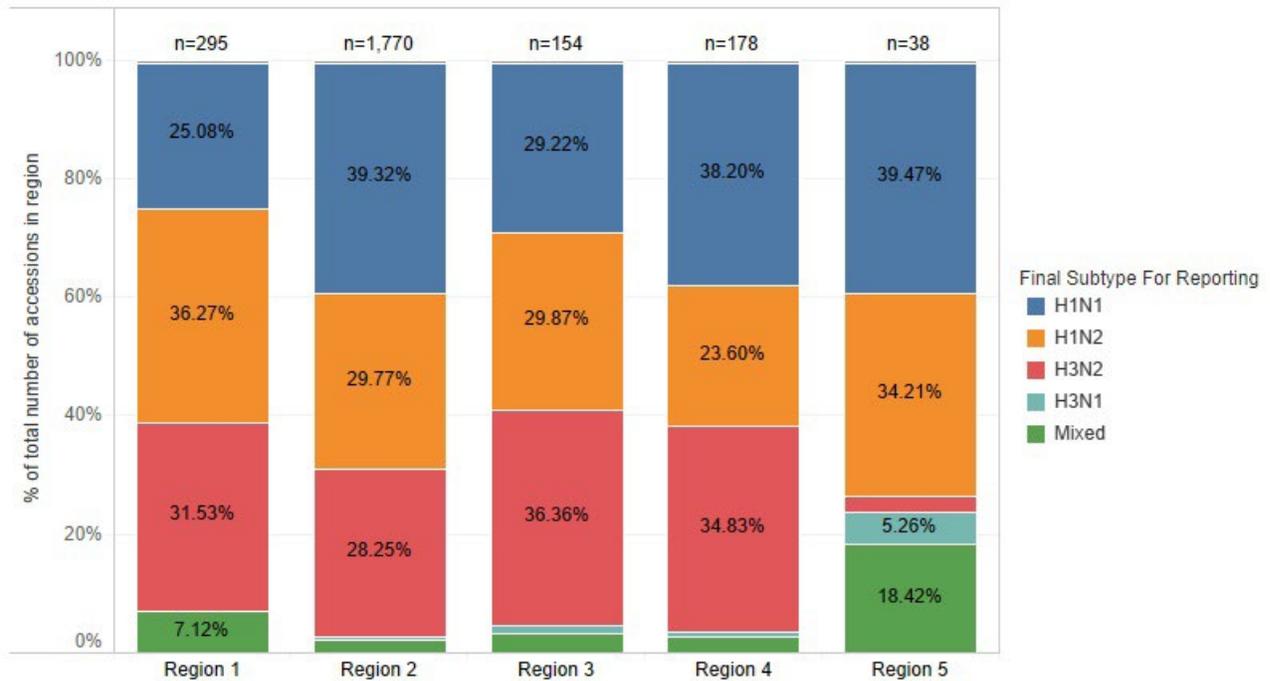
Figure 7. Distribution of rRT-PCR subtyped accessions across the five regions for Q4 FY2019 through Q4 FY2021

Regional phylogenetic analysis

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 201 isolates with published sequences in GenBank was characterized by phylogenetic analysis for the Q4 FY2021 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.

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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.

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.
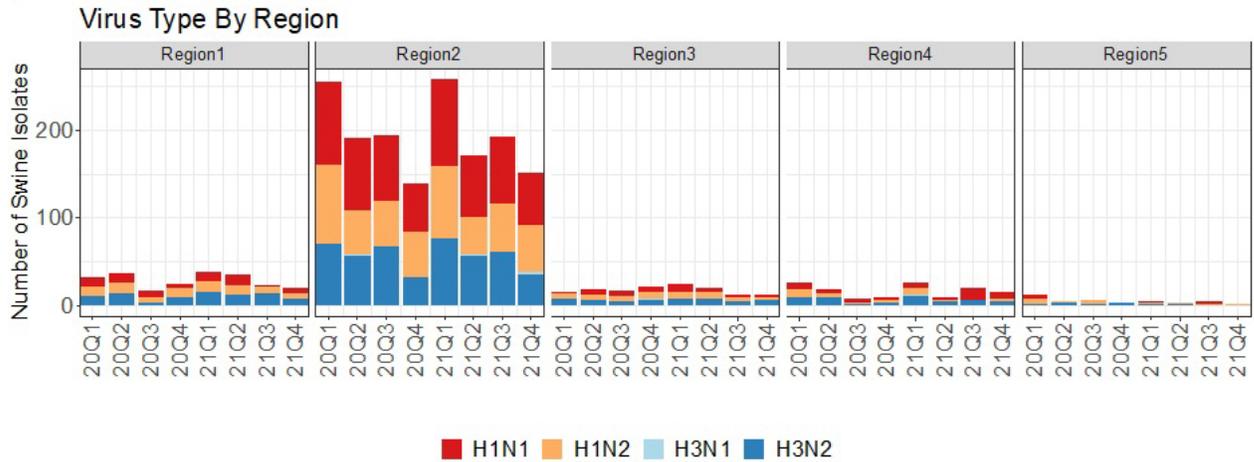
Figure 8. Temporal distribution of Influenza A virus subtype by region for Q1 FY2020 to Q4 FY2021

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. 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.

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. 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 Q1 FY2020 through Q4 FY2021, H1-Gamma remained the predominant H1 HA gene (Figure 9) and H3-Cluster IV-A remained the predominant H3 HA gene (Figure 10).
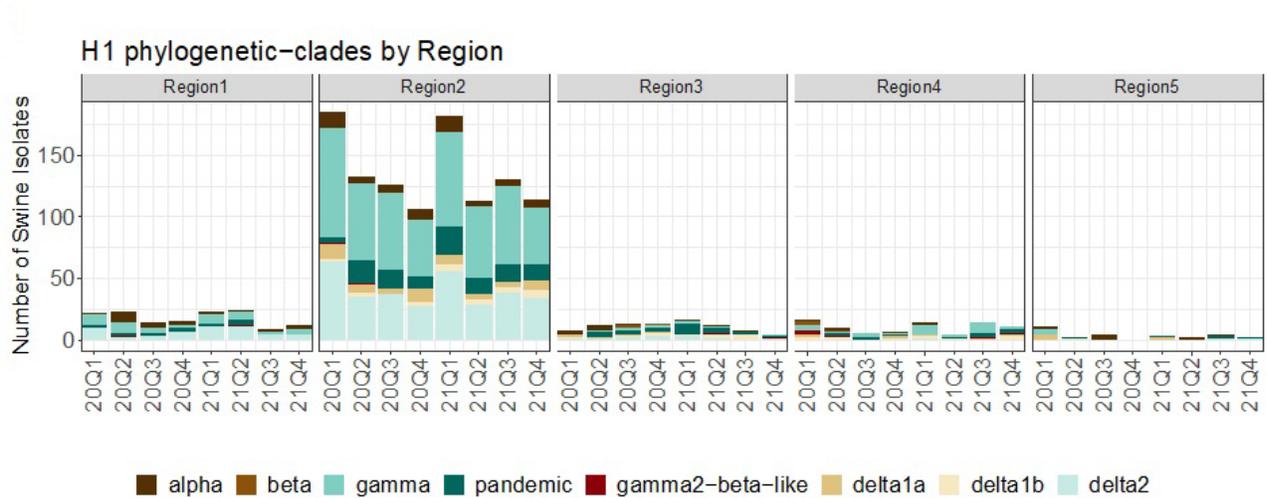
Figure 9. Temporal distribution of H1 phylogenetic clades by region for Q1 FY2020 to Q4 FY2021
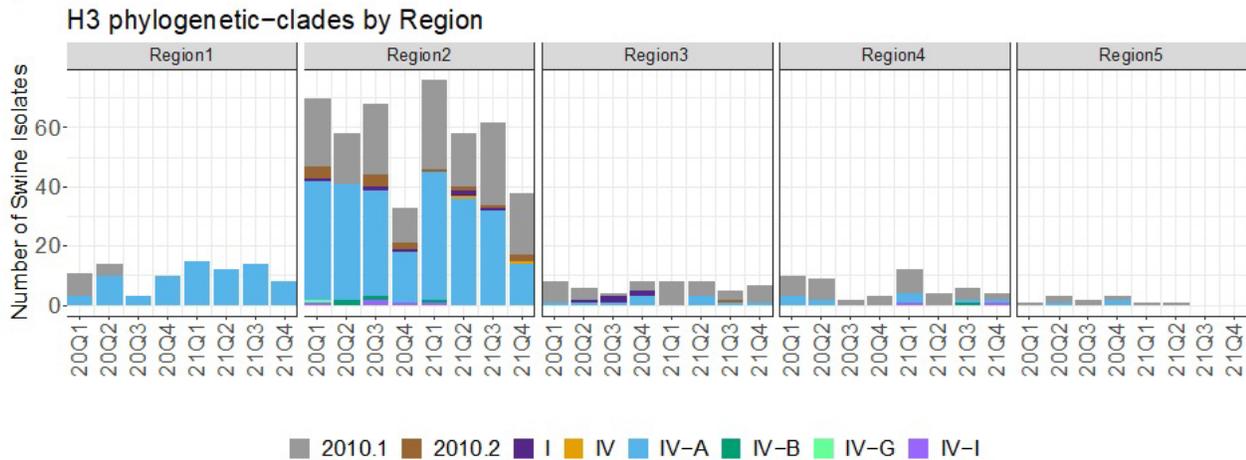
Figure 10. Temporal distribution of H3 phylogenetic clades by region for Q1 FY2020 to Q4 FY2021

National phylogenetic NA gene information

In Q4 FY2021, N1-Classical (n=55) was the most predominant N1 phylogenetic-clade (Figure 11) and was most commonly paired with H1-gamma (n=51), H1-pandemic (n=2, and H1-Alpha (n=2). N1-Pandemic (n=20) was the next most common N1 clade, paired with H1-Pandemic (n=15), H3-2010.1 (n=2), H1-Alpha (n=1), H1-Gamma (n=1), and H1-Delta2 (n=1). The final most common N1 phylogenetic-clade was N1-LAIV (n=4), paired with H1-LAIV (n=3) and H1-Delta1B (n=1).

In Q4 FY2021, the most predominant N2 phylogenetic-clade was N2-2002 (n=75) followed by N2-1998 (n=42), thenN2-LAIV (n=3) (Figure 12).

Gene constellations information

The most dominant internal gene constellations for FY2021 were TTTPPT (56%), TTTTPT (21%) and TTPPPT (8%). From October 2019 to July 2021, out of 583 strains with completed whole genomic sequencing that were analyzed, 35% were H1N1, 32% were H1N2, and 32% were H3N2, with 25 unique gene constellations and 49 unique HA/NA pairs.

N1 phylogenetic−clades by Region
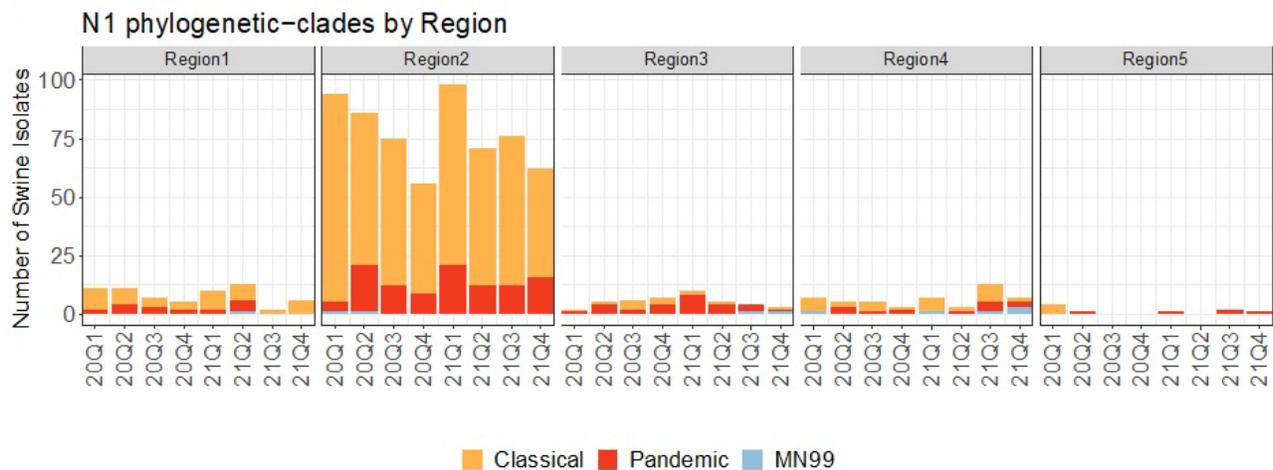
Figure 11. Temporal distribution of N1 phylogenetic clades by region for Q1 FY2020 to Q4 FY2021

N2 phylogenetic−clades by Region
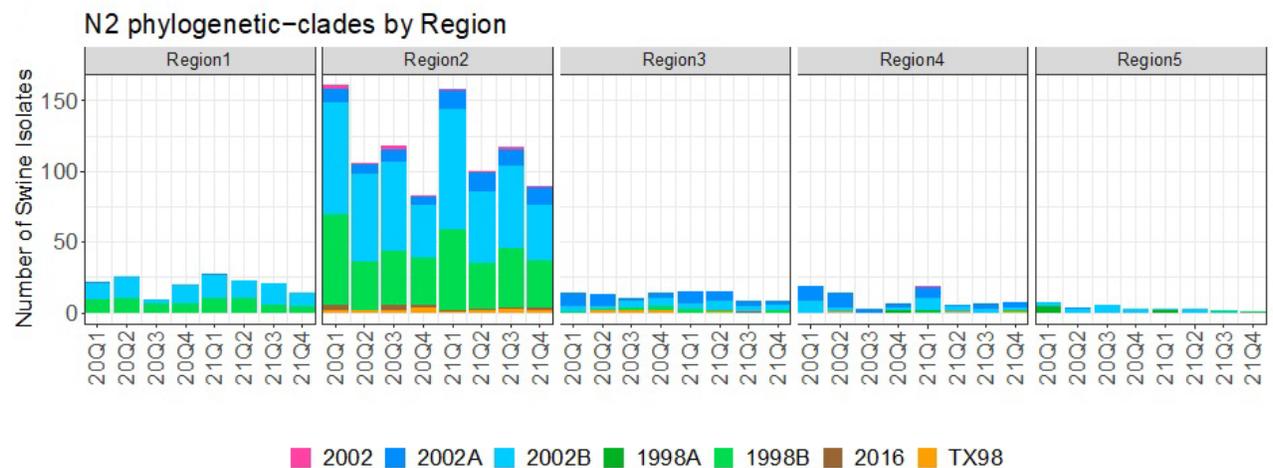
Figure12. Temporal distribution of N2 phylogenetic clades by region for Q1 FY2020 to Q4 FY2021

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 FY2021, the NVSL Diagnostic Virology Laboratory provided 69 isolates to five academic institutions, three government entities, and three pharmaceutical requestors. NVSL received 324 isolates into the repository in Q4 FY2021. Table 4 reports the total number of virus isolates received into the repository each year from FY2014 through Q4 of FY2021. Table 5 reports the total number of isolates by subtype available in the repository for sharing.

Table 4. Virus isolates received in NVSL repository by year

Fiscal Year	Number of isolates
FY2021	1,108
FY2020	1,074
FY2019	1,055
FY2018	994
FY2017	844
FY2016	1,046
FY2015	883
FY2014	765

Table 5. Total number of subtyped isolates collected from 2009-present and available through the NVSL repository

Subtyped isolates available	
H3N2	2,617
H3N1	24
H1N1	3,351
H1N2	3,097
Mixed	302
TOTAL	9,391

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 2020 through September 2021. 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 – Oct 2020 to Sep 2021

HA Clade	N1.Classical	N1.MN99	N1.Pandemic	N2.1998B	N2.2002A	N2.2002B	HA.Total
H1.alpha	2.6			0.9		3.4	6.9
H1.delta2				27.4			27.4
H1.gamma	17.1						17.1
H1.gamma2-beta-like		0.9					0.9
H1.pandemic			6				6
H3.IV-A					0.9	41	41.9
NA.Total	19.7	0.9	6	28.3	0.9	44.4	

NA Clade

Total HA & NA combinations – 117

Region 2

Percentage of HA and NA combinations − Oct 2020 to Sep 2021

HA Clade	N1.Classical	N1.Pandemic	N2.1998B	N2.2002	N2.2002A	N2.2002B	N2.2016	N2.TX98	HA.Total
H1.alpha		0.1	0.5	0.1	0.3	2.3		0.4	3.7
H1.delta1a					0.1	2.5		0.3	2.9
H1.delta1b					2.6	0.1			2.7
H1.delta2		0.1	20.1			0.3			20.5
H1.gamma	31.2	0.1	0.1		0.1	0.1			31.6
H1.pandemic	0.6	7.1			0.3	0.3			8.3
H3.2010.1		0.4	0.3		2.3	9.6			12.6
H3.2010.2							0.6		0.6
H3.I								0.4	0.4
H3.IV				0.3					0.3
H3.IV−A			0.1	0.1	1	14.9			16.1
H3.IV−B						0.1			0.1
H3.IV−I			0.1						0.1
NA.Total	31.8	7.8	21.2	0.5	6.7	30.2	0.6	1.1	

NA Clade

Total HA & NA combinations – 773

Region 3

Percentage of HA and NA combinations – Oct 2020 to Sep 2021

HA Clade	N1.Classical	N1.MN99	N1.Pandemic	N2.1998B	N2.2002A	N2.2002B	N2.2016	N2.TX98	HA.Total
H1.alpha					4.3	2.9			7.2
H1.delta1b					10	4.3			14.3
H1.delta2				7.1					7.1
H1.gamma	5.7								5.7
H1.gamma2–beta–like		1.4						1.4	2.8
H1.pandemic			22.9						22.9
H3.2010.1		1.4		1.4	15.7	12.9			31.4
H3.2010.2							1.4		1.4
H3.IV–A						7.1			7.1
NA.Total	5.7	2.8	22.9	8.5	30	27.2	1.4	1.4	

NA Clade

Total HA & NA combinations – 70

Region 4

Percentage of HA and NA combinations – Oct 2020 to Sep 2021

HA Clade	N1.Classical	N1.MN99	N1.Pandemic	N2.1998A	N2.1998B	N2.2002	N2.2002A	N2.2002B	N2.TX98	HA.Total
H1.alpha								4.3		4.3
H1.delta1a			1.4					2.9		4.3
H1.delta1b		1.4					5.7		1.4	8.5
H1.delta2					1.4					1.4
H1.gamma	25.7						1.4			27.1
H1.gamma2–beta–like		4.3								4.3
H1.pandemic			10				1.4		1.4	12.8
H3.2010.1		1.4					10	14.3		25.7
H3.IV–A							4.3	2.9		7.2
H3.IV–B								1.4		1.4
H3.IV–I					1.4	1.4				2.8
NA.Total	25.7	7.1	10	1.4	2.8	1.4	22.8	25.8	2.8	

NA Clade

Total HA & NA combinations – 70

Region 5

Percentage of HA and NA combinations – Oct 2020 to Aug 2021

HA Clade	N1.Pandemic	N2.1998A	N2.1998B	N2.2002B	HA.Total
H1.alpha				23.1	23.1
H1.delta1a		15.4			15.4
H1.delta2			15.4		15.4
H1.pandemic	30.8				30.8
H3.2010.1				15.4	15.4
NA.Total	30.8	15.4	15.4	38.5	

NA Clade

Total HA & NA combinations – 13