United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

May 2021

Influenza A Virus in Swine Surveillance

Fiscal Year 2021 Quarterly Report

Surveillance Summary for Second Quarter Fiscal Year 2021: January 1 to March 31, 2021

Report Summary[1]

- This report covers the second quarter (Q2) of fiscal year (FY) 2021 from January 1 through March 31, 2021.
- Where relevant, the report also includes previous years' data for historical perspective.
- The report provides data from both national and regional levels.
- In Q2 of FY2021, there were 468 samples submitted for influenza A virus (IAV) surveillance in swine from 442 accessions.
- H1N1 was the predominant subtype reported in USDA data in Q2 FY2021.
- Over the past 8 quarters, H1N2 was the main subtype in Regions 1, 3 and 5, H1N1 in Region 2 and Region 4 isolated H3N2 most frequently.
- 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 May 14, 2021, 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 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 does not entirely represent the total U.S. domestic swine population. Therefore, the data cannot be used to determine

[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.

IAV-S prevalence or other epidemiologic measures in the swine population. However, the data may help identify trends of influenza 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 genomic 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 FY2016 resulted in decreased laboratory accessions and samples, but a higher percentage of accessions resulting in a virus isolate that can be sequenced and analyzed. Through Q2 FY2021, a total of 1,100 samples have been tested from 1,006 accessions (Figure 1), with 468 samples being tested from 442 accessions in Q2. Figure 2 shows the overall trends in total accessions, rRT-PCR and VI positive accessions, and subtyped accessions.

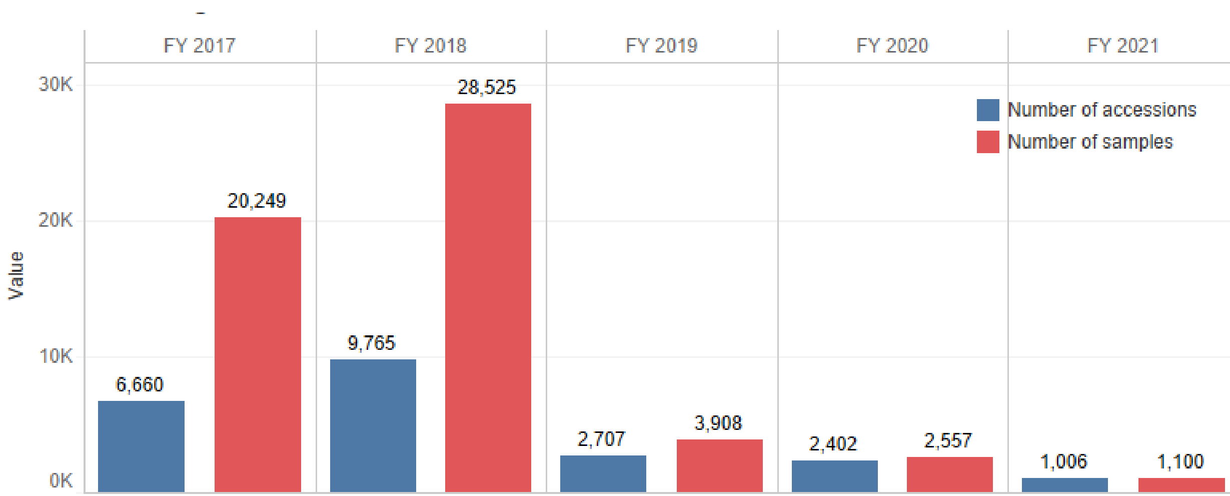


Figure 1. Number of IAV laboratory accessions and samples tested in swine FY2017 through Q2 FY2021

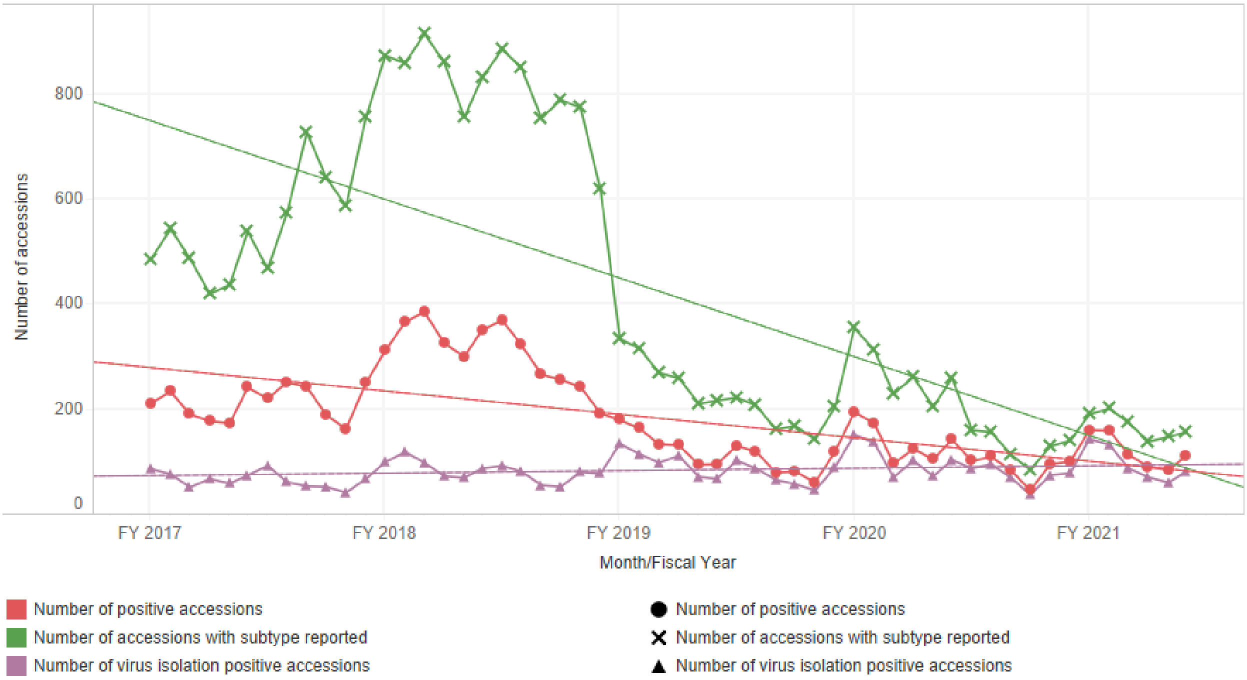


Figure 2. Accessions submitted, subtyped accessions, rRT-PCR positive accessions, and virus isolation positive accessions over time with trend lines for IAV-S, FY2017 through Q2 FY2021

Figure 3 shows the number of subtype detections in Q2 FY2021. The total number of samples subtyped was 231, including H1N1 (n=84), H1N2 (n=62), H3N2 (n=79), H3N1 (n=1), and mixed (n=5).

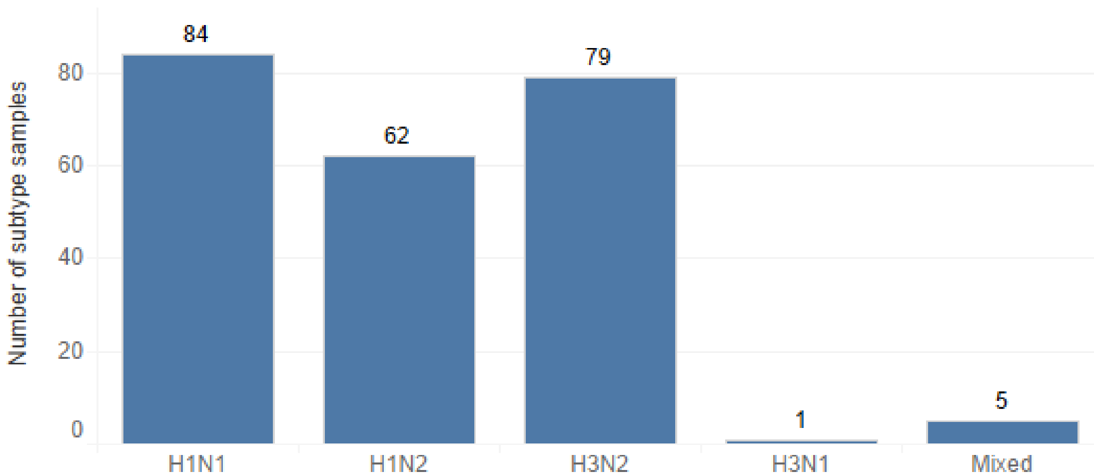


Figure 3. Number of IAV-S subtype detections in Q2 FY2021

Figure 4 breaks down accessions by rRT-PCR subtype from FY2017 through Q2 FY2021. H1N1 was the predominant subtype detected in 2018, 2020 and through Q2 FY2021. H1N2 was detected most often in 2017 and 2019.

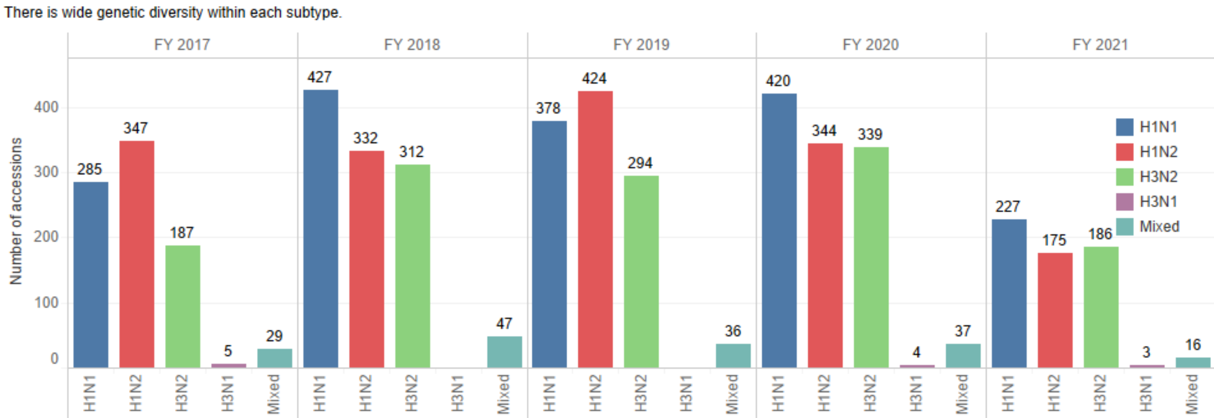


Figure 4. Breakdown of accessions by subtype rRT-PCR from FY2017 through Q2 FY2021

Figure 5 displays the number of times VI was conducted in blue, the number of times VI was conducted and was positive in purple, and the number of viral isolates submitted to GenBank in green. Since the implementation of the June 2016 modifications to the program, almost all VIs attempted now yield a virus and the sequences are submitted to Genbank for analysis.

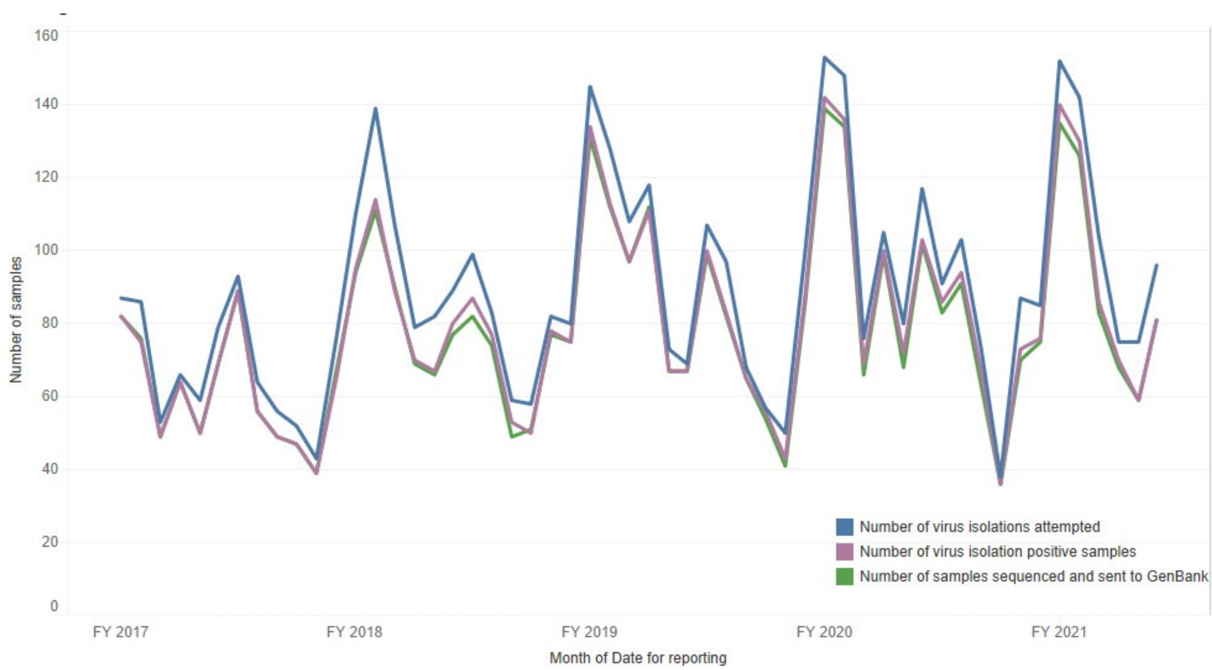


Figure 5. Number of virus isolations attempted, positive virus isolations, and GenBank submissions from FY2017 through Q2 FY2021

Laboratory accessions were evaluated by age-class for the second quarter. The most common subtype isolated among the nursery class was H1N1. Among the suckling age class, H1N1 and H1N2 were the predominant isolated subtypes. The sow/boar class had limited testing, with one isolate of H3N2 reported. Among isolates for which the age class was unknown or not recorded, H1N2 was the predominant subtype. The grow/finish class had H3N2 isolated the most. (Table 1). Excluding specimen types with less than 10 percent of total submissions, lung samples 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 FY2021

Age Class (group)	Number of H1N1	Number of H1N2	Number of H3N1	Number of H3N2	Number of Mixed
Suckling	21	21	1	20	0
Nursery	36	22	0	32	2
Sow/Boar	0	0	0	1	0
Not Recorded/Unknown	8	9	0	4	1
Grow/Finish	18	10	0	22	2

Table 2. Number of positive accessions* tested for IAV-S by specimen type and by viral subtype, Q2 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	222	76%	82	57	1	77	5	201
Nasal or Nasal Swab	7	83%	2	3	0	2	0	6
Oral Fluids	2	100%	0	2	0	0	0	2

*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 in five different regions (Figure 6). These regions are based on former USDA administrative districts only and do not represent specific industry distribution. Submissions are voluntary, as is any identifying information accompanying the submission (except the State of animal origin), and therefore no sampling strategies can be applied to the regions.

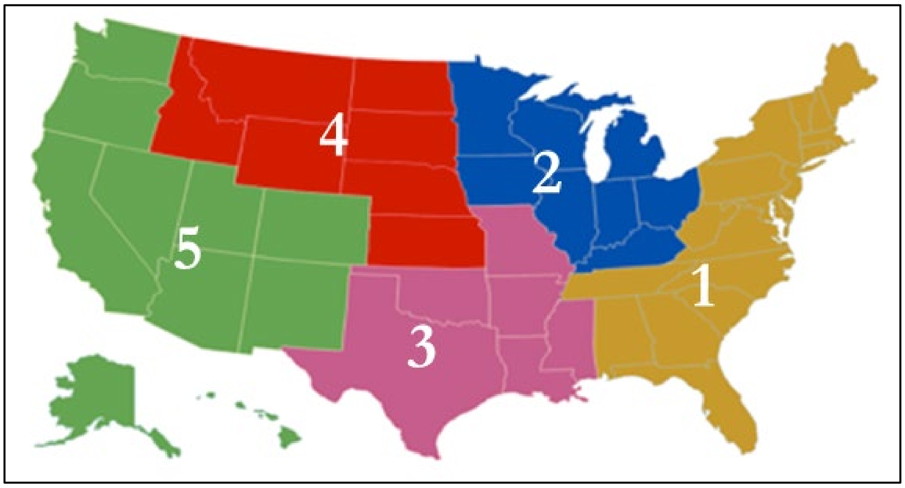


Figure 6. A map of the regions for national IAV-S surveillance

Table 3. Summary of predominant subtypes by region in a 1 year window from April 2020 through March 2021.

Region	Total number HA/NA pairs*	Most Predominant HA/NA subtype
1	117	H3N2 (H3-Cluster IV-A / N2-2002) (n=40) H1N2 (H1-Delta2 / N2-1998) (n=31) H1N1 (H1-Gamma / N1-Classical) (n=20) H1N1 (H1-Pandemic / N1-Pandemic) (n=12)
2	779	Most diversity of all regions H1N1 (H1-Gamma / N1-Classical) (n=226) H1N2 (H1-Delta2 / N2-1998) (n=143) H3N2 (H3-Cluster IV-A / N2-2002) (n=124) H3N2 (H3-2010.1 / N2-2002) (n=76)
3	81	H1N1 (H1-Pandemic / H1-Pandemic) (n=17) H3N2 (H3-2010.1 / N2-2002) (n=16) H1N2 (H1-Delta2 / N2-1998) (n=9) H1N2 (H1-Delta1b / N2-2002) (n=8)
4	63	H3N2 (H3-2010.1 / N2-2002) (n=15) H1N1 (H1-Gamma / N1-Classical) (n=12)
5	18	Low participation H1N2 (H1-Alpha / N2-2002) (n=6) H3N2 (H3-2010.1 / N2-2002) (n=5)

Most Predominant HA/NA phylo-types overall:
H1N1 (H1-Gamma / N1-Classical) (n=226)
H1N2 (H1-Delta2 / N2-1998) (n=143)
H3N2 (H3-Cluster IV-A / N2-2002) (n=124)
H3N2 (H3-2010.1 / N2-2002) (n=76)

*HA/NA pairs are included in count if they comprise over 10% and more than 5 observations from a region

Summary of Regional Data from ARS

Figure 7 shows the distribution of rRT-PCR subtyped accessions among the five regions for Q2 FY2019 through Q2 FY2021. Regions 1, 3 and 5 demonstrate H1N2 as the predominant subtype, Region 2 demonstrates H1N1 as predominant and Region 4 isolated H3N2 most frequently. For regions recorded as "unknown," H1N2 was the only subtype found with an n of 1.

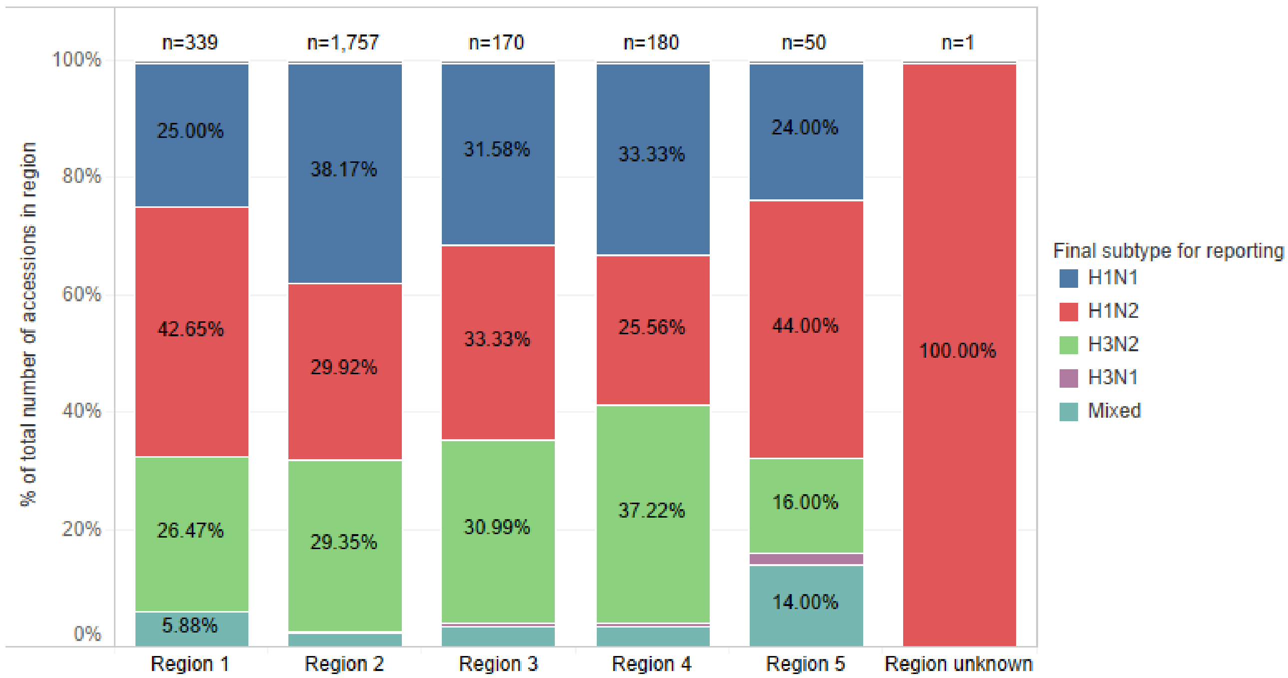


Figure 7. Distribution of rRT-PCR subtyped accessions among the five regions Q2 FY2019 through Q2 FY2021

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 200 isolates with published sequences in GenBank was characterized by phylogenetic analysis for the Q2 FY2021 report. This analysis provides information on the genetic diversity and evolution 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 subtypes for each region, followed by charts further describing the phylogenetic clades of H1 and H3 subtypes. Regional charts depicting the various combinations of HA and NA are in Appendix 1.

[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.

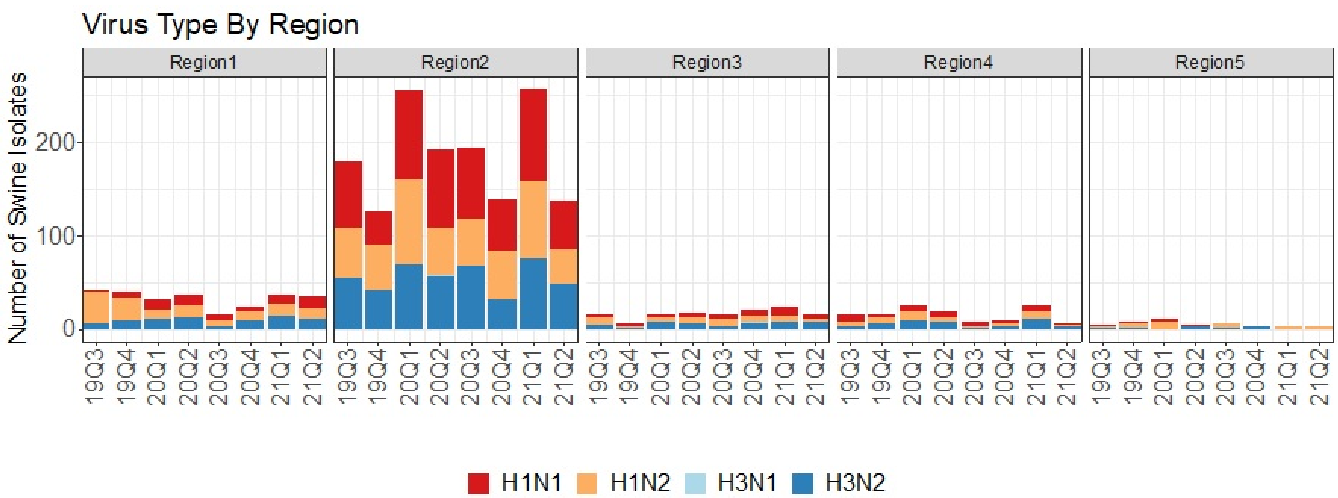


Figure 8. Temporal distribution of Influenza A virus subtype by region for Q3 FY2019 to Q2 FY2021

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 and 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 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 FY2021: H1-Gamma remained the predominant H1 (Figure 9); H1-LAIV/N1-MN99 observed in North Carolina; and H1-delta1b/N2-TX98 was observed in Nebraska.

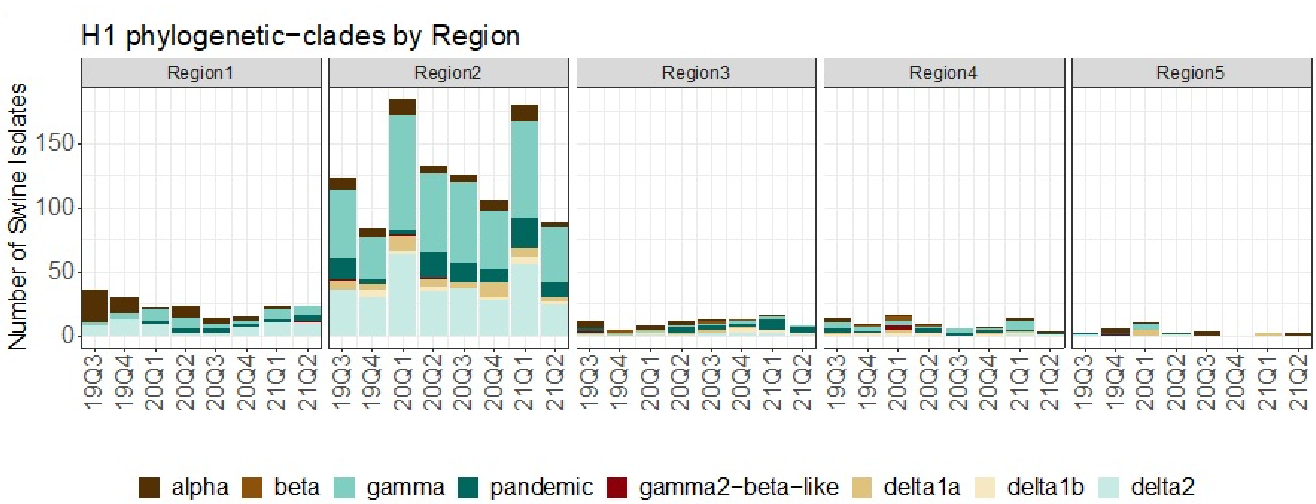


Figure 9. Temporal distribution of H1 phylogenetic clades by region for Q3 FY2019 to Q2 FY2021

In Q2 FY2021: H3-Cluster IV-A was the predominant H3 (Figure 10); only one observation of H3-2010.2/N2-2016; and H3-Cluster I/N2-TX98 observed in Illinois and Iowa.

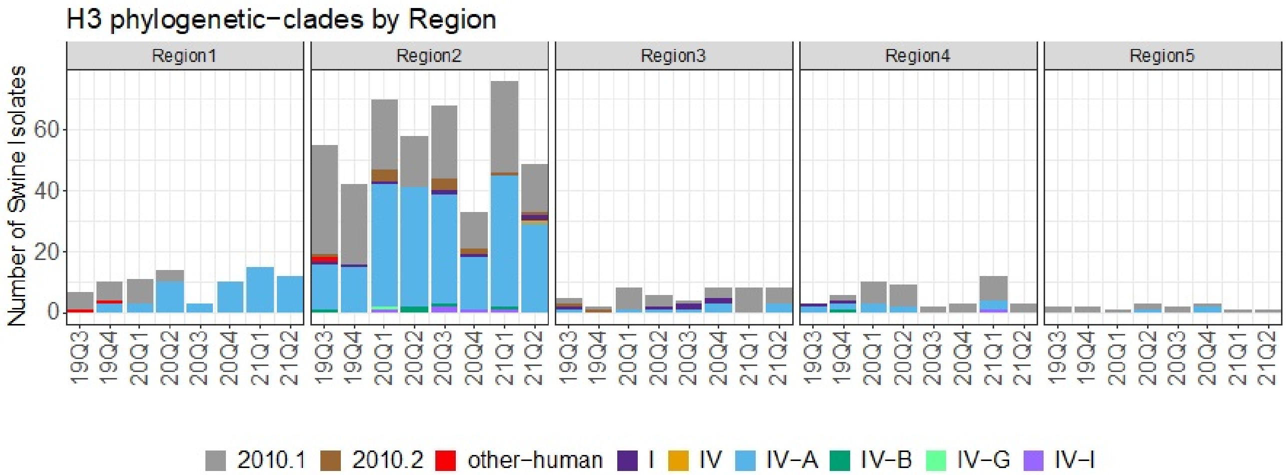


Figure 10. Temporal distribution of H3 phylogenetic clades by region for Q3 FY2019 to Q2 FY2021

National phylogenetic NA gene information

Whole genome sequencing with HA/NA pairs with internal gene lineages were still pending at the time this report was created.

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 IAV swine surveillance 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 FY2021, the NVSL Diagnostic Virology Laboratory provided 48 isolates to four academic institutions, two government requestors, and 1 pharmaceutical requestor. NVSL received 298 isolates into the repository in Q2 FY2021. Table 4 reports the total number of virus isolates received into the repository each year from FY2014 through Q2 of FY2021. Table 5 reports the total number of isolates available in the repository by subtype for sharing.

Table 4. Virus isolates received in repository

Virus isolates in the repository	
FY2021	617
FY2020	1,074
FY2019	1,055
FY2018	994
FY2017	844
FY2016	1,046
FY2015	883
FY2014	765
TOTAL TO DATE	7,278

Table 5. Total number of subtyped isolates available through repository from 2009-Present

Subtyped isolates available through repository	
H3N2	2,462
H3N1	22
H1N1	3,173
H1N2	2,941
Mixed	302
TOTAL	8,900

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.

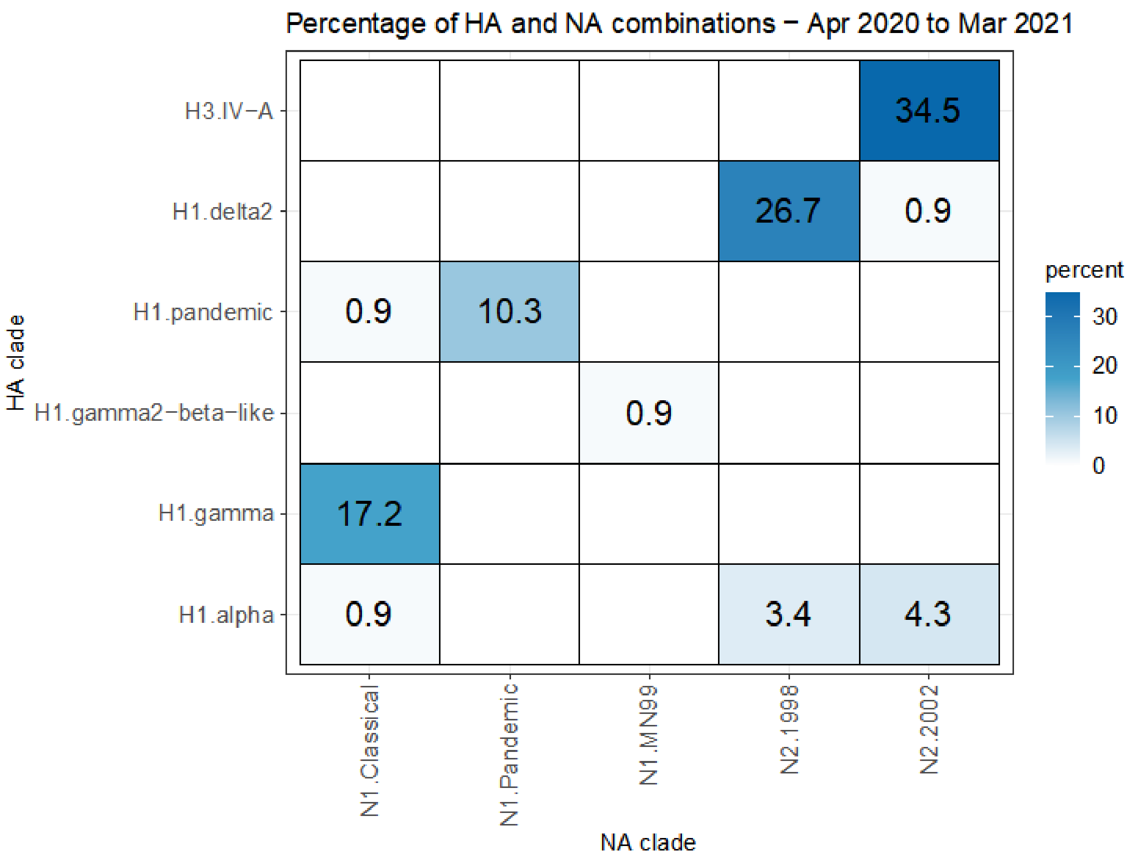
Conclusion

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

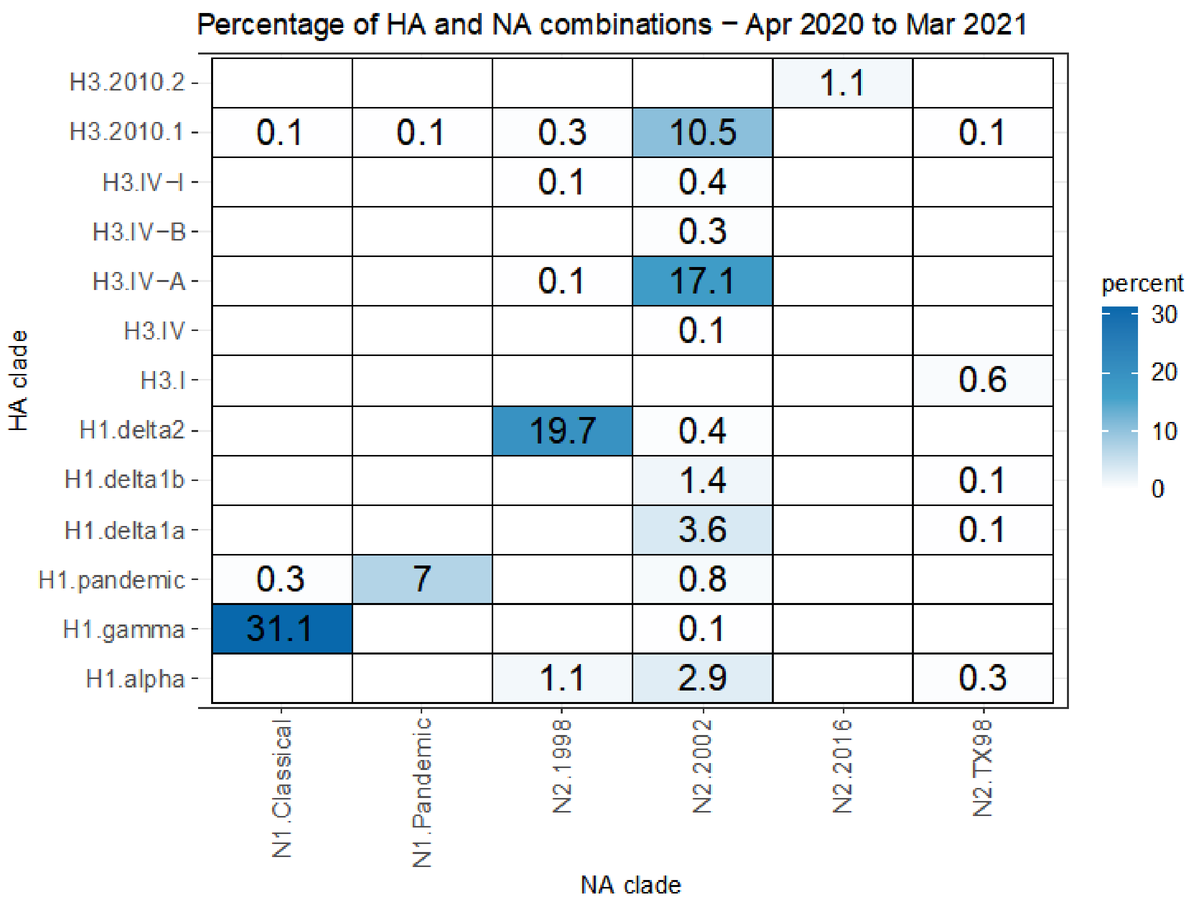
The following charts present the percentages of combinations of HA and NA on the national and regional scales based on ARS-NADC phylogenetic analyses. The results are reported from April 2020 through March 2021. These “heat maps” represent the percentage of combinations by using a color gradient where 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

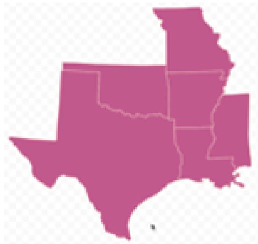


Total HA & NA combinations – 116

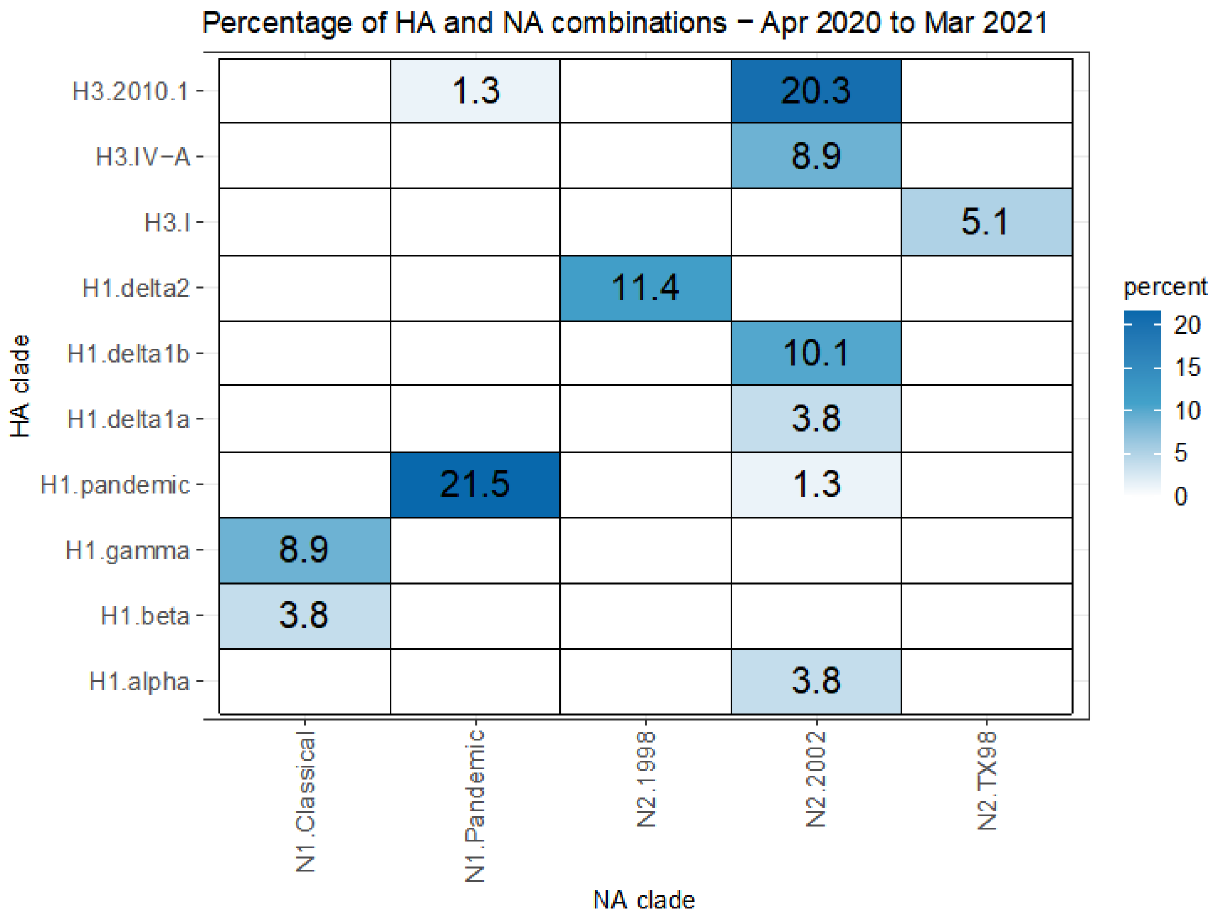
Region 2



Total HA & NA combinations – 726

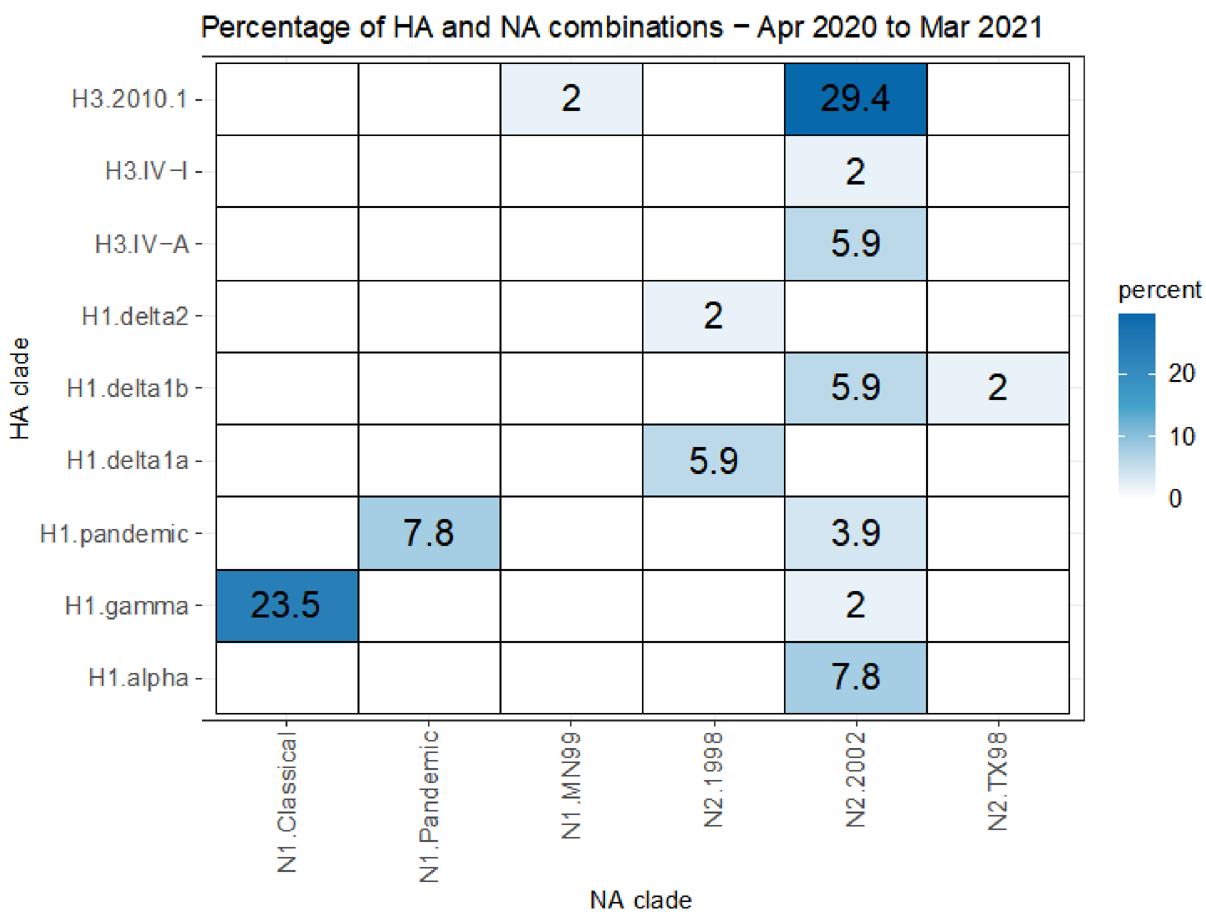


Region 3



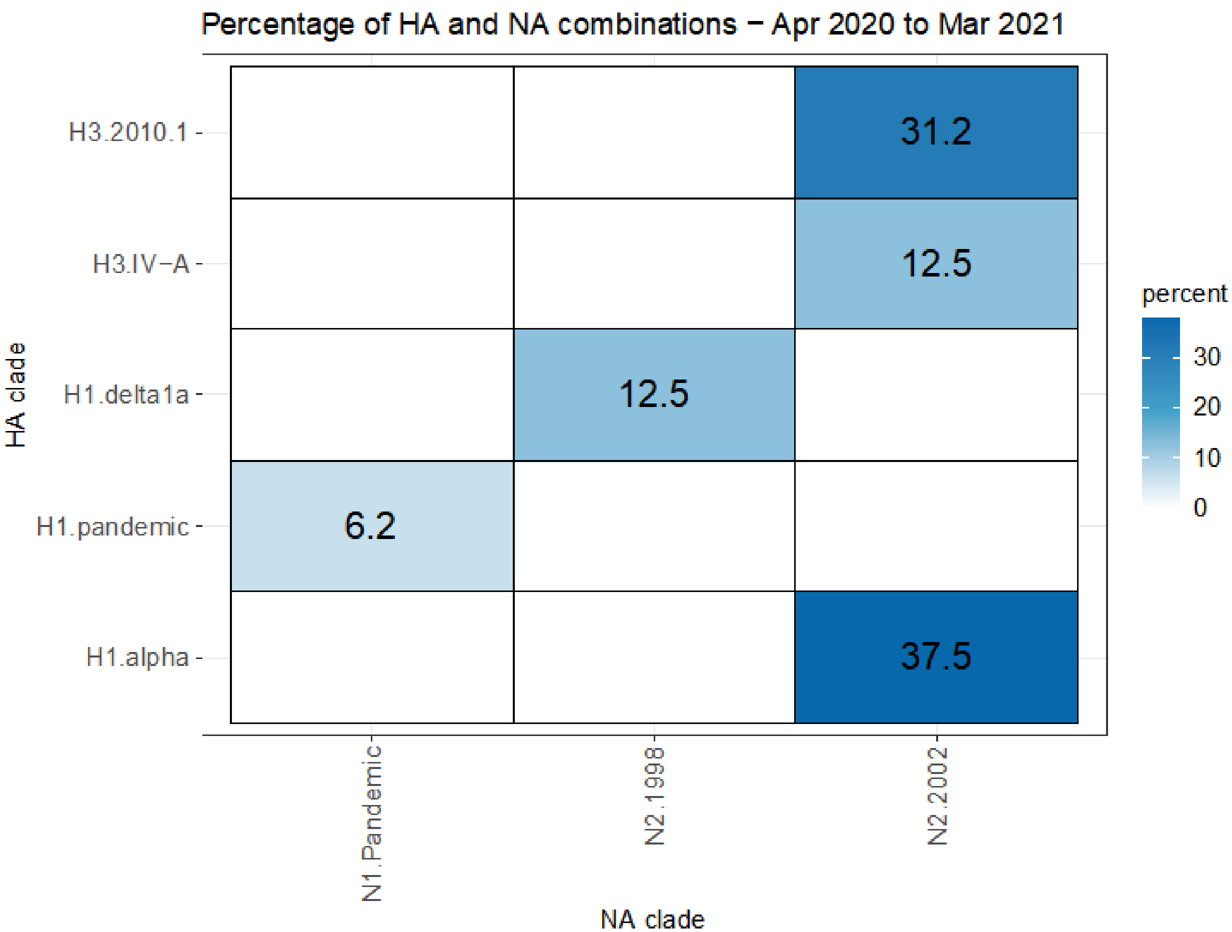
Total HA & NA combinations – 79

Region 4



Total HA & NA combinations – 51

Region 5



Total HA & NA combinations – 16