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Veterinary
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July 2020

Influenza A Virus in Swine Surveillance

Fiscal Year 2020 Quarterly Report

Surveillance Summary for Second Quarter Fiscal Year 2020: January 1 to March 31, 2020

Report Summary[1]

- This report covers the second quarter (Q2) of fiscal year 2020 from January 1 to March 31, 2020.
- Where relevant, the report also includes previous years' data for historical perspective.
- The report provides data from both national and regional levels.
- In fiscal year 2020 Q2, there were 1,709 samples submitted for influenza A virus (IAV) surveillance in swine from 1,620 accessions.
- H1N1was the predominant subtype reported in USDA data in the first half of FY2020.
- Over the past 7 quarters, H1N2 was the main subtype in Regions 1, 3 and 5 while Region 2 and Region 4 most frequently isolated H1N1 and H3N2, respectively. For regions recorded as "unknown," H1N2 was the most frequent subtype.
- 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 15, 2020, 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

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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.

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 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:

https://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/downloads/appendix_c_testing_guidelines.pdf

and

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 fiscal year 2010 through fiscal year 2016, the total number of accessions and samples submitted increased. Changes initiated in fiscal year 2016 resulted in decreased laboratory accessions and samples, but a higher percentage of accessions resulting in a virus isolate that can be sequenced and analyzed. In fiscal year 2020, 1,709 samples have been tested from 1,620 accessions (Figure 1). Figure 2 shows the overall increasing trends in total accessions, rRT-PCR and VI positive accessions, and subtyped accessions.
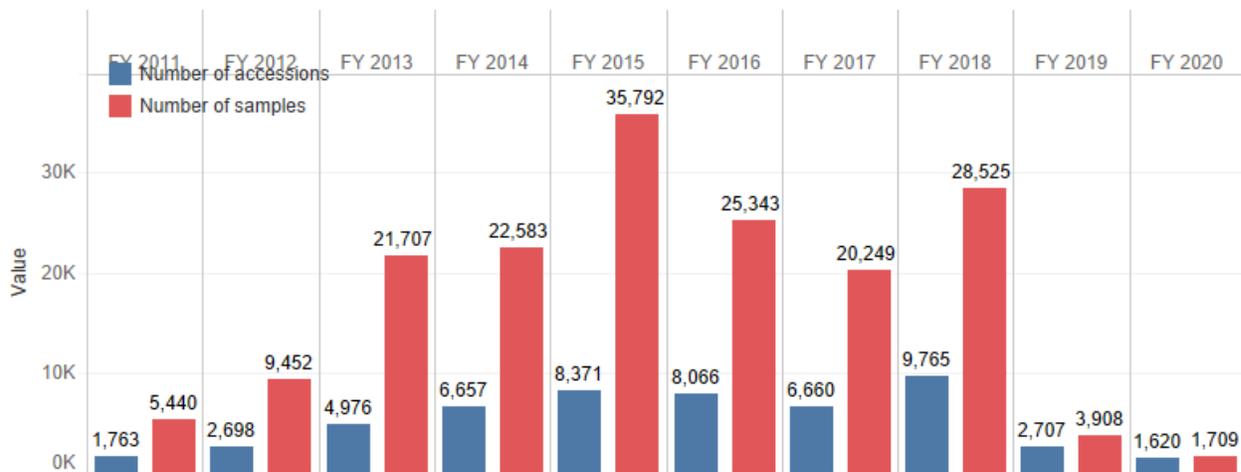

Figure 1. Number of IAV-S laboratory accessions and samples tested in swine, fiscal year 2011 through fiscal year 2020 Q2.
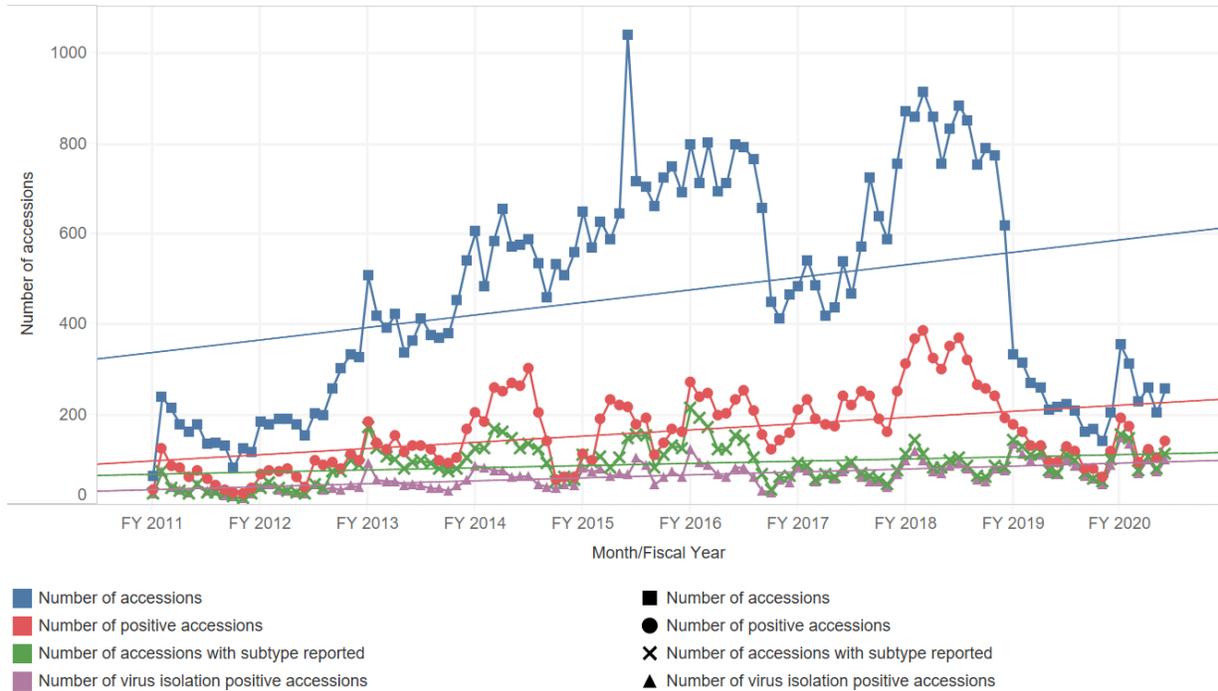
Figure 2. Accessions submitted, subtyped accessions, rRT-PCR positive accessions, and virus isolation positive accessions over time with trend lines for influenza A virus in swine, fiscal year 2011 to fiscal year 2020 Q2.

Figure 3 shows the number of subtype detections in fiscal year 2020 Q2. The total number of samples subtyped was 306, including H1N1 (n=111), H1N2 (n=82), H3N2 (N=103), H3N1 (n=2), and mixed (N=8).
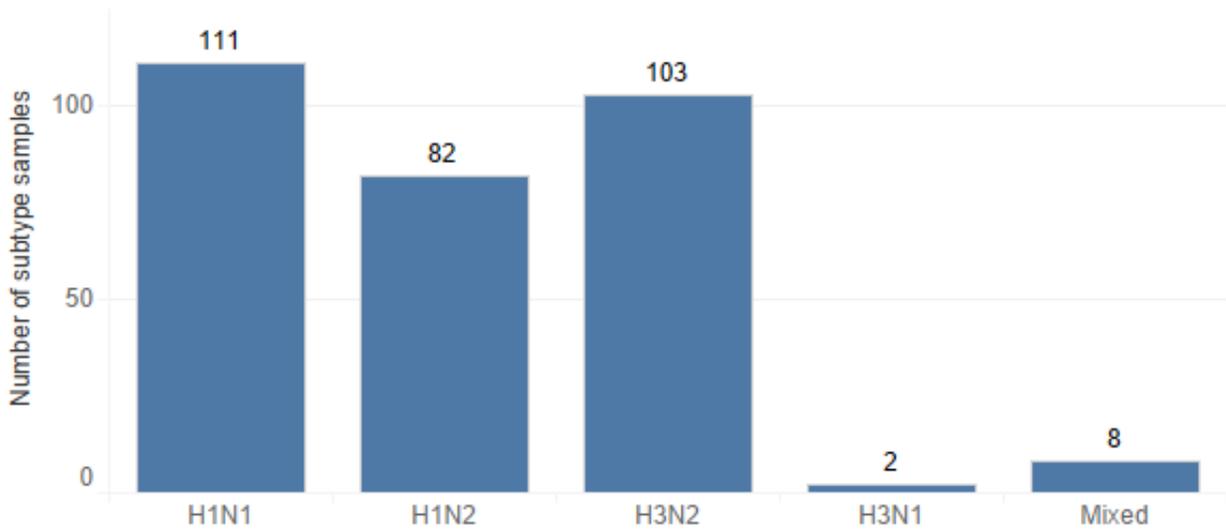
Figure 3. Number of influenza A subtype detections in swine fiscal year 2020 Q2.

Figure 4 breaks down accessions by rRT-PCR subtype from fiscal year 2011 to fiscal year 2020 Q2. H1N1 was the predominant subtype detected in 2012, 2013, 2014, 2018, and 2020. H1N2 was detected most often in 2011, 2015, 2016, 2017, and 2019.
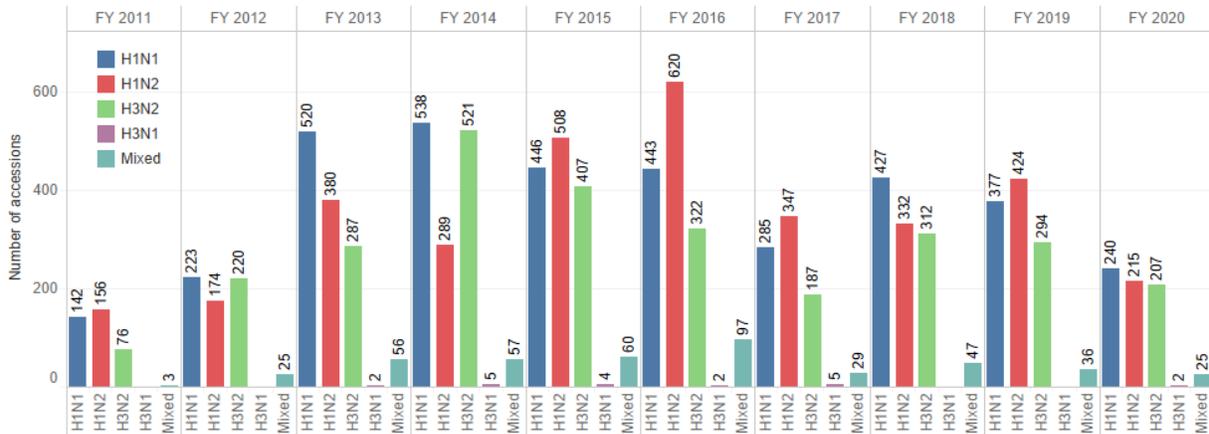
Figure 4. Number of subtypes, fiscal year 2011 through fiscal year 2020 Q2.

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. Due to an unresolvable data processing coding error, samples sequenced and sent to Genbank during FY2019 Q1 appear greatly reduced in Figure 5. As with other fiscal years since FY2016, the samples sent during that timeframe closely follow the number of attempted virus isolations as well as the number of positive virus isolation samples.
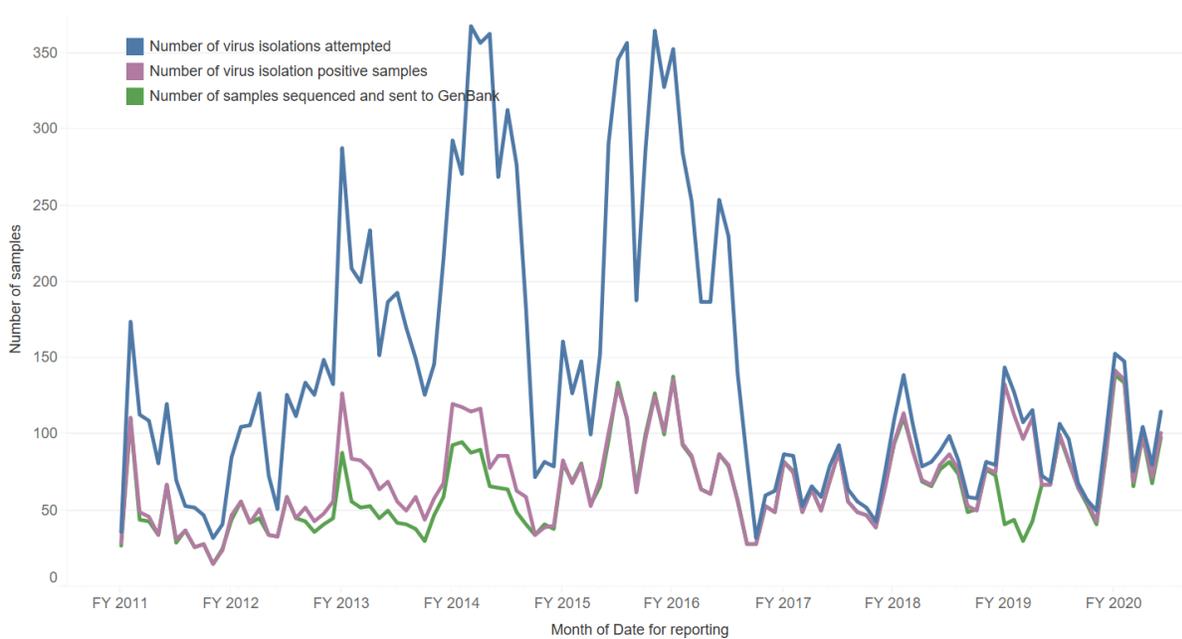
Figure 5. Number of influenza A virus isolations attempted, number of virus isolations that were positive, and the number of viruses submitted to GenBank from fiscal year 2011 to fiscal year 2020 Q2.

Laboratory accessions were evaluated by age-class for the second quarter. The most common subtype isolated among the suckling age class was H3N2 and among the nursery age class it was H1N2. Among the grow-finish age class, H1N1 was the predominant isolated subtype. The sow/boar class had limited testing, with one isolate of H1N1 and one isolate of H3N2. Among isolates for which the age class was unknown or not recorded, H3N2 was the predominant subtype (Table 1). Nasal specimens (swabs or

wipes) were the most successful at providing a virus isolate which enables sequence submission to GenBank (Table 2).

Table 1. Number of positive accessions tested for influenza A virus in swine by age class and subtype fiscal year 2020, Q2.

Age class	Number of accessions with subtype reported	Number of H1N1	Number of H1N2	Number of H3N1	Number of H3N2	Number of Mixed
Suckling	71	24	19	1	28	0
Nursery	126	37	42	1	40	7
Grow/finish	58	33	10	0	15	0
Sow/Boar	2	1	0	0	1	0
Unknown	47	16	11	0	18	1

Table 2. Number of positive accessions* tested for influenza A virus in swine by specimen type** and subtype fiscal year 2020 Q2.

Specimen Type	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	253	90%	100	65	2	82	6	230
Nasal	39	92%	8	17	0	13	0	35
Oral Fluid	11	100%	3	0	0	6	2	9
Other	1	100%	0	0	0	1	0	2

*Accessions may include samples with multiple specimen types. In these cases, individual accessions are counted in more than one specimen type category.

** Other includes specimen types recorded as swab, mixed tissue, or unknown.

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.

Summary of Regional Data from ARS

Table 3. Summary of predominant subtypes in each region in a 2 year window (fiscal year 2018 Q3 through fiscal year 2020 Q2).

Region	Total number	HA/NA subtype
1	272	Gamma H1/Classical N1
		Delta2 H1/1998-N2
		2010.1 H1/2002-N2
2	1,367	Gamma H1/Classical N1
		2010.1 H3/2002-N2
		Delta2 H1/1998-N2
3	155	Alpha H1/2002-N2
		Gamma H1/Classical N1
		2010.1 H3/2002-N2
4	169	IV-A H3/2002-N2
		Gamma H1/Classical N1
		2010.1 H3/2002-N2
5	49	Low participation
		2010.1 H3/2002 N2
		Alpha H1/2002-N2
		Delta1a H1/1998 N2

Most Predominant HA/NA phylotypes overall: H1N1 (Gamma H1/Classical N1), H3N2 (2010.1 H3/2002-N2) and H1N2 (Delta2 H1/1998-N2)

Figure 7 shows the distribution of rRT-PCR subtyped accessions among the five regions for Q4 fiscal year 2018 through Q2 fiscal year 2020. Regions 1, 3 and 5 demonstrate H1N2 as the predominant subtype while Region 2 and Region 4 isolated H1N1 and H3N2 most frequently, respectively. For regions recorded as "unknown," H1N2 was the most frequent subtypes.
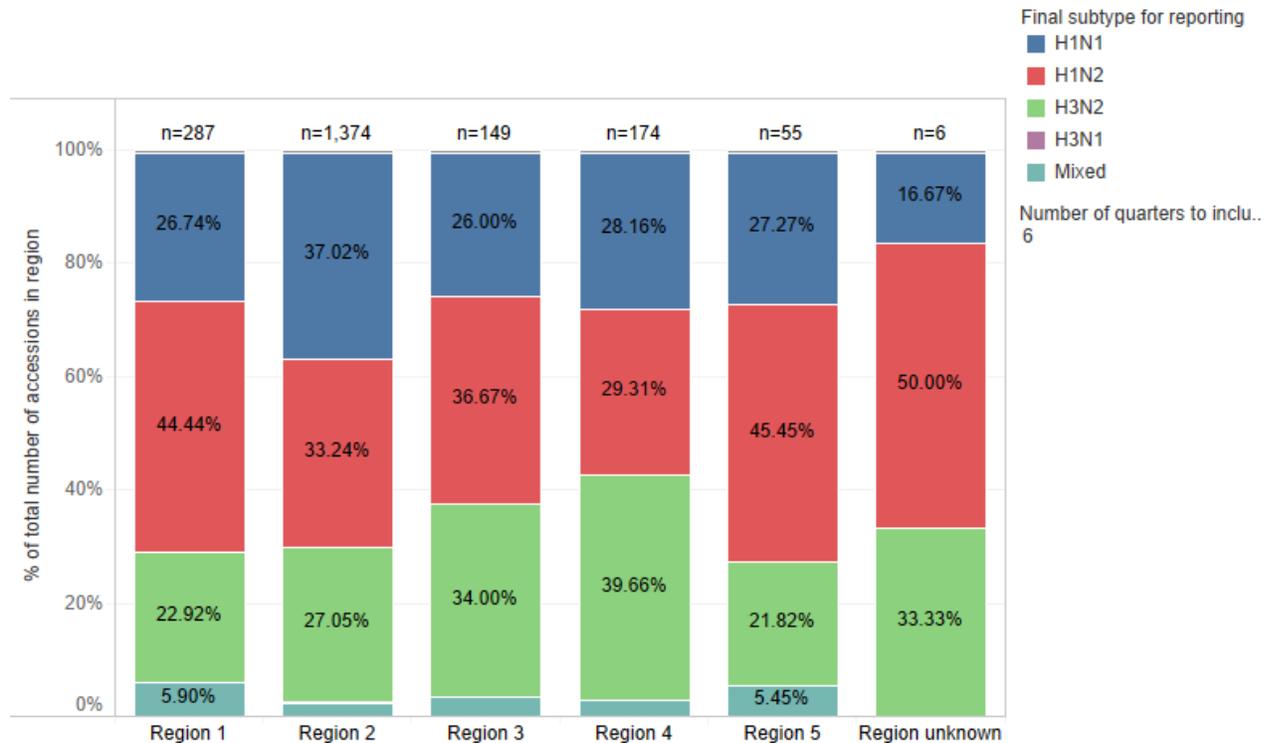

Figure 7. Percentage of influenza A subtyped accessions for swine by region for fiscal year 2018 Q2 to fiscal year 2020 Q2.

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 379 isolates with published sequences in GenBank was characterized by phylogenetic analysis for the Q2 fiscal year 2020 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.

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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 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.
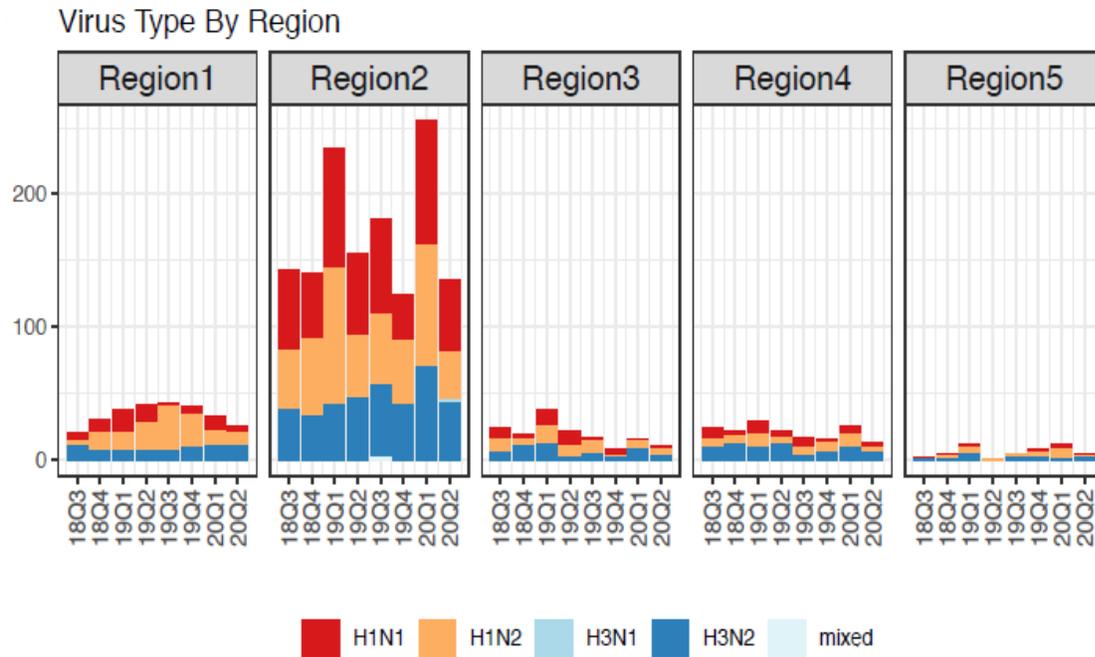

Figure 8. Temporal distribution of Influenza A virus subtype by region for Q3 fiscal year 2018 to Q2 fiscal year 2020

Figure 8 demonstrates the four subtypes H1N1, H1N2, H3N2 and mixed subtypes 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 fiscal year 2020, Q2, the H1 subtypes gamma viruses are the predominant H1, with a total of 122 detected, with delta2 and alpha also frequently being detected. Gamma2-beta-like continue to be detected at low levels and is paired with MN99 N1 (Figure 9).

H1 phylogenetic–clades by Region
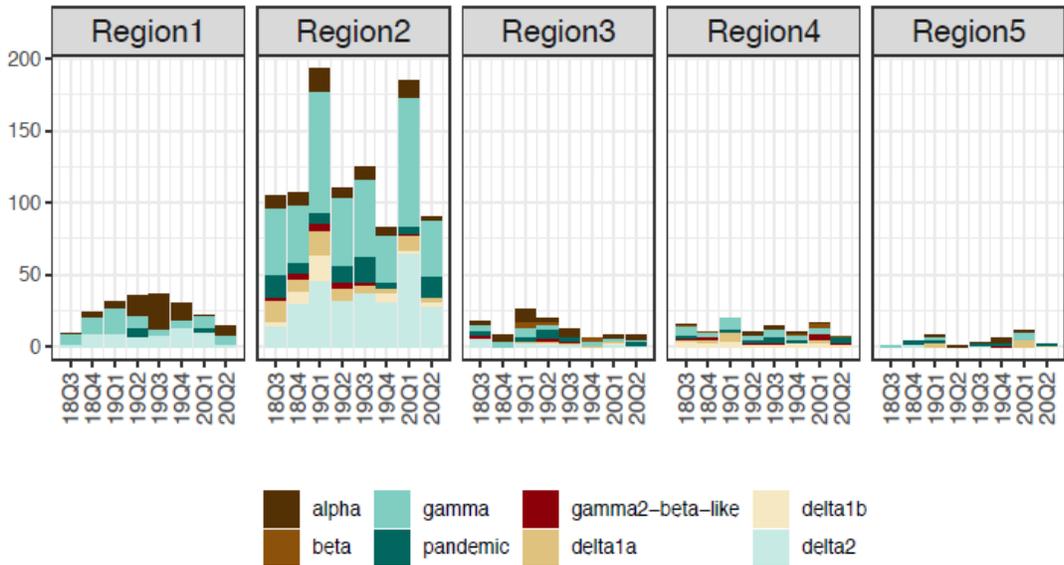
Figure 9. Temporal distribution of H1 phylogentic clades by region for fiscal year 2018 Q3 to fiscal year 2020 Q2.

In Q2 fiscal year 2020, there were 67 detections of H3s and human-like_2010.1 H3 was the predominant H3 (Figure 10). 2010.2 H3 was detected in Arkansas, Iowa, Illinois, Indiana, Ohio, and Oklahoma and is paired with human seasonal N2. Cluster I H3 continues to be detected at low levels. TX98 N2 detected at low levels and predominantly paired with H3 Cluster I. Human-seasonal N2 is paired with a human-seasonal H3

H3 phylogenetic–clades by Region
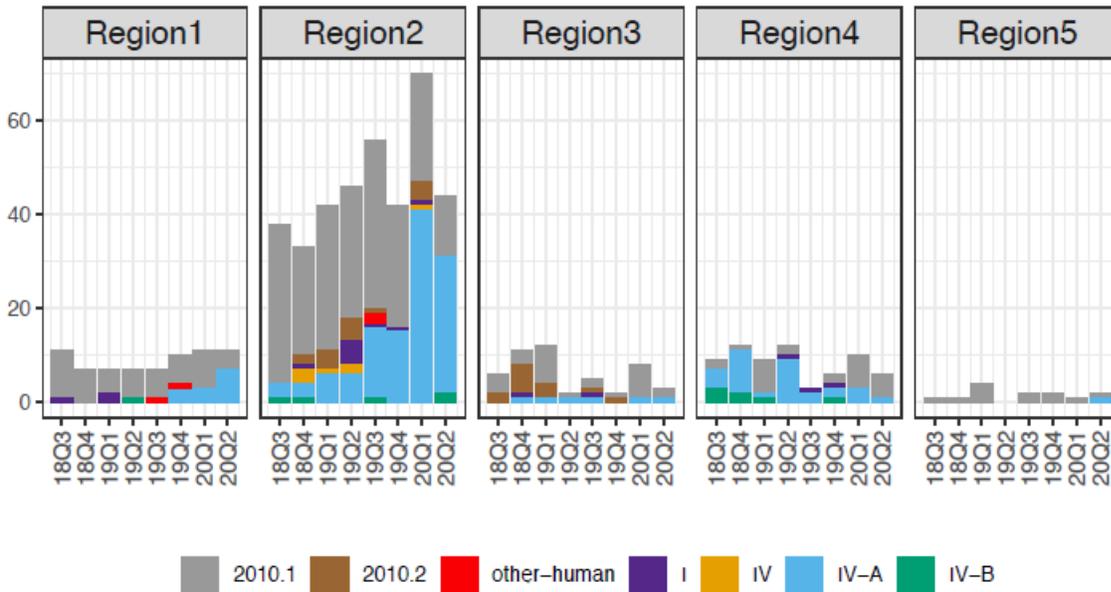
Figure 10. Temporal distribution of H3 phylogentic clades by region for fiscal year 2018 Q3 to fiscal year 2020 Q2.

National phylogenetic NA gene information
Whole genome patterns with HA/NA pairs were reported and the dominant patterns are in descending order below with most frequent pattern by HA/NA pair:
- TTTTPT: H3.2010.1/N2-02, H1.gamma/cN1, H1.delta1a/N2-02,
- TTTPPT: H1.delta2/N2-98, H1.gamma/cN1, H3.IVA/N2-02.
- TTPPPT: H1.gamma/cN1, H1.alpha/N2-02, H1.delta1a/N2-02.

Leading vaccine constellation is TVVTPT, with other patterns circulating. Both the N1 and N2 subtypes are found in circulating swine viruses. Classical N1 continued to be the dominant cluster at 81.1 percent; and the 2002-lineage N2 represents 64 percent of N2 collections.

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 the second quarter of fiscal year 2020, the NVSL Diagnostic Virology Laboratory provided 30 isolates to two government, and one academic institutions. NVSL received 323 isolates into the repository in the second quarter. Table 4 reports the total number of virus isolates received into the repository each year FY2014 to FY2020. 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	
2020	600
2019	1,055
2018	994
2017	844
2016	1,046
2015	883
2014	765
TOTAL TO DATE	6,187

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.

Table 5. Total number of subtyped isolates available through repository.

Subtyped isolates available through repository	
H3N2	2,120
H3N1	17
H1N1	2,767
H1N2	2,603
Mixed	302
TOTAL	7,809

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 2018 through March 2020. 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 –272

Percentage of HA and NA combinations – Apr 2018 to Mar 2020

HA Clade	N1.Classical	N1.Pandemic	N2.1998	N2.2002	N2.TX98	N2.Human_N2
H3.other–human	0	0	0	0	0	0.7
H3.2010.1	0	0	0	19.1	0	0
H3.IV–B	0	0	0	0.4	0	0
H3.IV–A	0	0	0	4.8	0	0
H3.I	0	0	0	0	1.1	0
H1.delta2	0	0.4	21.3	0.4	0	0
H1.pandemic	0	2.6	0	0	0	0
H1.gamma	23.5	0	0	0.4	0	0
H1.alpha	0	0	17.6	7.7	0	0

NA clade

percent

20
15
10
5
0
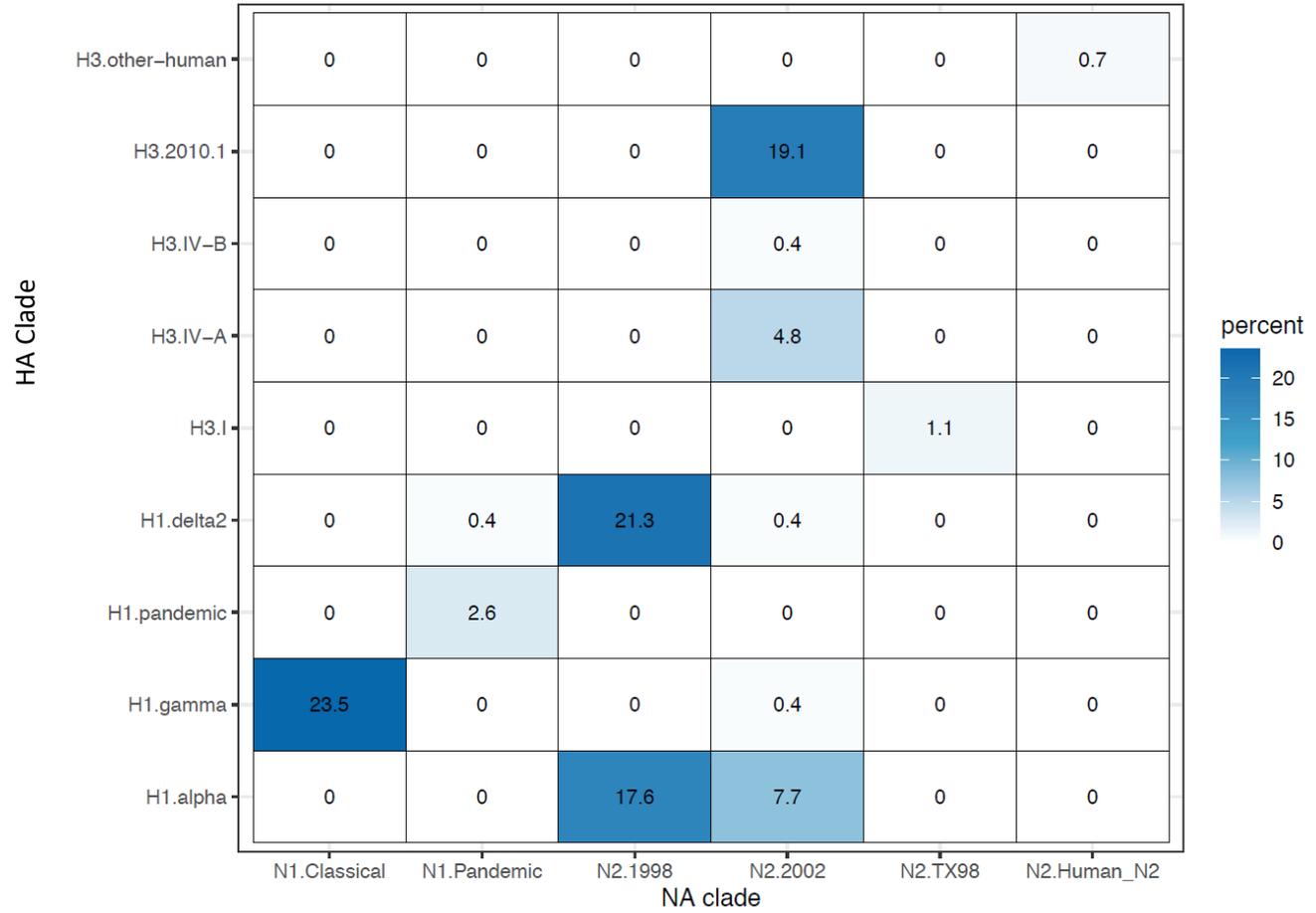
Region 2. Total HA & NA combinations –1,370

Percentage of HA and NA combinations – Apr 2018 to Mar 2020

HA Clade	N1.Classical	N1.Pandemic	N1.MN99	N2.1998	N2.2002	N2.2016	N2.TX98	N2.Human_N2
H3.other–human	0	0	0	0	0	0	0	0.1
H3.2010.2	0	0	0	0	0.1	1.1	0	0
H3.2010.1	0.1	0	0	0.1	15	0	0.4	0
H3.IV–B	0	0	0	0.1	0.3	0	0	0
H3.IV–A	0	0.1	0	0.1	8.5	0	0	0
H3.IV	0	0	0	0	0.5	0	0	0
H3.I	0	0	0	0	0	0	0.7	0
H1.delta2	0.1	0	0	19.4	0.6	0	0.1	0
H1.delta1b	0	0	0	0	2.9	0	0	0
H1.delta1a	0.1	0	0	0.1	5.8	0	0	0
H1.pandemic	0.1	5.2	0	0	0.1	0	0.1	0
H1.gamma2–beta–like	0	0.1	0.8	0	0.1	0	0.4	0
H1.gamma	31.3	0	0	0.1	0.1	0	0.1	0
H1.alpha	0	0.1	0	0.4	4.9	0	0.1	0

NA clade

percent
- 30
- 20
- 10
- 0

Region 3. Total HA & NA combinations - 155

Percentage of HA and NA combinations – Apr 2018 to Mar 2020

HA Clade	N1.Classical	N1.MN99	N1.Pandemic	N2.1998	N2.2002	N2.2016	N2.TX98
H3.IV–A	0	0	0	0	3.9	0	0
H3.I	0	0	0	0	0	0.6	0.6
H3.2010.2	0	0	0	0	0	8.4	0
H3.2010.1	0	0	0	0.6	17.4	0	0
H1.pandemic	0	0	8.4	0	0	0	0
H1.gamma2–beta–like	0	2.6	0	0	0	0	0.6
H1.gamma	12.9	0	0	0	0	0	0
H1.delta2	0	0	0	7.1	0	0	0
H1.delta1b	0	0	0.6	0	0.6	0	0
H1.delta1a	0	0	0	0	6.5	0	0
H1.beta	5.8	0	0	0	0	0	0
H1.alpha	0	0	0	0	23.2	0	0

NA clade

percent

20

15

10

5

0
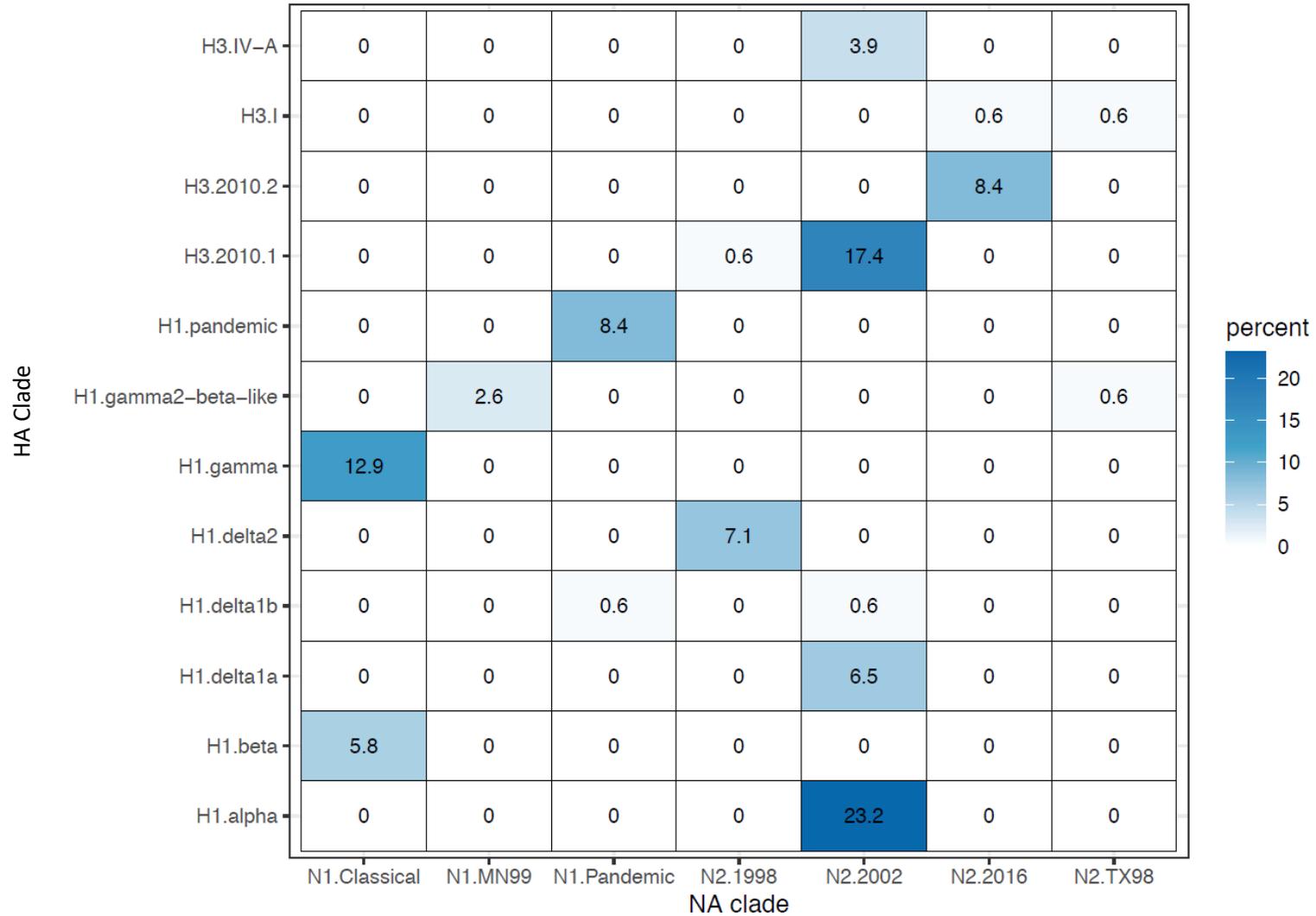
Region 4. Total HA & NA combinations – 169

Percentage of HA and NA combinations – Apr 2018 to Mar 2020

HA Clade	N1.Classical	N1.MN99	N1.Pandemic	N2.1998	N2.2002	N2.TX98
H3.IV–B	0	0	0	0	3.6	0.6
H3.IV–A	0	0	0	0	18.3	0
H3.I	0	0	0	0	0	1.8
H3.2010.1	0	0	0	0	15.4	0
H1.pandemic	0	0	5.3	0	0.6	0
H1.gamma2–beta–like	0	2.4	0	0	1.8	0.6
H1.gamma	17.8	0	0	0	1.8	0
H1.delta1b	0	0	0	0	10.1	0
H1.delta1a	0	0	0	0	10.1	0
H1.beta	4.1	0	0	0	0	0
H1.alpha	0	0	0	0.6	5.3	0

NA clade
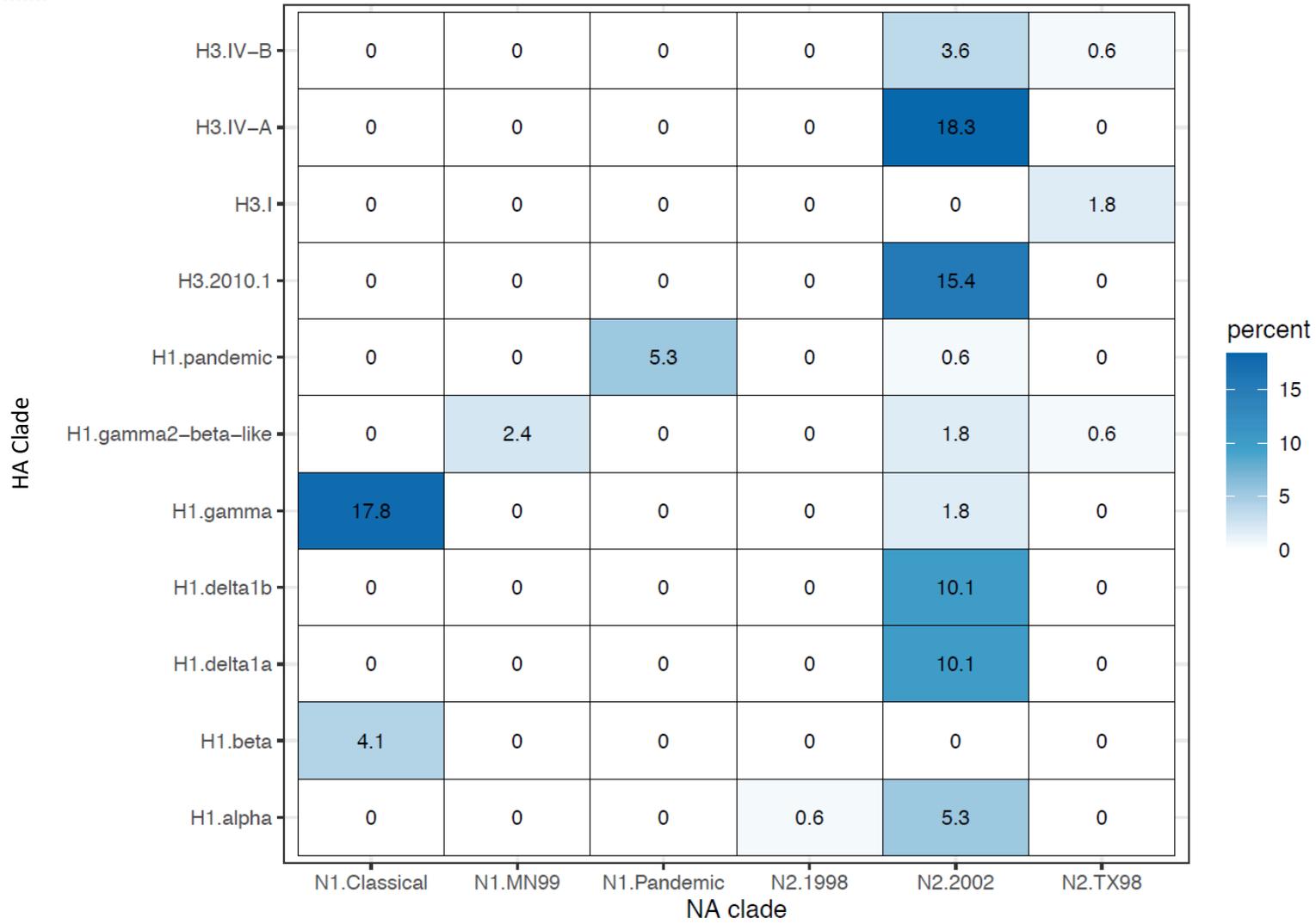
Region 5. Total HA & NA combinations – 49

Percentage of HA and NA combinations – Apr 2018 to Mar 2020

HA Clade	N1.Classical	N1.MN99	N1.Pandemic	N2.1998	N2.2002	N2.TX98
H3.IV–A	0	0	0	0	2	0
H3.2010.1	0	0	0	0	22.4	2
H1.pandemic	2	0	10.2	0	0	0
H1.gamma2–beta–like	0	2	0	0	0	0
H1.gamma	12.2	0	2	0	0	0
H1.delta2	0	0	0	6.1	0	0
H1.delta1b	0	0	0	0	2	0
H1.delta1a	0	0	0	14.3	2	0
H1.alpha	0	0	0	0	20.4	0

percent
- 20
- 15
- 10
- 5
- 0

NA clade