United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

April 2017

Influenza A Virus in Swine Surveillance

Fiscal Year 2017 Quarterly Report

Surveillance Summary for First Quarter FY 2017:
October 1 – December 31, 2016

*** In November 2016, VS modernized the process that prepares and stages lab results data for reporting. As a consequence of this modernization effort 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***

Report Summary

- This report covers the first quarter (Q1) of fiscal year (FY) 2017, from October 1– December 31, 2016.
- Where relevant, the report also includes previous years' data for historical perspective.
- The report provides data from both national and regional levels.
- In this quarter, 2,265 samples were submitted for IAV-S surveillance from 996 accessions.
- The H1N2 was the predominant subtype for all regions.
- Over the past eight quarters, Region 1 demonstrates H1N2 as the predominant subtype. Regions 2 and 3 demonstrate H1N2 as the predominant subtype. H1N2 was the predominant in Region 4, and Region 5 and Region reported as unknown saw H1N1 as the predominant subtype.
- Limited accessions from a region can skew data and lead to misinterpretation. Therefore, less inference can be applied to results from Regions 3 and 5.
- All IAV-S submissions are voluntary and based on clinical case submissions to veterinary diagnostics 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 trends.

Introduction

This report, based on data received as of April 25, 2017, 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 labs. Reporting months are based on the month sample was collected. The USDA-APHIS Web site provides general information about the IAV-S surveillance program at http://www.aphis.usda.gov/animal-health/swine-health.

The IAV-S surveillance program is voluntary and, as a result, the accessions and samples submitted represent a subset of the swine population. Samples submitted should only be collected from animals displaying influenza-like illness. 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 in influenza in swine.

When the submitter does not report relevant information, data are recorded as "unknown." Summaries in this report may differ from those provided in past reports due to the ongoing addition of data from participating labs. Reporting months are based on the month when the sample was collected.

A laboratory accession is generally a set of samples collected at a single premises on a single day and received at the laboratory. A maximum of 10 samples of any kind is allowed per accession for reimbursement under the USDA IAV-S system. However, no more than five of the 10 samples may be oral fluid for any given accession. This does not prevent additional samples from being tested at the owner's expense. 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). The subtype result is based on the rRT-PCR based subtyping assays. Virus isolation (VI) and sequencing are only attempted on rRT-PCR positives meeting criteria listed below. Phylogenetic analyses are based on successful sequencing results, with sequences deposited into GenBank, the public sequence database.

Program Updates

On June 27, 2016, the modifications to IAV-S surveillance testing algorithm were implemented. They are:

- Sample screening costs for the Matrix PCR have flowed to industry
- Matrix PCR test CT cutoff values have been lowered for samples to be accepted into USDA funded further testing
    - For lung and nasal swab samples, only one sample (per accession) moves forward for Subtyping PCR (H1/H3 and N1/N3) if CT <25
    - For oral fluid samples only one sample moves forward for subtyping PCR if CT < 20
- The M gene is to be monitored through NVSL whole genome sequencing while payment to NAHLN labs for M gene sequencing is discontinued

IAV-S Surveillance Objectives

USDA's National Surveillance Plan for Swine Influenza Virus in Pigs (July 2010) describes the current surveillance system for IAV-S 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 to 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, updating 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 by voluntary laboratory submissions to NAHLN laboratories; select samples undergo further analysis by the National Veterinary Services Laboratories (NVSL) for VI and submission banking. USDA's Agricultural Research Service (ARS) National Animal Disease Center (NADC) provides phylogenetic analysis for select isolates under a cooperative agreement with USDA-APHIS-VS.

National Surveillance Data Summary

The total number of accessions and samples submitted continues to rise over time. For FY 2017's first quarter, 2,265 samples have been tested from 996 accessions (Figure 1). Figure 2 shows the overall increasing trends in total accessions, PCR positive accessions, subtyped accessions and VI positive accessions.

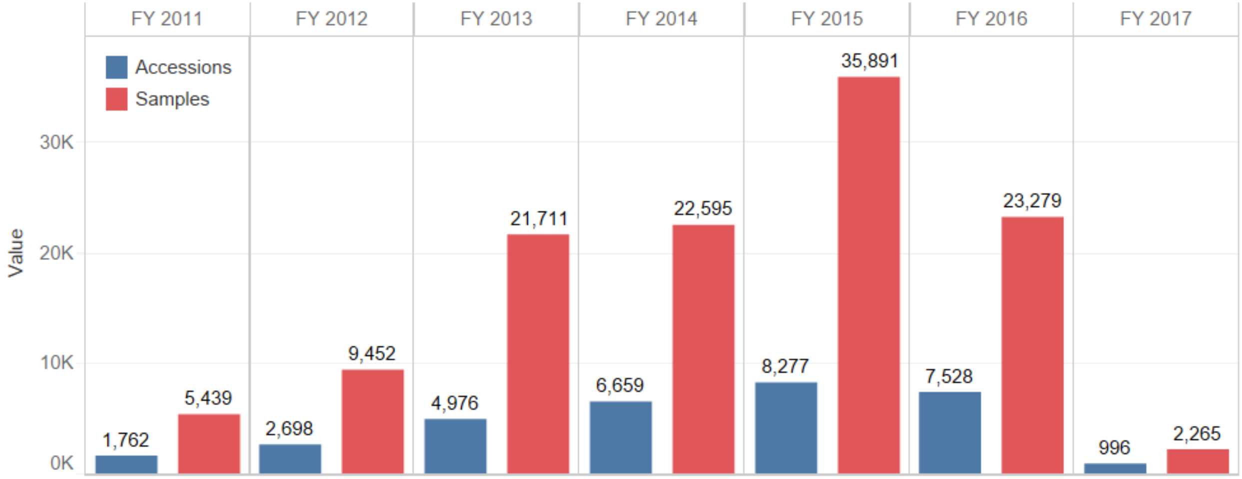


Figure 1. Surveillance for IAV in swine: Number of laboratory accessions and samples tested, FY 2011 – FY 2017 Q1

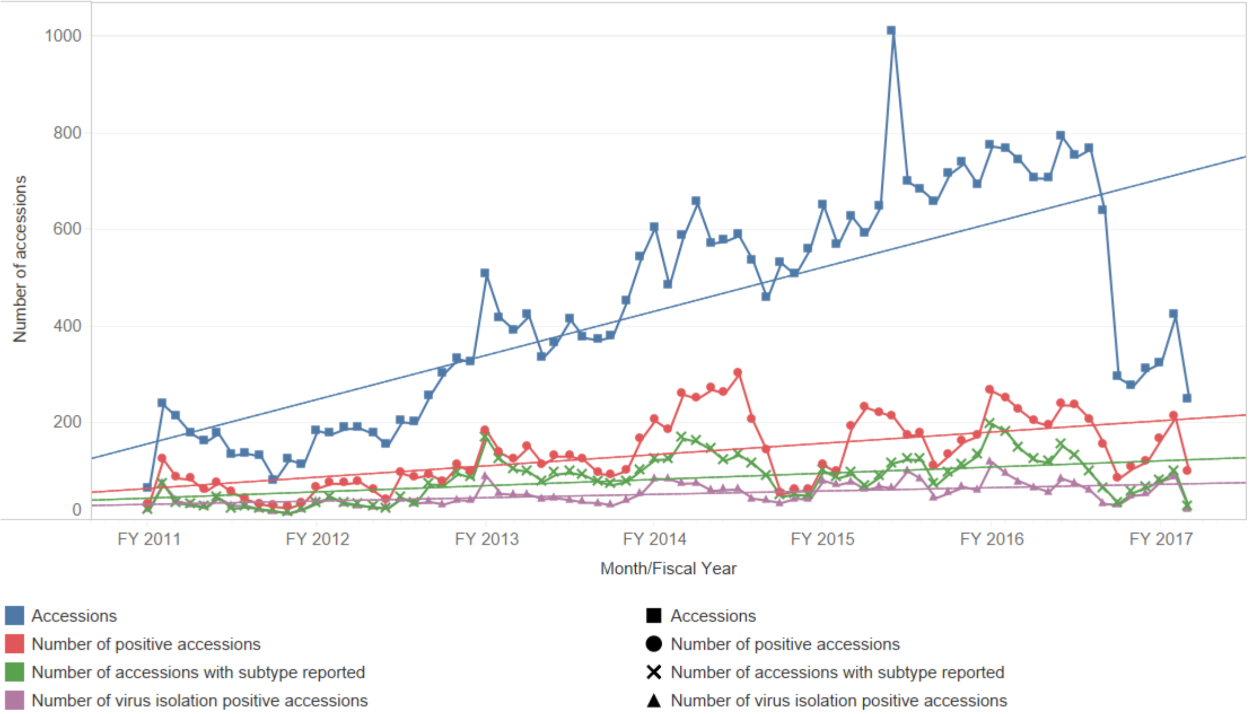


Figure 2. Accessions submitted, subtyped accessions, positive accessions, and VI positive accessions over time with trend lines, FY 2011-FY2017 Q1

Figure 3 shows the number of subtype detections in FY 2017 Q1. The total number of samples subtyped was 209, including 65 H1N1, 91 H1N2, 40 H3N2, 4 H3N1, and 9 mixed.

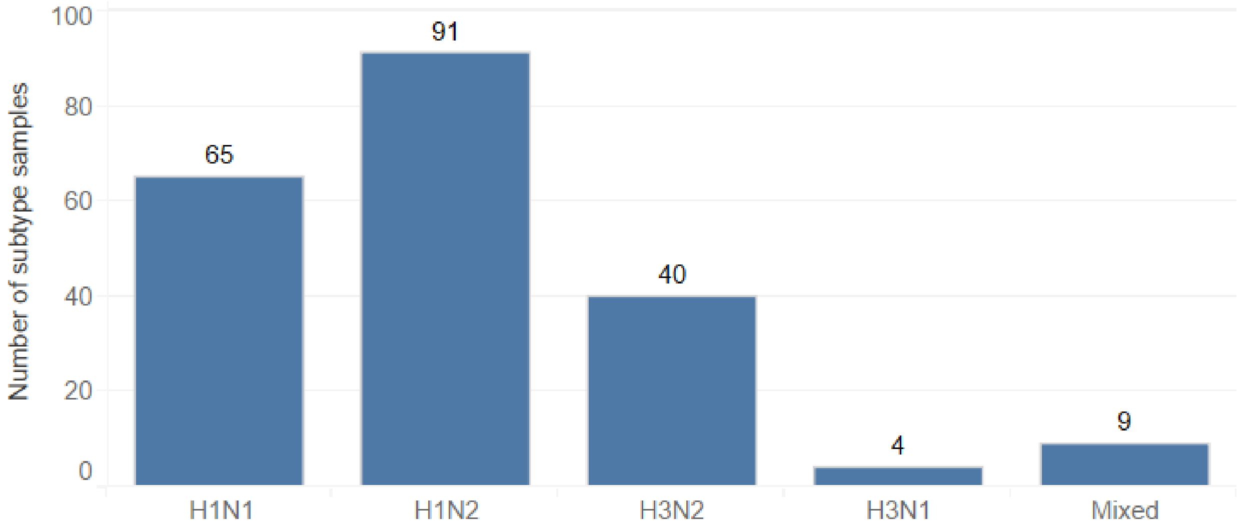


Figure 3. FY 2017 first quarter subtype detections

Figure 4 breaks down accessions by subtype rRT-PCR from FY 2011 to FY 2017 Q1. H1N1 remains the major subtype over the course of the surveillance; however, H1N2 and H3N2 detections have increased substantially since 2012 and H1N2 jumped to the common subtype in the first quarter. Figure 5 displays the number of VIs attempted, the number of those attempts that were positive, and the number of positive VIs that are submitted to GenBank.

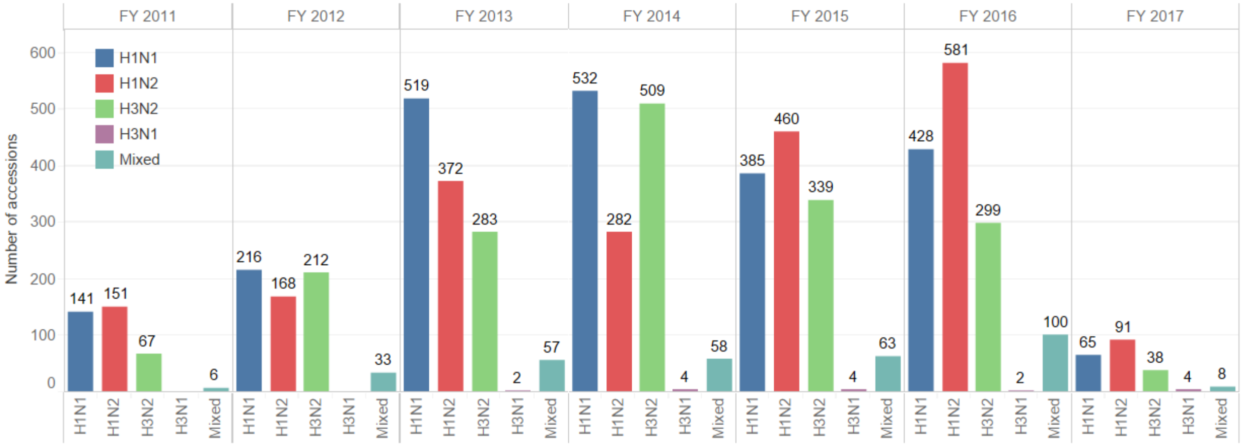


Figure 4. Number of subtypes, FY 2011 through FY 2017 Q1

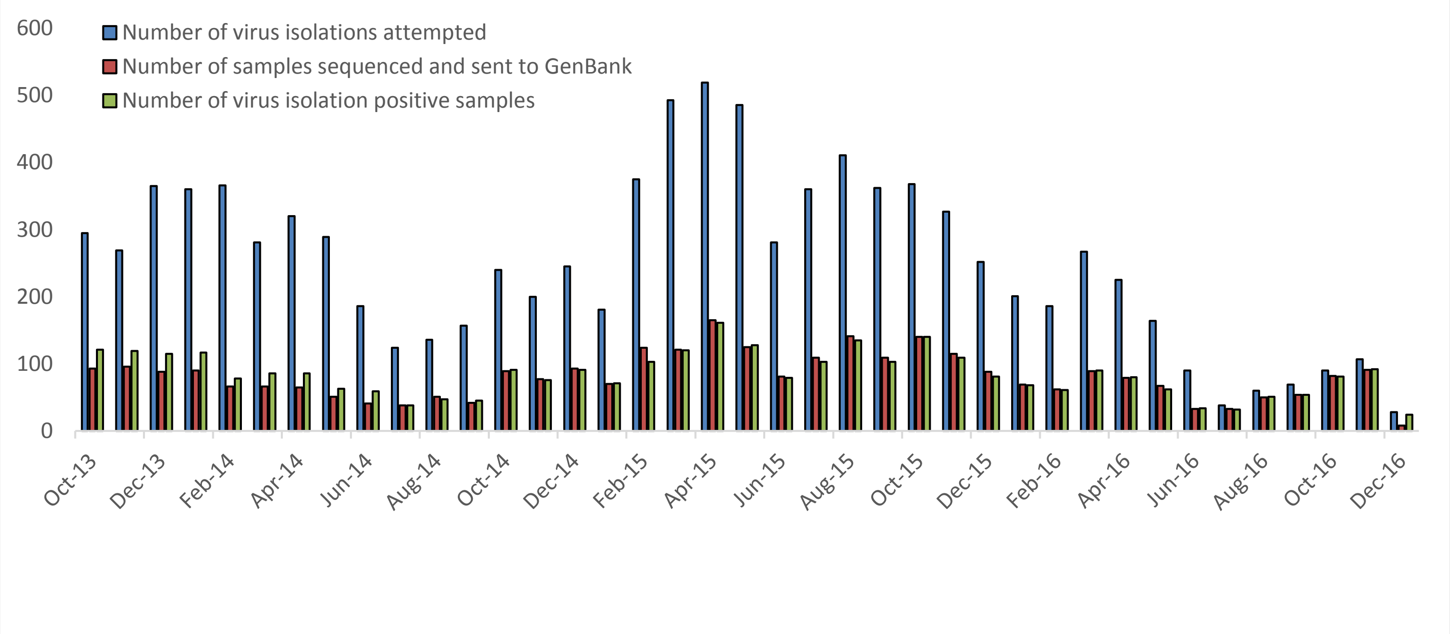
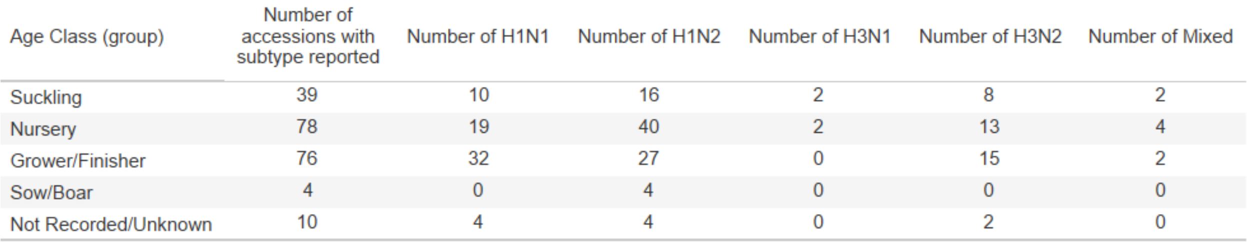


Figure 5. Number of virus isolations attempted, positive virus isolations, and GenBank submissions from FY 2013 through FY 2017 Q1

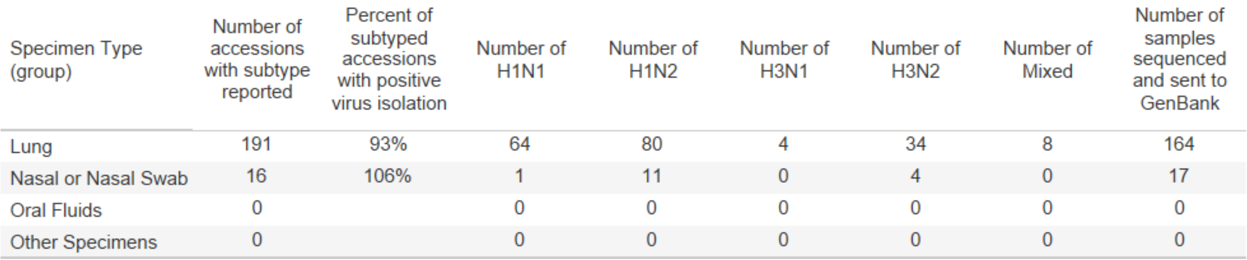
When accessions were evaluated by age-class for the first quarter, the following observations were noted. H1N2 was the most common subtype among Suckling and Nursery. H1N1 was the most common subtype among Grower/Finishers. Sow/Boar had limited testing, with four occurrences of H1N2. Among accessions for which the age class was unknown or not recorded, H1N1 and H1N2 were the equally predominant subtypes (Table 1). When looking at specimen type submitted, lung samples were the predominant accession type but the least successful at resulting in virus isolation and submission to GenBank. Nasal and nasal swab samples are 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 by viral subtype, Q1 FY 2017.



Age Class (group)	Number of accessions with subtype reported	Number of H1N1	Number of H1N2	Number of H3N1	Number of H3N2	Number of Mixed
Suckling	39	10	16	2	8	2
Nursery	78	19	40	2	13	4
Grower/Finisher	76	32	27	0	15	2
Sow/Boar	4	0	4	0	0	0
Not Recorded/Unknown	10	4	4	0	2	0

Table 2. Number of positive accessions tested for IAV-S by specimen type and by viral subtype, Q1 FY 2017.



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%	64	80	4	34	8	164
Nasal or Nasal Swab	16	106%	1	11	0	4	0	17
Oral Fluids	0		0	0	0	0	0	0
Other Specimens	0		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.

**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) to parse the analysis across regions. These regions are based on current USDA administrative districts for simplicity; these divisions 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.

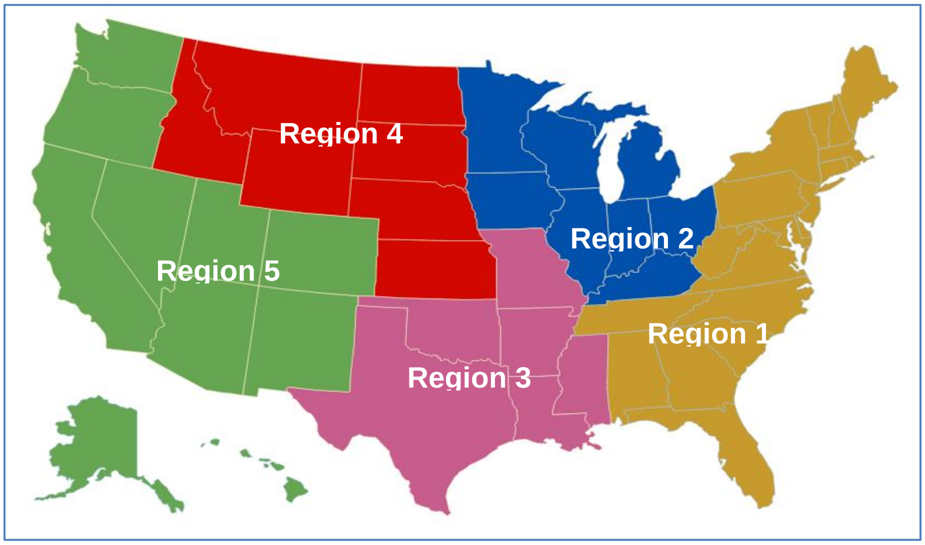


Figure 6. A map of the regions for national IAV-S surveillance

Summary of Regional Data and Predominant Subtypes from ARS

Table 3. Summary of predominant subtypes in each region for FY 2015 Q2 through FY 2017 Q1

Most Predominant HA/NA phylo-types overall:

H1N1 (Gamma H1/Classical N1)
H1N2 (Delta2 H1/1998-N2)
H3N2 (hu-like H3/2002-N2)
H1N2 (Delta1 H1/2002-N2)
H3N2 (IV-A H3/2002-N2)

Region 1 (Total HA/NA: 367)

Gamma H1/Classical N1
IV-A H3/2002-N2
Delta2 H1/1998-N2
Low frequency but consistent detections of IV-B H3/2002-N2, pdm H1/pdm N1, delta2 H1/2002-N2, alpha H1/2002-N2

Region 4 (Total HA/NA: 169)

Delta1 H1/2002-N2
IV-A H3/2002-N2
Gamma H1/Classical N1
Pdm H1/Pdm N1
Low frequency but consistent detections of beta H1/2002-N2, Alpha H1/2002-N2

Region 2 (Total HA/NA: 997)

Most diversity of all regions
Gamma H1/Classical N1
Delta1 H1/2002-N2
IV-A H3/2002-N2
Hu-like H3/2002-N2
Low frequency but consistent detections of IV-E H3/2002-N2, delta2 H1/1998-N2, IV-B H3/2002-N2, hu-like H3/2002-N2, pdm H1/pdm N1, alpha H1/2002-N2

Region 3 (Total HA/NA: 133)

Delta1 H1/2002-N2
Gamma H1/Classical N1
IV-A H3/2002-N2
Hu-like-H3/2002-N2
Low frequency but consistent detections of Beta H1/Classical N1, pdm H1/pdm N1

Region 5 (Total HA/NA: 5)

Two IV-B H3/2002-N2
One IV-A H3/2002-N2
One Delta1 H1/Classical N1
One Gamma H1/Classical N1

Figure 7 shows the distribution of rRT-PCR subtyped accessions among the five regions for Q1 FY 2015 through Q1 FY 2017. Region 1 demonstrates H1N2 as the predominant subtype. Regions 2 and 3 demonstrate H1N2 as the predominant subtype. H1N2 was the predominant in Region 4, and Region 5 and Region reported as unknown saw H1N1 as the predominant subtype.

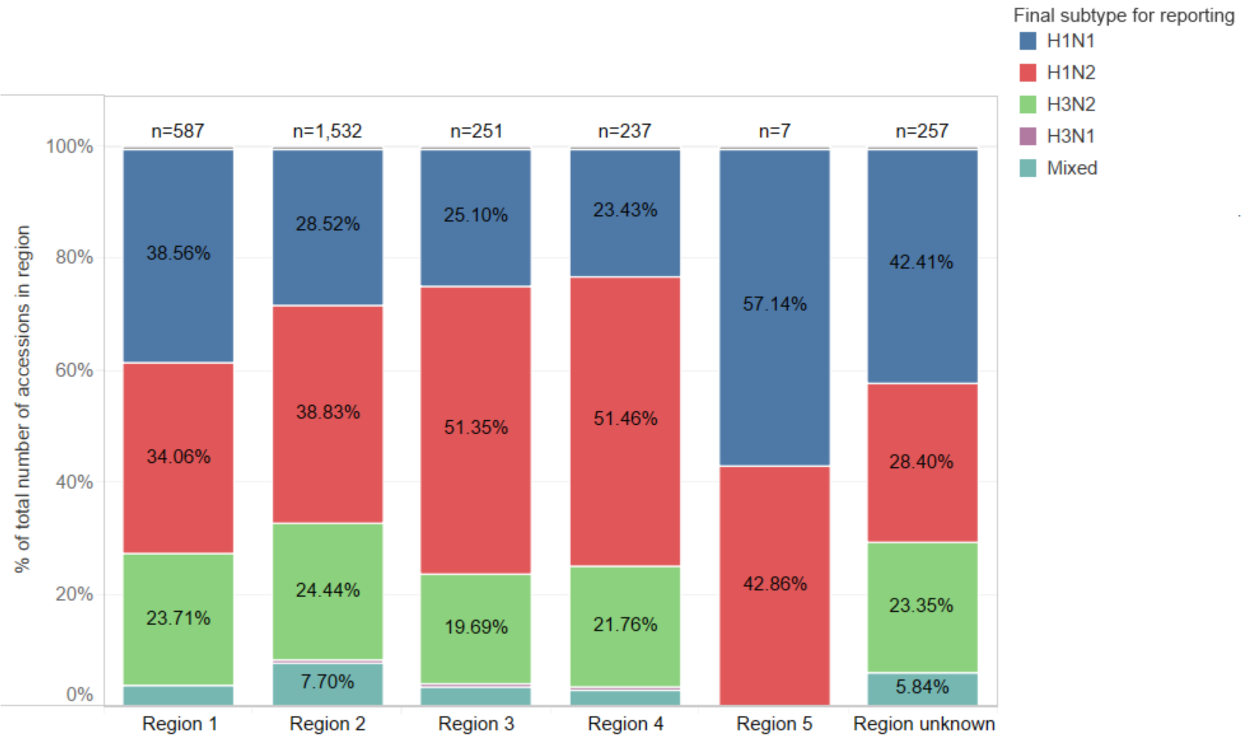


Figure 7. Percentage of subtyped accessions by region for Q1 FY 2015 through Q1 FY 2017 Q1

Regional phylogenetic analysis

Phylogenetic analysis of sequences from the IAV-S surveillance system

Phylogenetic analysis of gene sequences of the influenza A virus in swine is conducted to further examine the genetic changes that occur in HA, NA, and M genes of this rapidly changing virus. Through collaboration with ARS, a dataset[1] of 273 isolates with published sequences in GenBank was characterized by phylogenetic analysis in Q1 FY 2017. 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 parses the data into an approximately 2-year window by quarters and subtypes for each region, followed by charts further parsing the H1 and H3 subtypes into phylogenetic clades. Regional charts depicting the various combinations of HA and NA are available in Appendix 1.

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[1] 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.

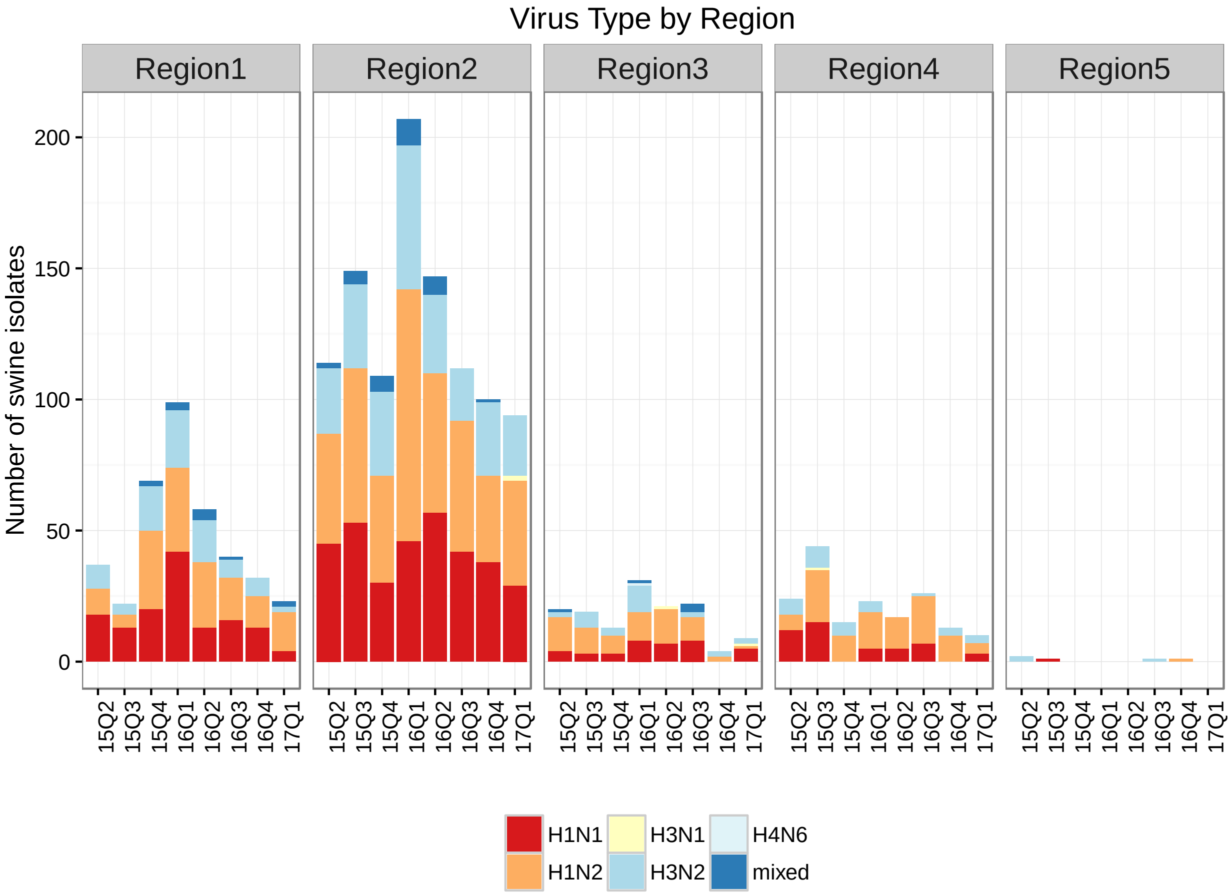


Figure 8. Virus type by region 2-year summary Q2 FY 2015 to Q1 FY 2017

Figure 8 demonstrates the four subtypes H1N1, H1N2, H3N1, 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 the H1 subtypes (see Figure 9), there were slightly more alpha detections in Q1 of FY 2017 than in the previous quarter and there was a relative increase in delta H1 compared to delta 2 and gamma.

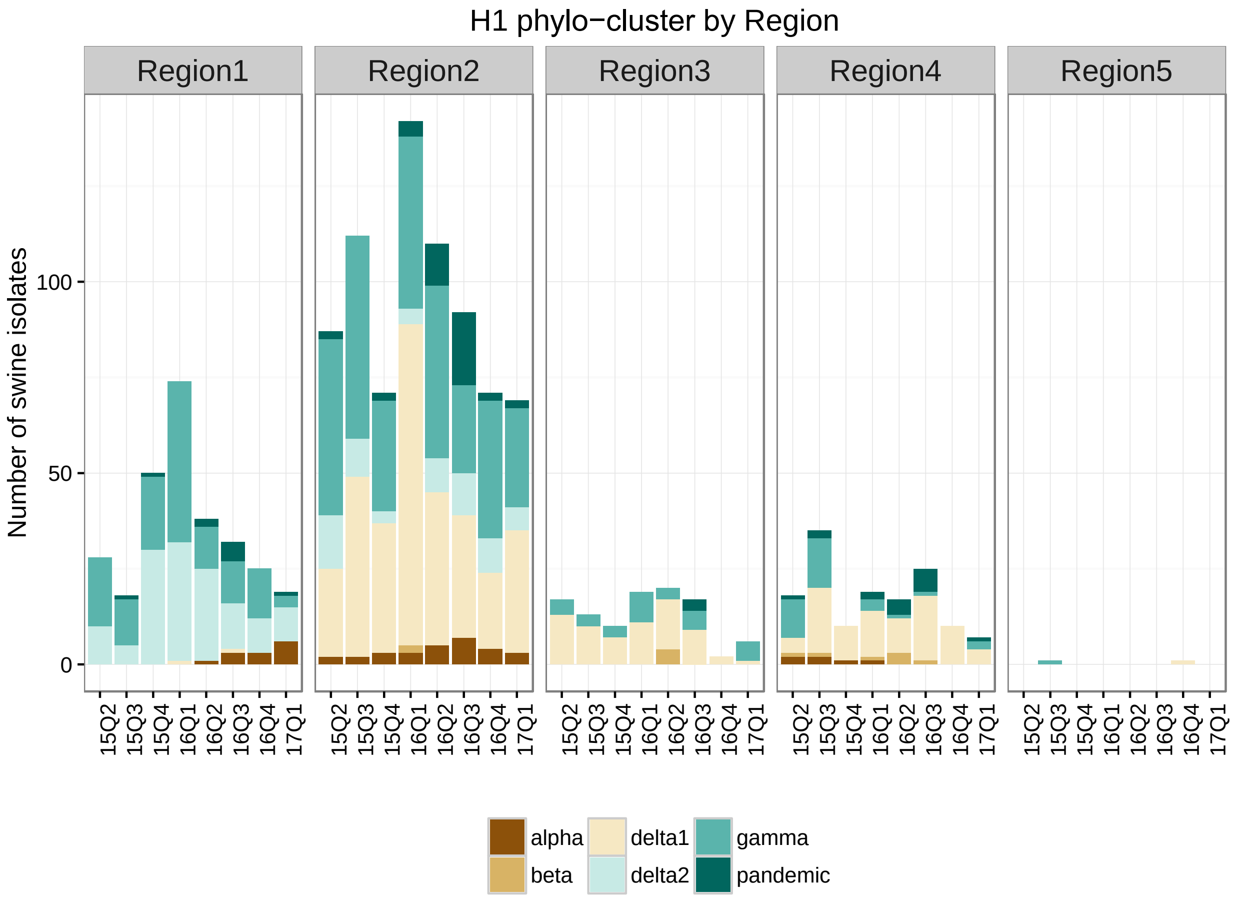


Figure 9. H1 phylo-cluster by region – 2-year summary Q2 FY 2015 to Q1 FY 2017

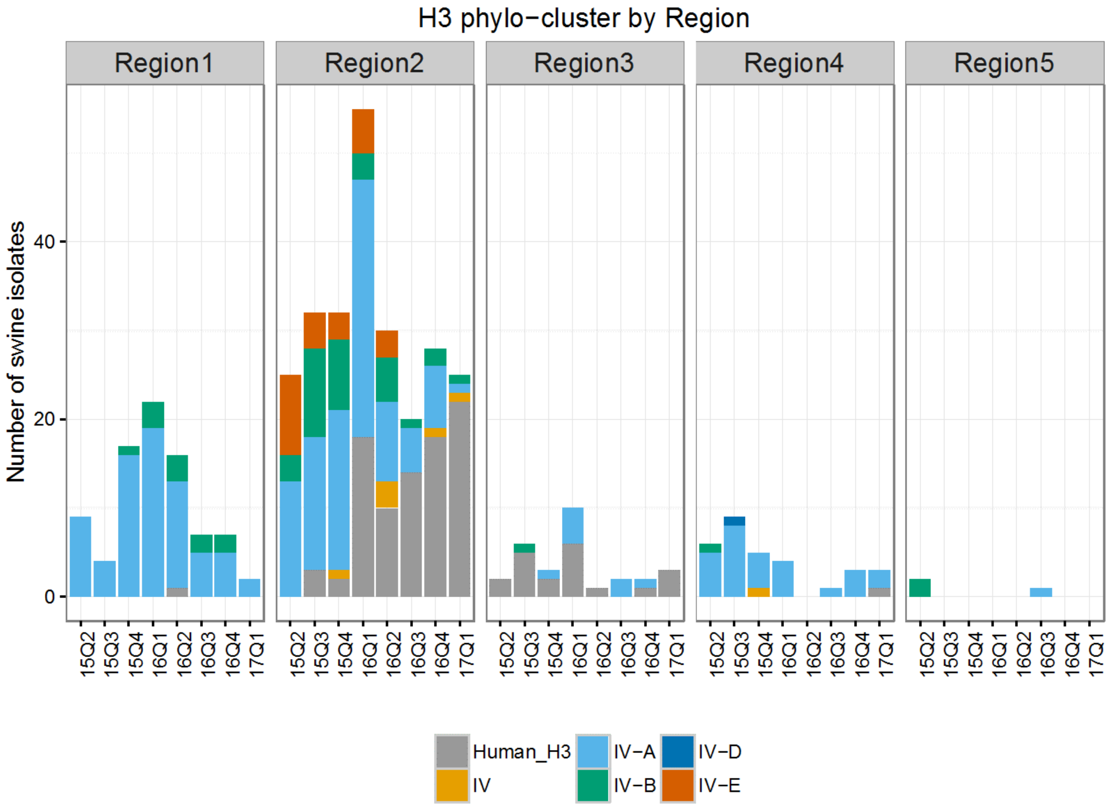


Figure 10. H3 phylo-cluster by region, 2-year summary Q2 FY 2015 to Q1 FY 2017

In Q1 FY 2016, there was an increase in human-like H3 detections (Figure 10) with spread to other States. Some clusters of IV-B and IV-E were detected. Cluster IV-A remains the predominant cluster.

National phylogenetic NA gene information

NA gene information remained the same in Q1 FY 2017. Both the N1 and N2 subtypes are found in circulating swine viruses. Classical N1 continued to be the dominant cluster. The 2002-lineage N2 continued to be more frequently detected than the 1998-lineage. The 1998-lineage N2 is most frequently paired with the delta2 H1.

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-S surveillance is to share selected virus isolates obtained from the surveillance system with public health, animal health, and academic researchers to facilitate genetic analysis and research on viruses of interest. In the first quarter of FY 2017, the NVSL Diagnostic Virology Laboratory provided 28 isolates to four institutions, one governmental, two academic, and one pharmaceutical. NVSL received 230 isolates into the repository (Table 3).

Table 3. Virus isolates received in repository

Virus isolates in the repository	
2017 YTD	230
2016	1046
2015	883
2014	765
2013	820
TOTAL TO DATE	3,744

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. Table 4 reports the total number of isolates available in the repository by subtype. ARS-NADC conducts research on isolates obtained from the repository and sequences generated from the surveillance system. Genetic sequencing 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 4. Total number of subtyped isolates available through repository

Subtyped isolates available through repository	
H3N2	1,475
H3N1	12
H1N1	1,877
H1N2	1,734
Mixed	300
TOTAL	5,398

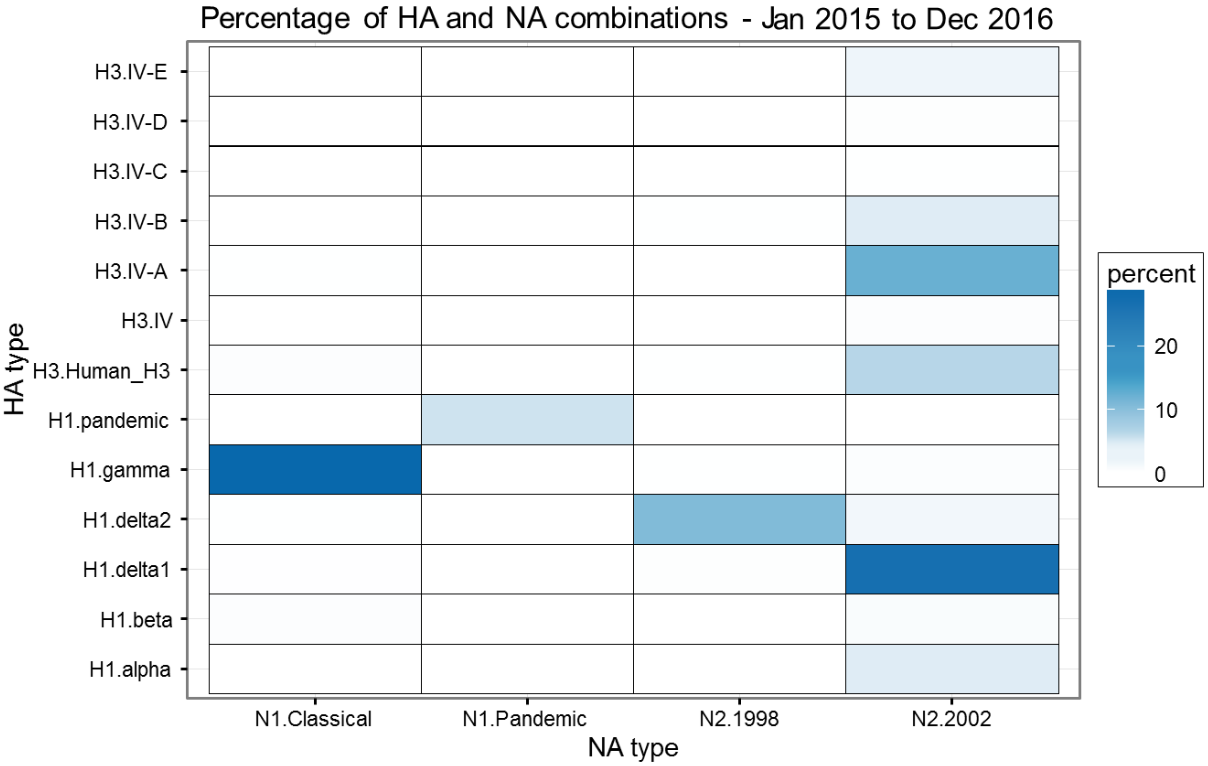
Conclusion

The IAV-S voluntary surveillance system continues to provide insight into the genetic makeup of circulating influenza 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.

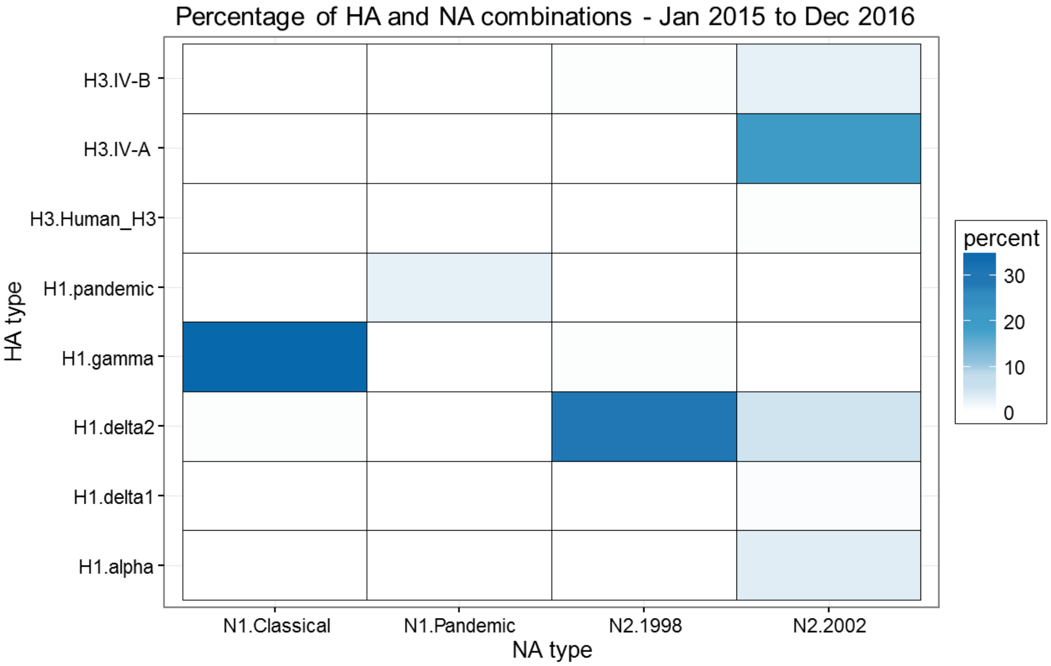
Appendix 1. Regional Charts of HA and NA Combinations by Percentage

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 January 2015 to December 2016. These “heat maps” represent the percentage of combinations by using a color gradient where a deeper gradient 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 percentage of occurrence of that particular combination.

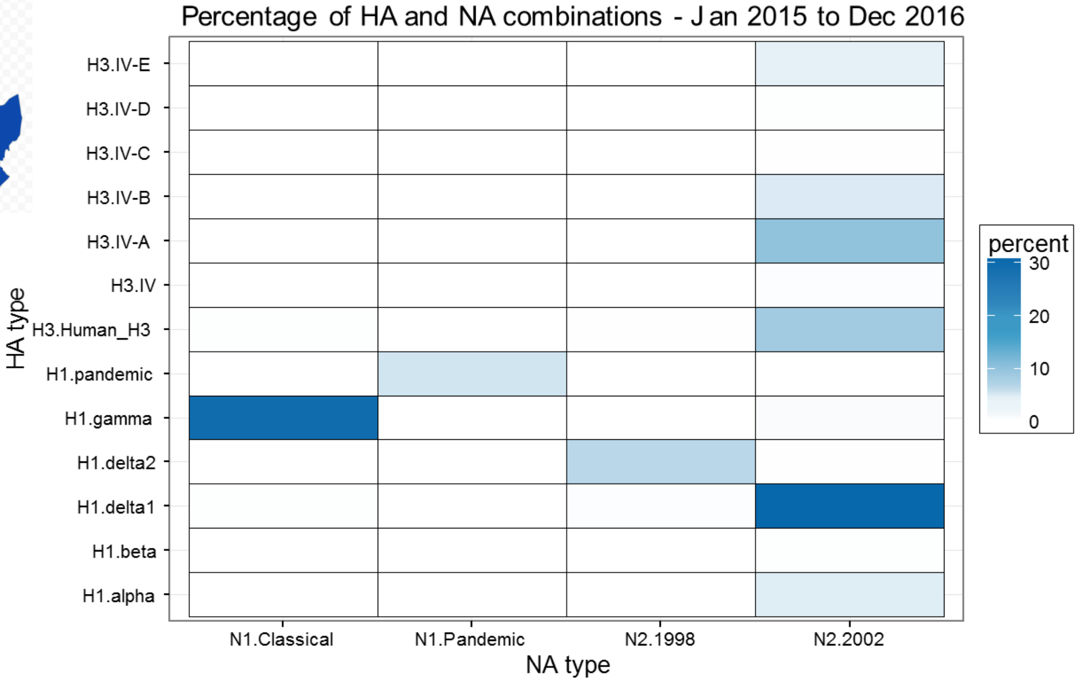
Total HA & NA combinations – 1,672

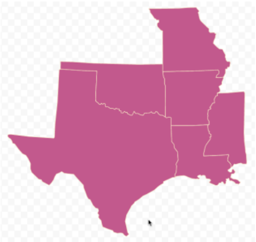


Region 1: Total HA & NA combinations – 367

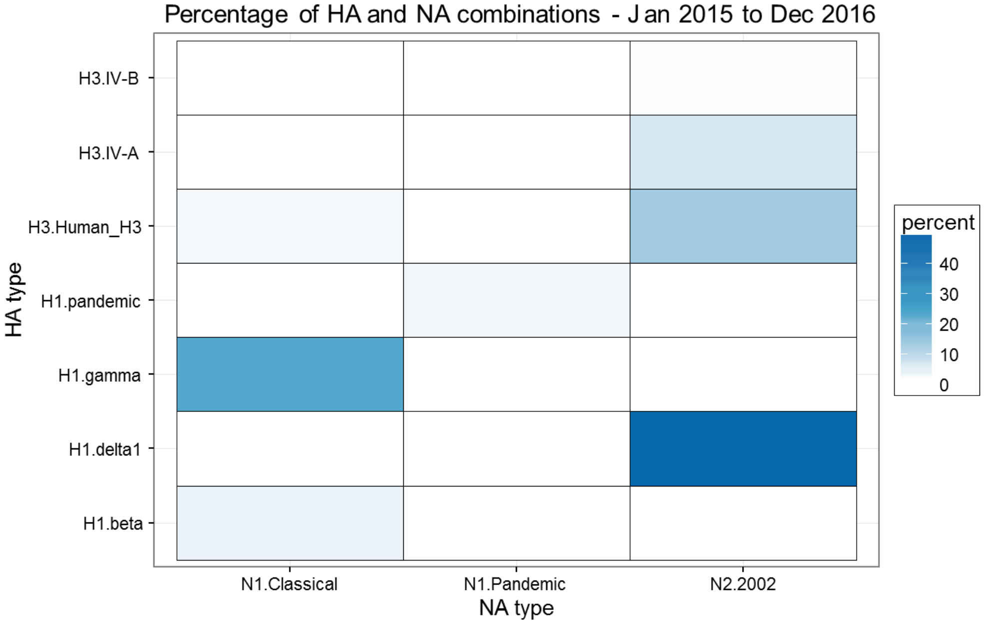


Region 2: Total HA & NA combinations – 997

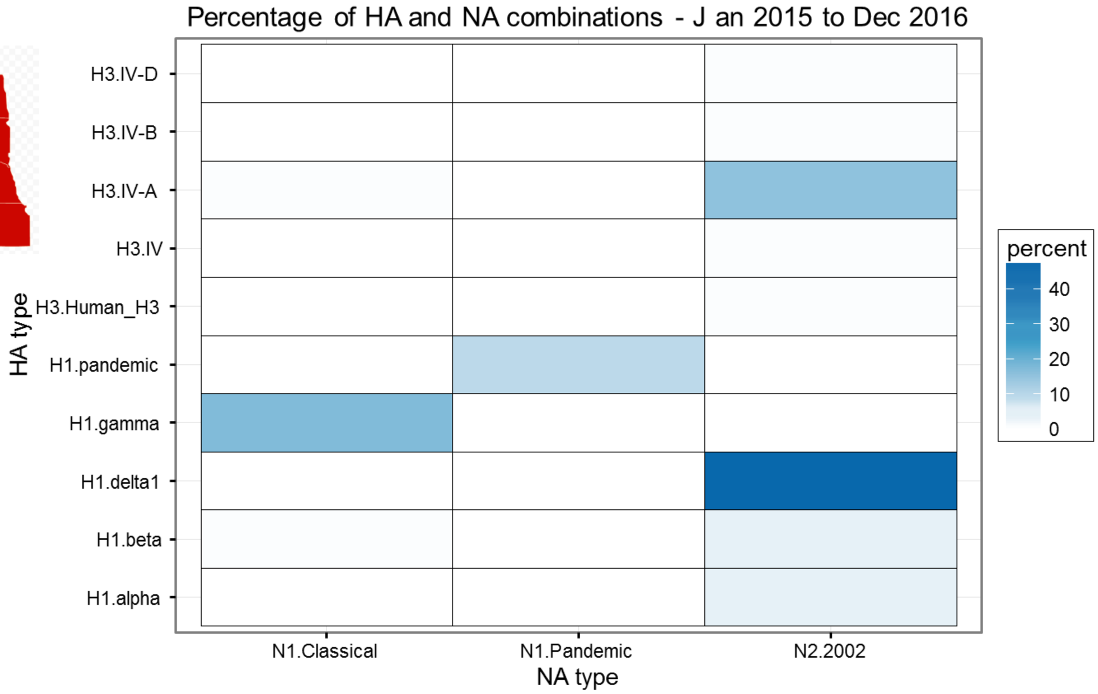




Region 3: Total HA & NA combinations – 133



Region 4: Total HA & NA combinations – 169



Region 5: Total HA & NA combinations – 5