Animal and
Plant Health
Inspection
Service

Veterinary
Services

April 2017

Influenza A Virus in Swine Surveillance

Fiscal Year 2016 Quarterly Report

Surveillance Summary for Second Quarter FY 2016:
January 1 – March 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 second quarter (Q2) of fiscal year (FY) 2016, from January 1, 2016 – March 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 FY 2016 Q2, 6,794 samples were submitted for IAV-S surveillance from 2,205 accessions.
- H1N2 was the predominant subtype.
- Over the past 8 quarters, H1N1 predominated in Regions 1 and 5. Regions 2, 3, and 4 observed H1N2 as the predominant subtype. Region 5 observed H1N1 as the predominant subtype. When Regions are unknown H1N1 predominates (see map in regional section).
- 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-S trends.

Introduction

This report, based on data received as of April 4, 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 https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/swine-disease-information/ct_siv_surveillance

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

IAV-S surveillance program review

Summaries of the APHIS-Policy and Program Development Assessment and the Technical Assessment of the IAV-S surveillance program are available online at:
http://www.aphis.usda.gov/animal-health/swine-health-surveillance

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 2016's second quarter, 6,794 samples were tested from 2,205 accessions (Figure 1) for a fiscal year-to-date total of 14,720 samples and 4,489 accessions. Figure 2 shows the overall increasing trends in total accessions, PCR positive accessions, subtyped accessions and VI positive accessions.

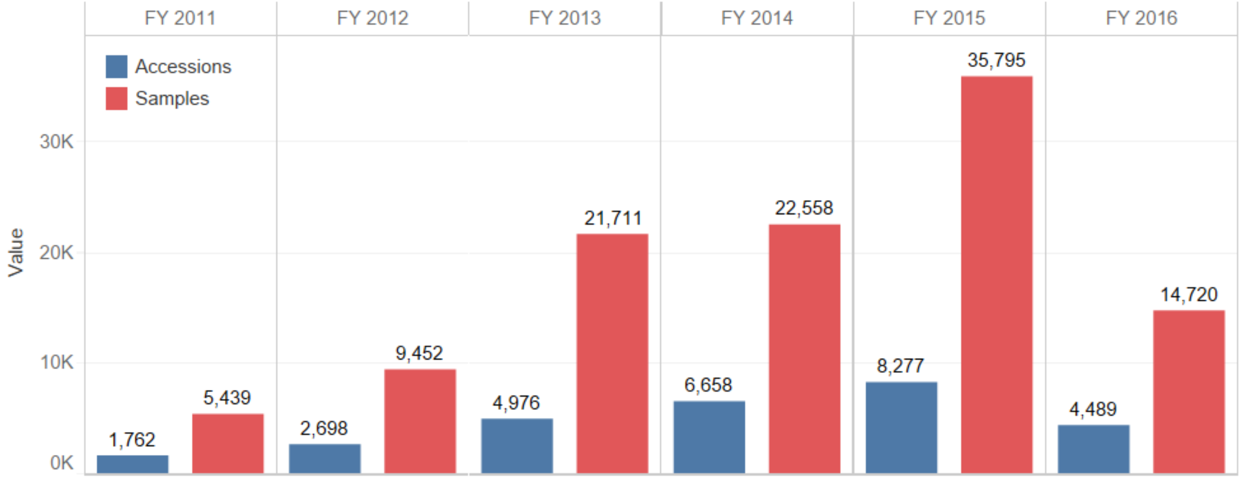

Figure 1. Number of IAV-S laboratory accessions and samples tested in swine FY2011 through FY2016 Q2.

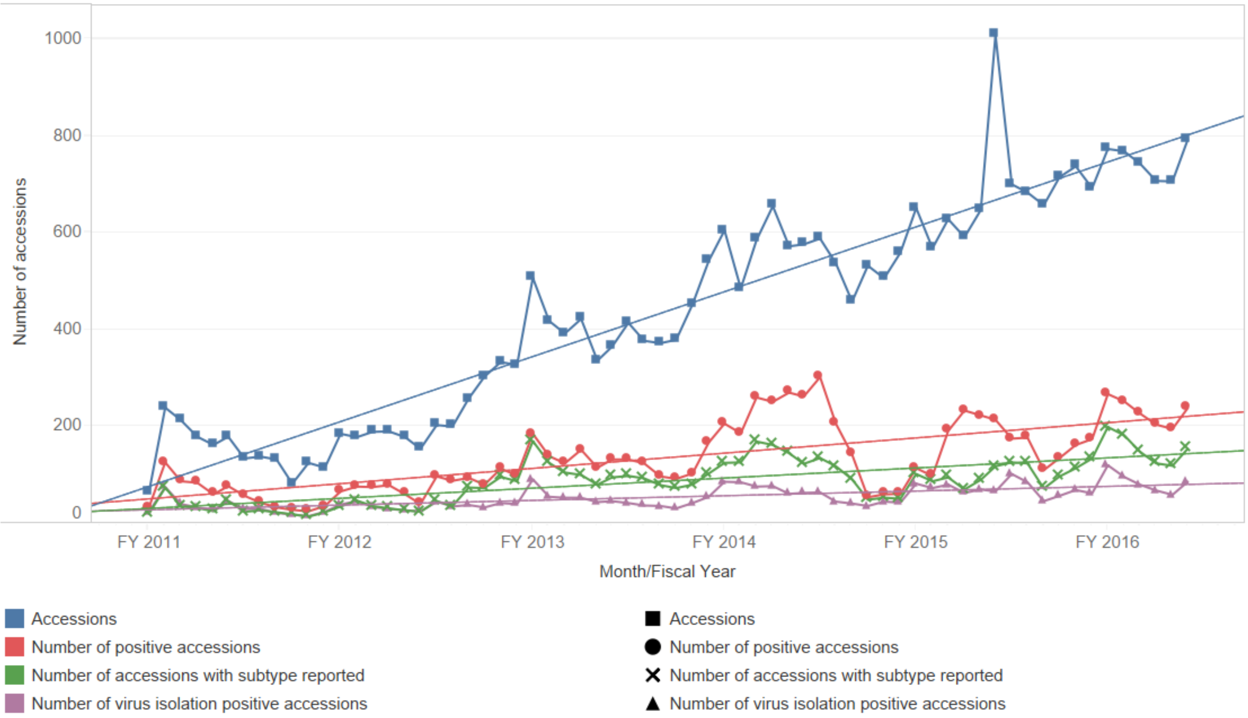

Figure 2. Accessions submitted, subtyped accessions, positive accessions, and VI positive accessions over time with trend lines, FY 2011 through FY2016 Q2.

Figure 3 shows the number of subtype detections in FY 2016 Q2. The total number of samples subtyped was 605, including 175 H1N1, 269 H1N2, 113 H3N2, 2 H3N1, and 46 mixed.

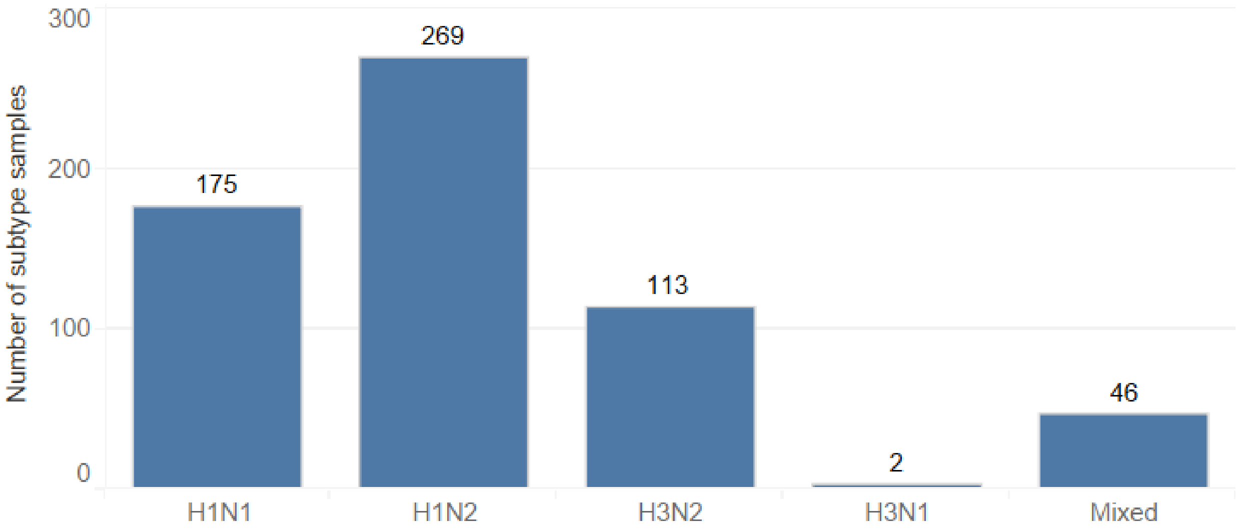

Figure 3. Number of subtype detections in FY 2016 Q2.

Figure 4 breaks down accessions by subtype rRT-PCR from FY 2010 to FY 2016 Q2. 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 most common subtype in the first and second quarter.

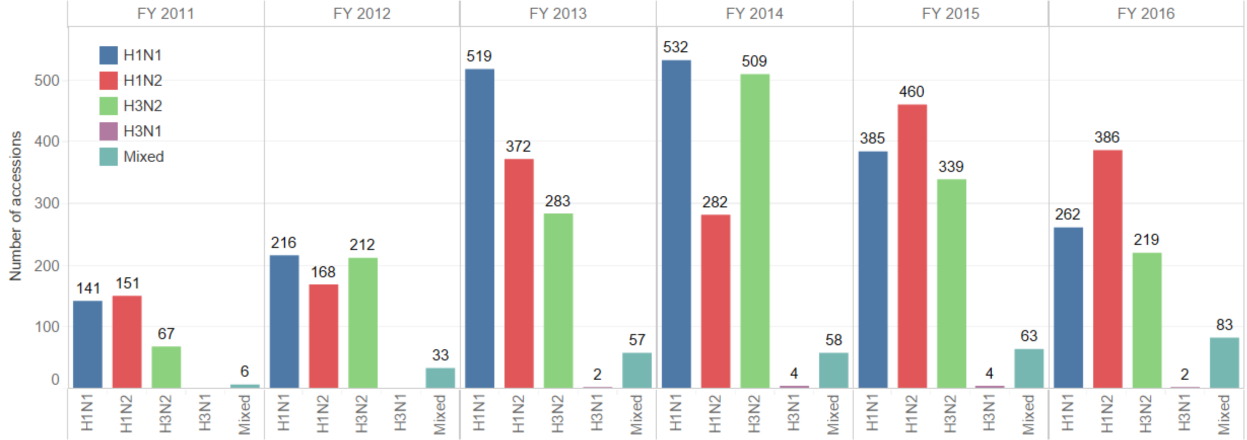

Figure 4. Number of subtypes, FY 2011 through FY 2016 Q2

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.

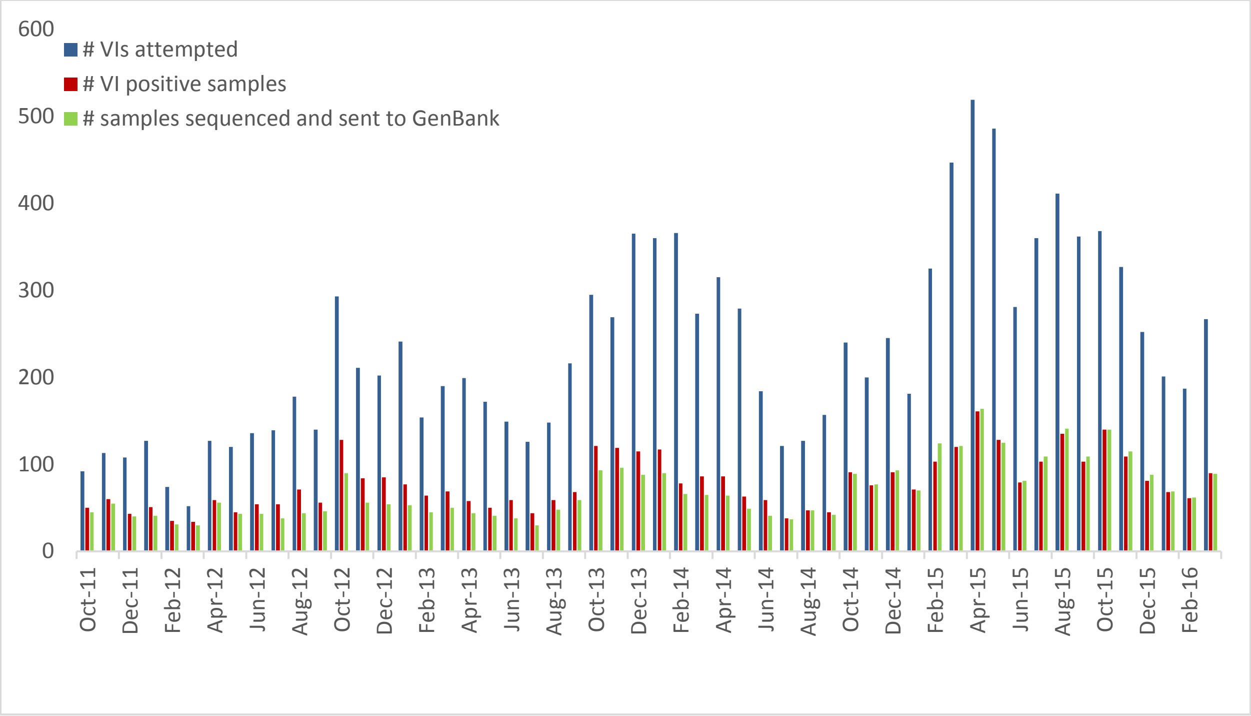

Figure 5. Number of virus isolations attempted, positive virus isolations, and GenBank submissions from FY 2012 through FY 2016 Q2

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 occurrence of H1N1, zero occurrences of H1N2, zero of H3N1, one of H3N2, and one mixed. Among accessions for which the age class was unknown or not recorded, H1N2 was the predominant subtype (Table 1).

When looking at specimen type submitted, oral fluids were the predominant accession type but the least successful at resulting in virus isolation and submission to GenBank. Samples collected from the lung 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, Q2 FY 2016.

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	57	16	28	0	13	1
Nursery	77	29	29	0	19	3
Grower/Finisher	39	20	11	0	7	1
Sow/Boar	6	4	0	0	1	1
Not Recorded/Unknown	218	54	104	2	39	26

Table 2. Number of positive accessions tested for IAV-S by specimen type and by viral subtype, Q2 FY 2016.

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	111	74%	42	42	0	24	3	83
Nasal or Nasal Swab	65	65%	16	42	0	9	0	42
Oral Fluids	231	39%	67	92	2	49	29	94
Other Specimens	0		0	0	0	0	0	1

*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.

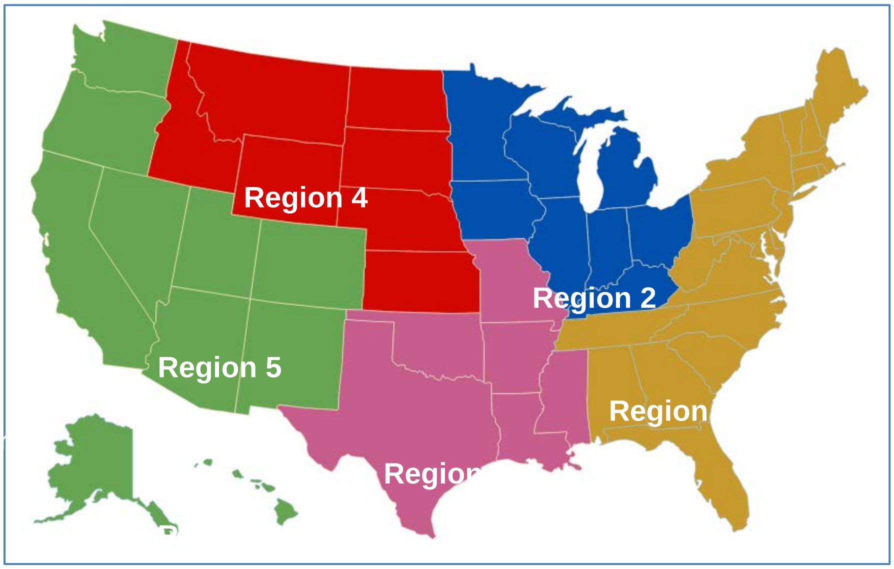

Figure 6. A map of the regions for national IAV-S surveillance

Summary of Regional Data from ARS

Table 3. Summary of predominant subtypes in each region for FY 2014 Q3 through FY 2016 Q2

Most Predominant HA/NA phylo-types overall:

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

Region 1 (Total HA/NA: 387)

Gamma H1/Classical N1
IV-A H3/2002-N2
Delta2 H1/1998-N2

Region 2 (Total HA/NA: 918)

Most diversity of all regions
Gamma H1/Classical N1
Delta1 H1/2002-N2
IV-A H3/2002-N2
IV-B H3/2002-N2
Consistent detections of 9 additional clusters of H1 and H3

Region 3 (Total HA/NA: 130)

Delta1 H1/2002-N2
Gamma H1/Classical N1
IV-A H3/2002-N2
Human-H3/2002-N2
Low frequency detections of IV-B H3/2002-N2, Beta H1/Classical N1

Region 4 (Total HA/NA: 174)

Delta1 H1/2002-N2
IV-A H3/2002-N2
Gamma H1/Classical N1
Pdm H1/Pdm N1
Low frequency detections of IV-D, IV-B H3/N2-2002, Delta1 H1/1998-N2 and Delta2 H1/1998-N2, Alpha H1/2002-N2

Region 5 (Total HA/NA: 5)

No new data since May 2015

Figure 7 shows the distribution of rRT-PCR subtyped accessions among the five regions for Q2 FY 2014 through Q2 FY 2016. Region 1 demonstrates H1N1 as the predominant subtype. Regions 2 and 3 demonstrate H1N2 as the predominant subtype. H3N2 was predominant in Region 4, and Region 5 saw H1N1 as the predominant subtype. For regions unknown, H1N1 was the predominant subtype.

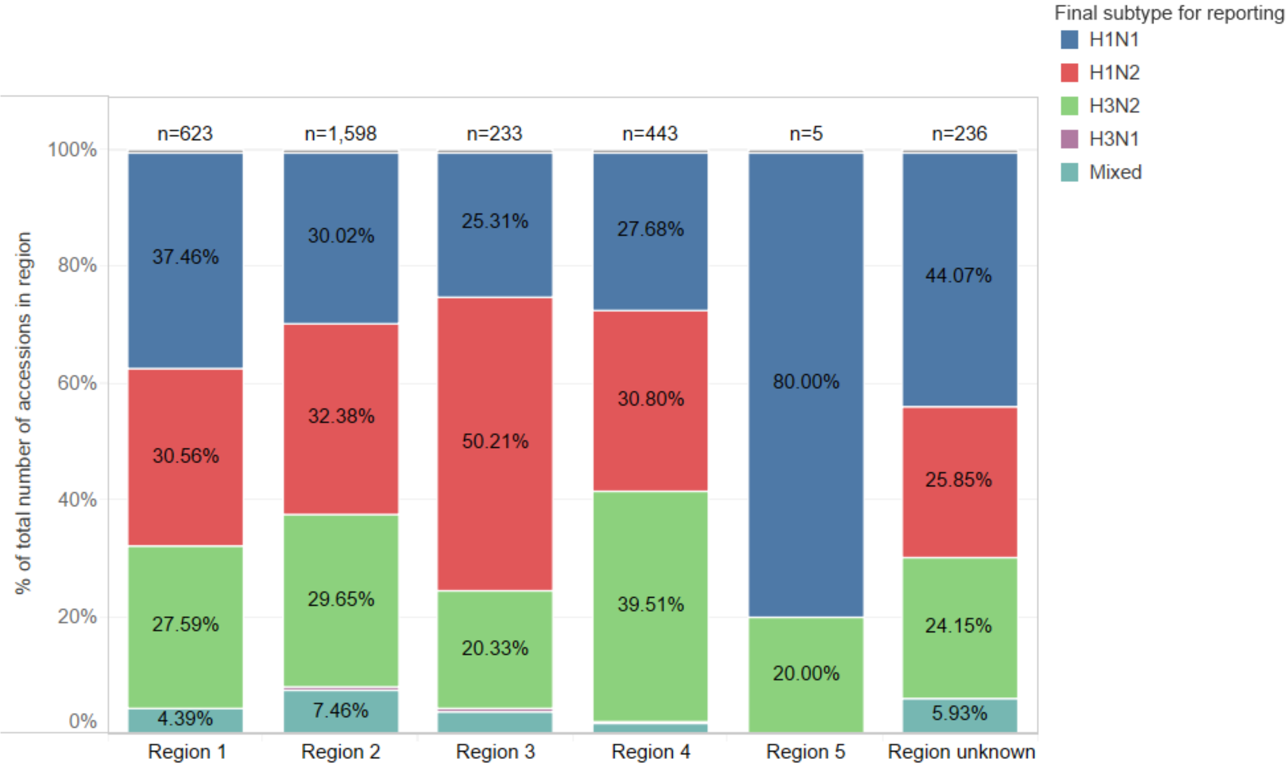

Figure 7. Percentage of subtyped accessions by region for FY 2014 Q2 through FY2016 Q2

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 382 isolates with published sequences in GenBank was characterized by phylogenetic analysis in Q2 FY 2016. 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 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.

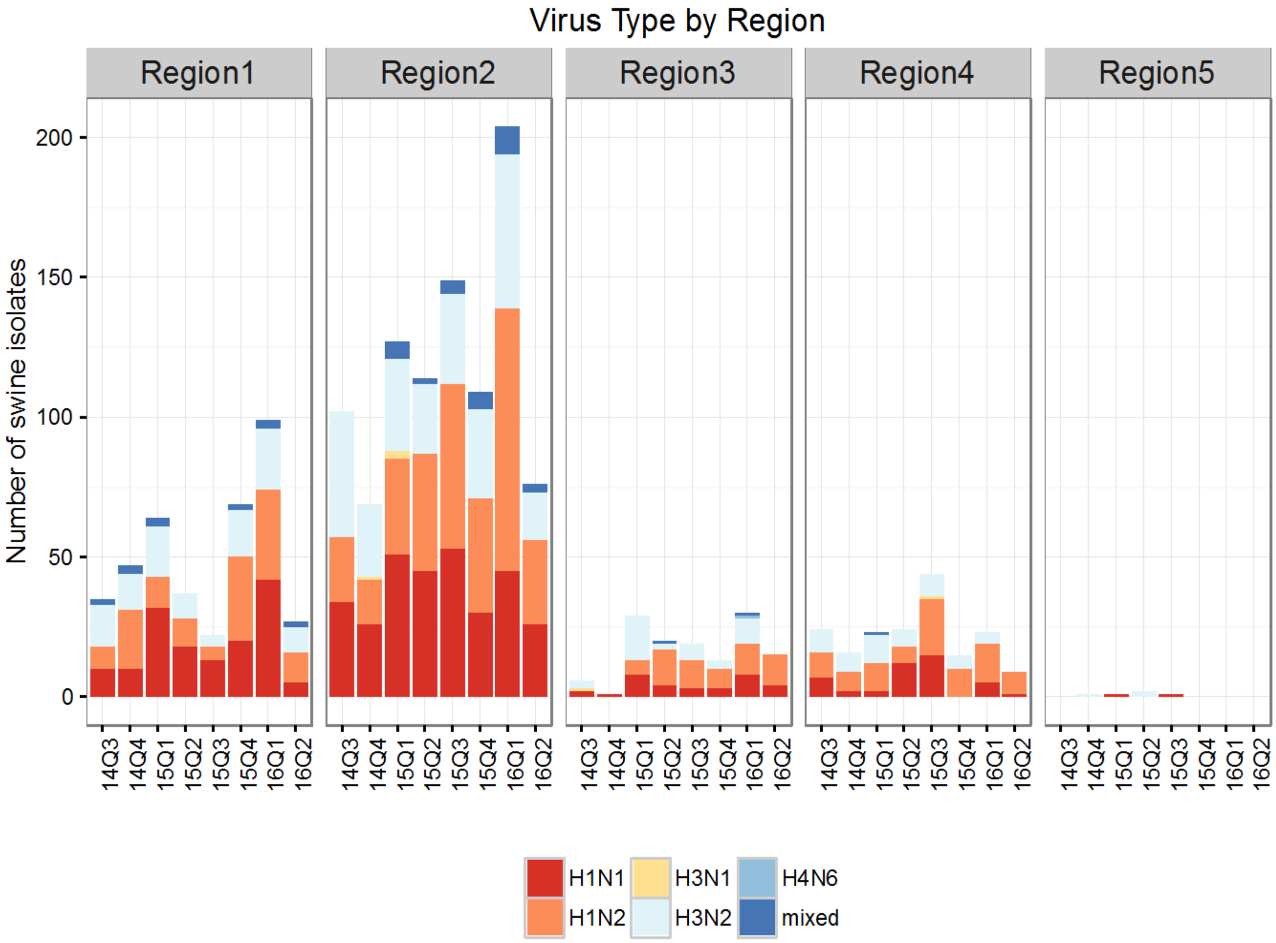


Figure 8. Virus type by region 2-year summary Q3 FY 2014 to Q2 FY 2016

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 continued to be detections of alpha with 2 aa deletions (n=3). Four human-to-swine PDM (IL, 2 in Jan, 2 in Feb) and 1 swine-to-swine PDM were detected. Delta 1, delta 2, and gamma viruses consistently account for 90% of H1.

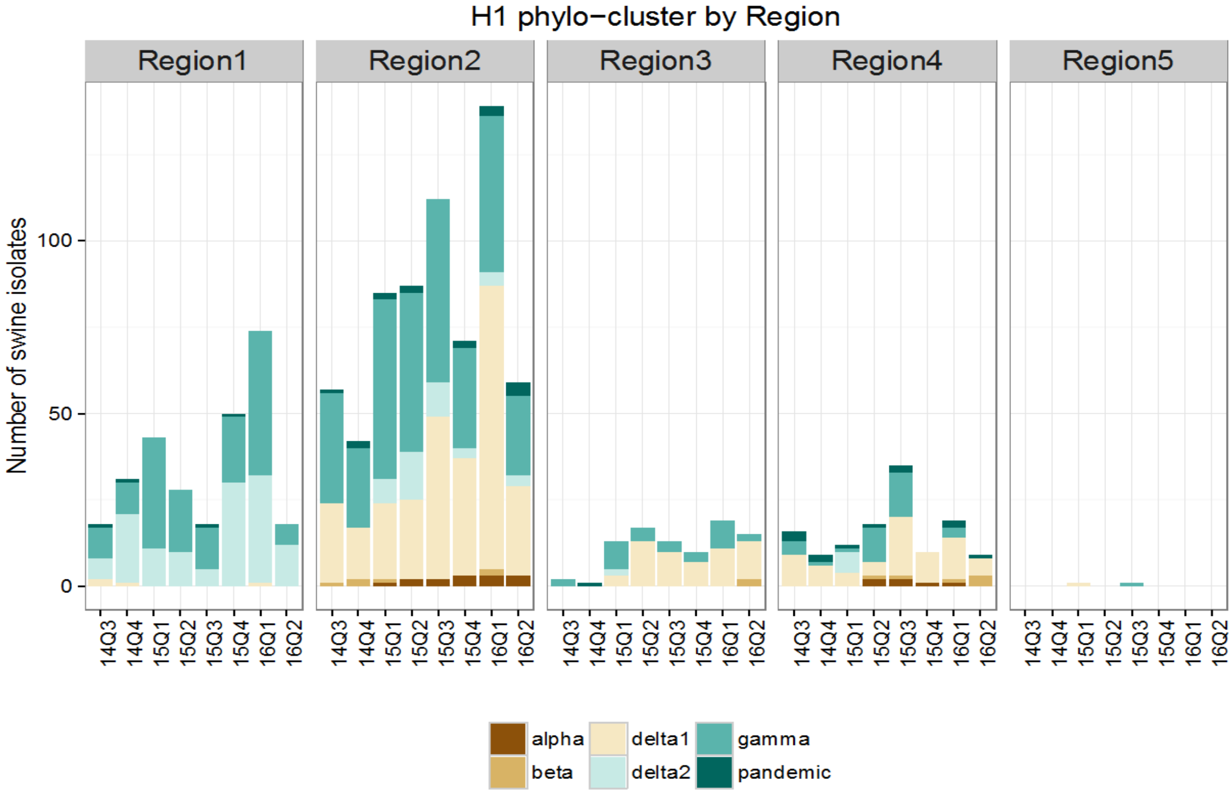


Figure 9. H1 phylo-cluster by region – 2-year summary Q3 FY 2014 to Q2 FY 2016

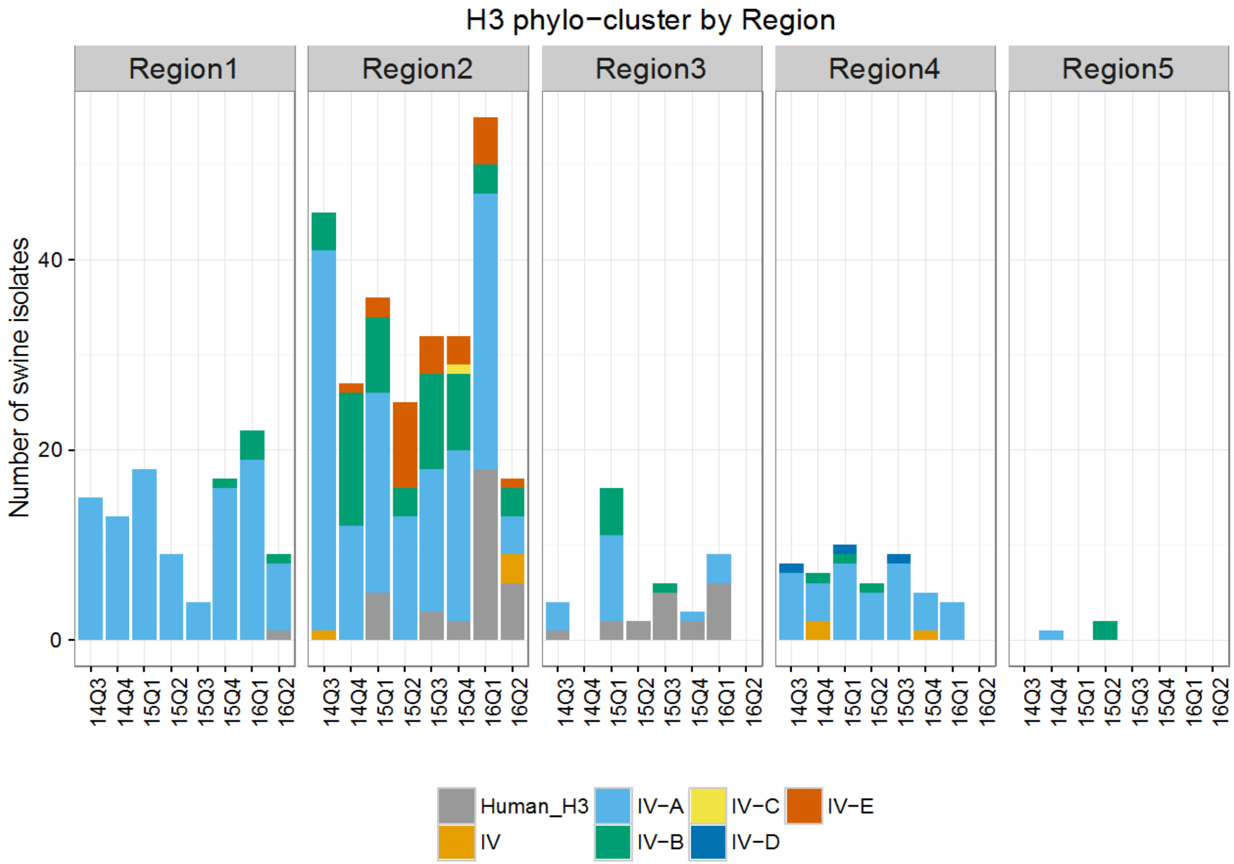


Figure 10. H3 phylo-cluster by region, 2-year summary Q3 FY 2014 to Q2 FY 2016

In Q2 FY 2016, there were seven new H3 detections, including expansion into a new State (Figure 10). Cluster IV-A remains the predominant cluster at around 66 percent of the detections. There were five detections of unclassified contemporary C-IV. Some clusters of IV-B and Cluster IV-E were detected. Cluster IV-A remains the predominant cluster.

National phylogenetic NA gene information

NA gene information remained the same in Q2 FY 2016. Both the N1 and N2 subtypes are found in circulating swine viruses. Classical N1 continued to be the dominant cluster. The 2002-lineage N2 represents 85% of N2 collections. The 1998-lineage N2 is most frequently paired with the delta2 H1.

National phylogenetic information M gene

Two North American M gene (Oklahoma, 2 beta H1- classical N1) were detected. The remaining data (n=1,661) were H1N1pdm09 M gene. There was a single detection of H4N6 in MO (Dec 2015) and was a probable wild-bird source.

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 second quarter of FY 2016, the NVSL Diagnostic Virology Laboratory provided 16 isolates to two institutions, one governmental and one pharmaceutical. NVSL received 238 isolates into the repository (Table 3). Table 4 reports the total number of isolates available in the repository by subtype.

Table 3. Virus isolates received in repository

Virus isolates in the repository	
2016 YTD	238
2015	883
2014	765
2013	820
2012	915
TOTAL TO DATE	3,220

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 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	1,300
H3N1	9
H1N1	1,649
H1N2	1,464
Mixed	287
TOTAL	4,709

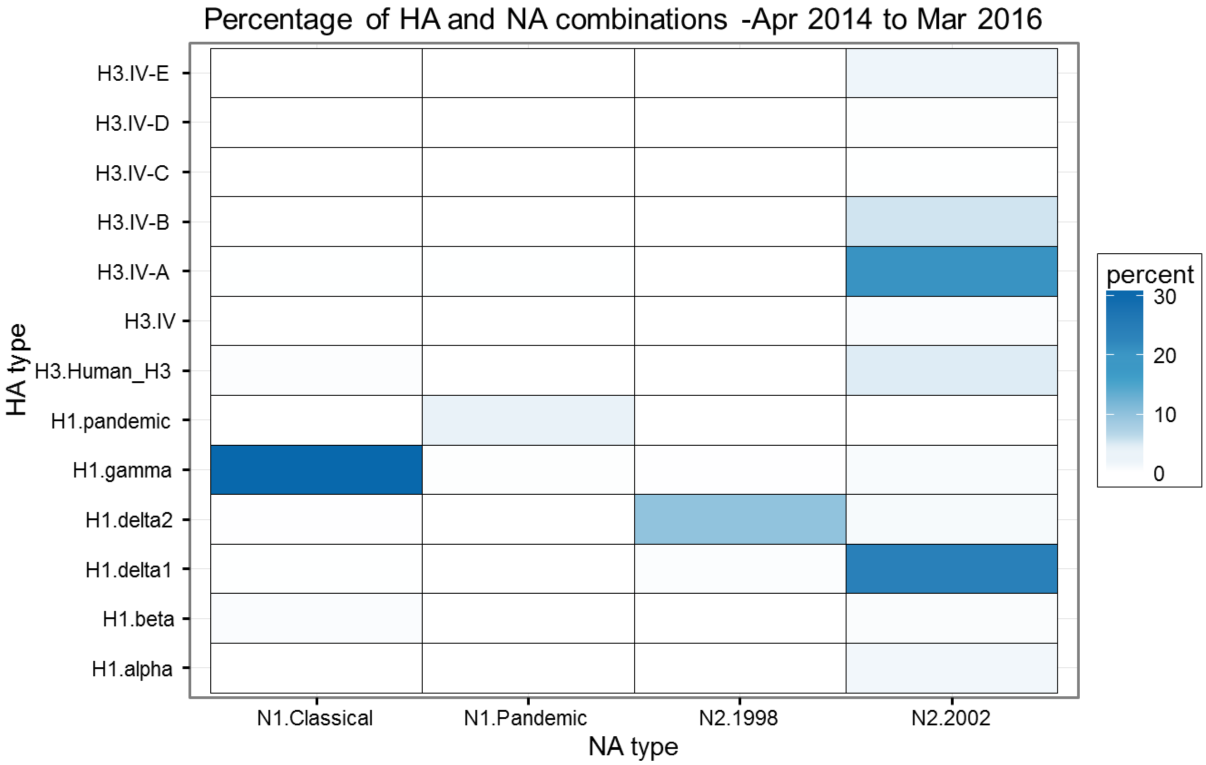
Conclusion

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.

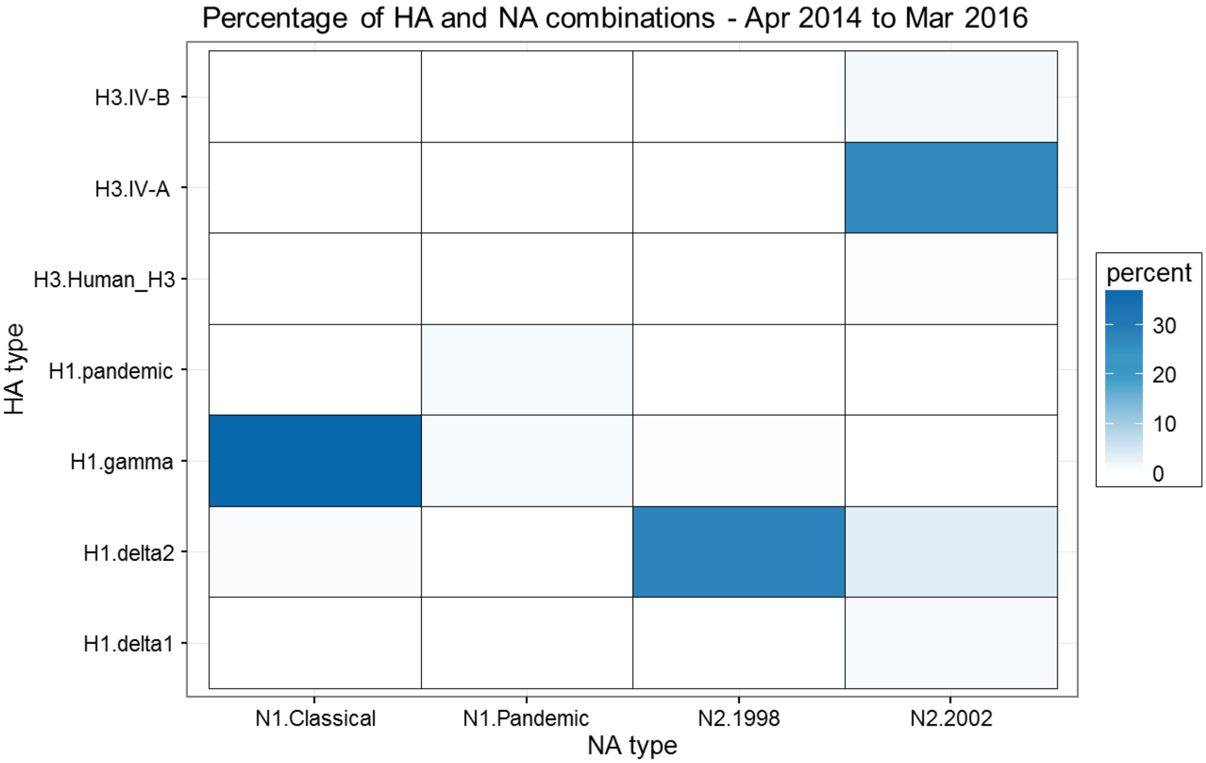
Appendix 1. Regional Charts of HA and NA Combinations by Percentage

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 April 2014 to March 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.

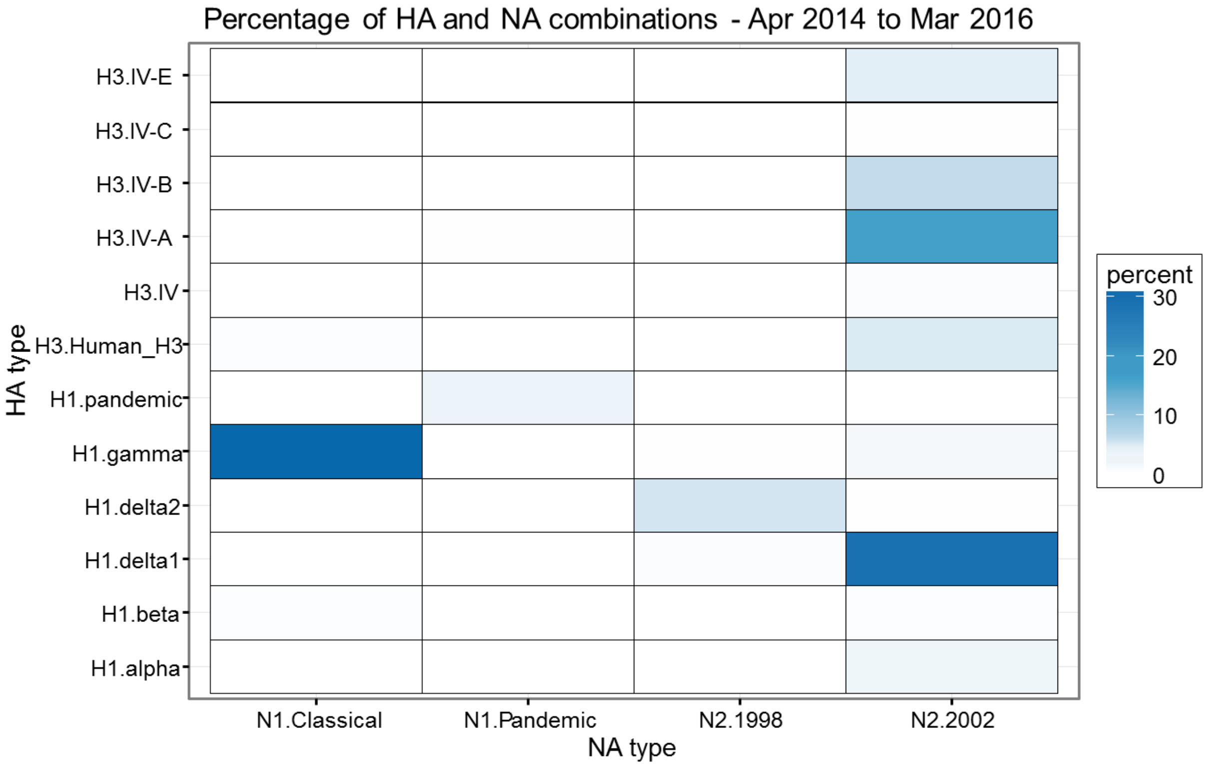
Total HA & NA combinations – 1,622



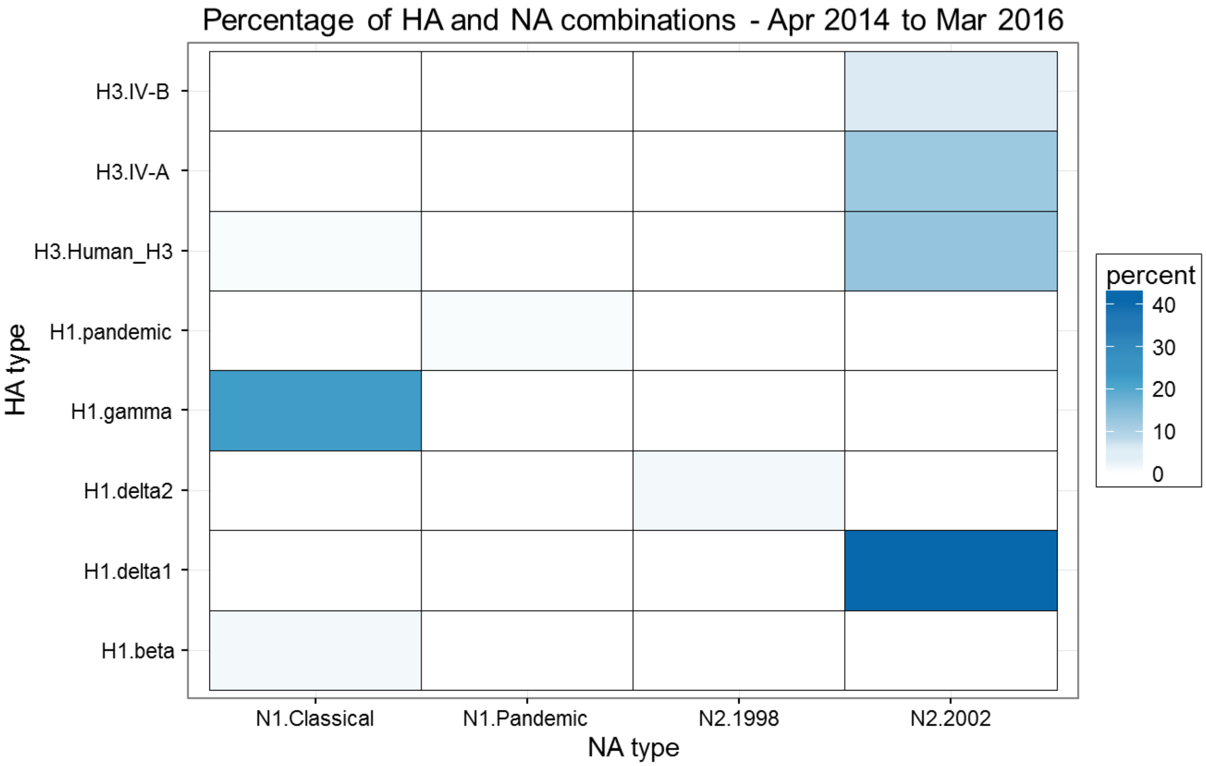
Region 1: Total HA & NA combinations – 387



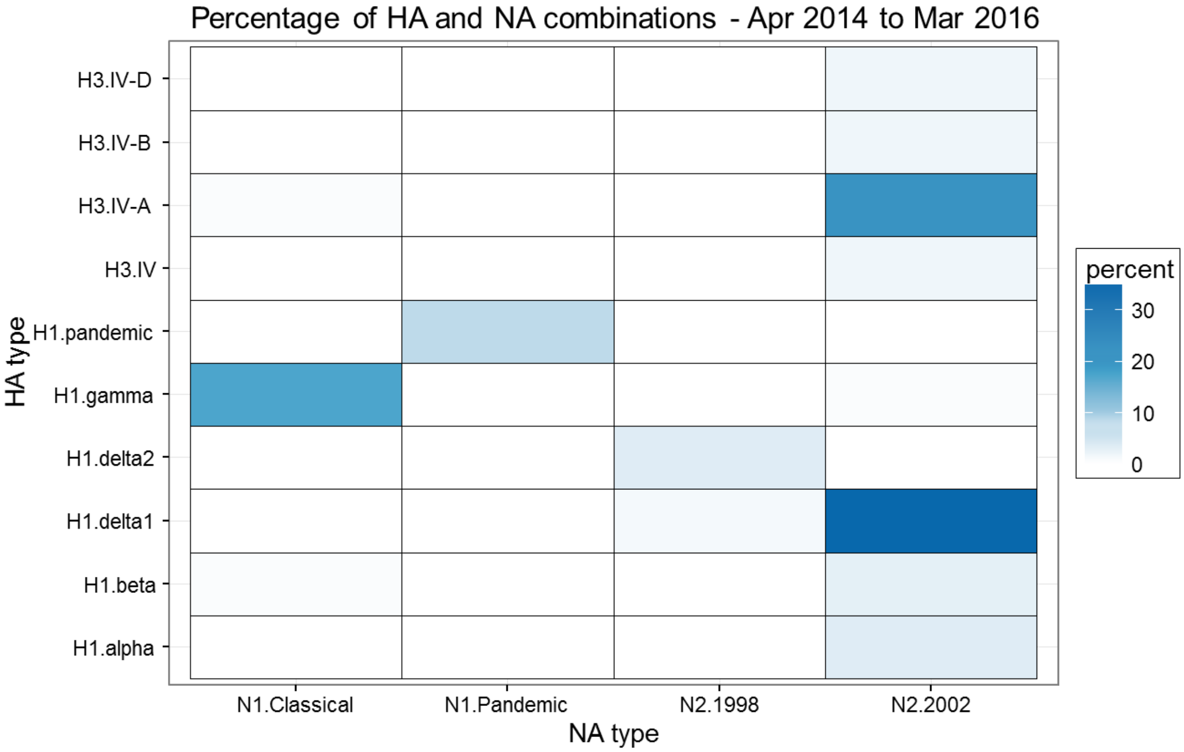
Region 2: Total HA & NA combinations – 918



Region 3: Total HA & NA combinations – 130



Region 4: Total HA & NA combinations – 174



Region 5: Total HA & NA combinations – 5