



Animal and  
Plant Health  
Inspection  
Service

Veterinary  
Services

April 2017

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# Influenza A Virus in Swine Surveillance

## Fiscal Year 2016 Quarterly Report

Surveillance Summary for First Quarter FY 2016:  
October 1 – December 31, 2015

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\*\*\* 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) 2016, from October 1– December 31, 2015.
- 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, 7,926 samples were submitted for IAV-S surveillance from 2,284 accessions.
- The H1N2 was the predominant subtype for all regions.
- Over the past eight quarters, Regions 1 and 2 observed H1N1 as the predominant subtype. Region 3 observed H1N2 as the predominate subtype. Region 4 observed H3N2 as the predominant subtype. Region 5 observed H1N1 as the predominant subtype. When a region was recorded as "unknown," H1N1 predominated (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.
- USDA surveillance identified an H4N6 subtype in a commercial swine herd in December 2015. H4N6 is a common influenza virus found in wild birds. H4N6 is not considered a highly pathogenic avian influenza (HPAI) or low pathogenic avian influenza (LPAI). Follow-up investigation found no indication of within-herd transmission.
- 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 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 <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

### 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 now posted to the Web at this address:

<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 first quarter, 7,926 samples have been tested from 2,284 accessions (Figure 1). Figure 2 shows the overall increasing trends in total accessions, PCR positive accessions, subtyped accessions and VI positive accessions.

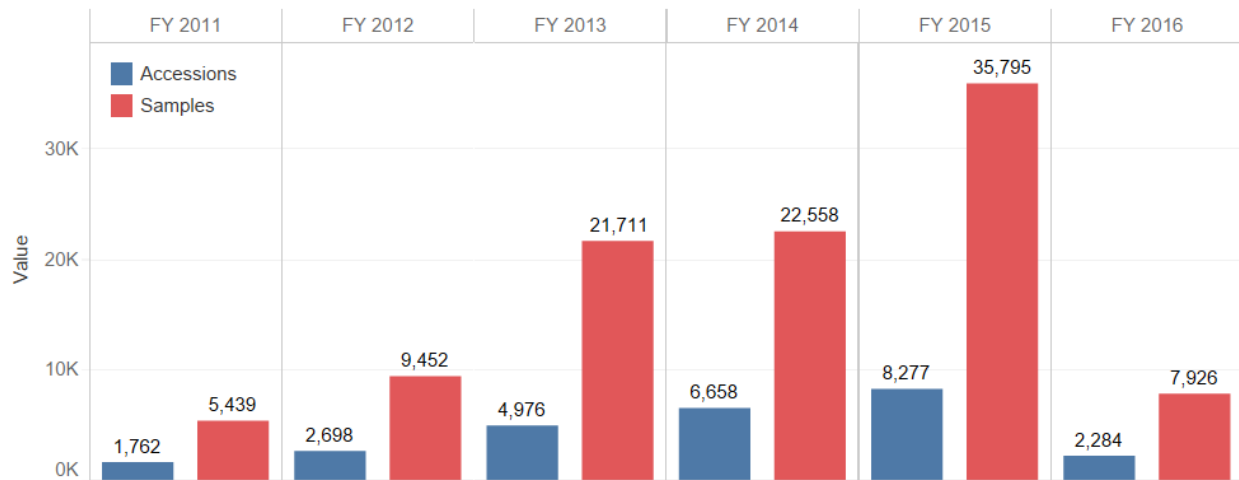
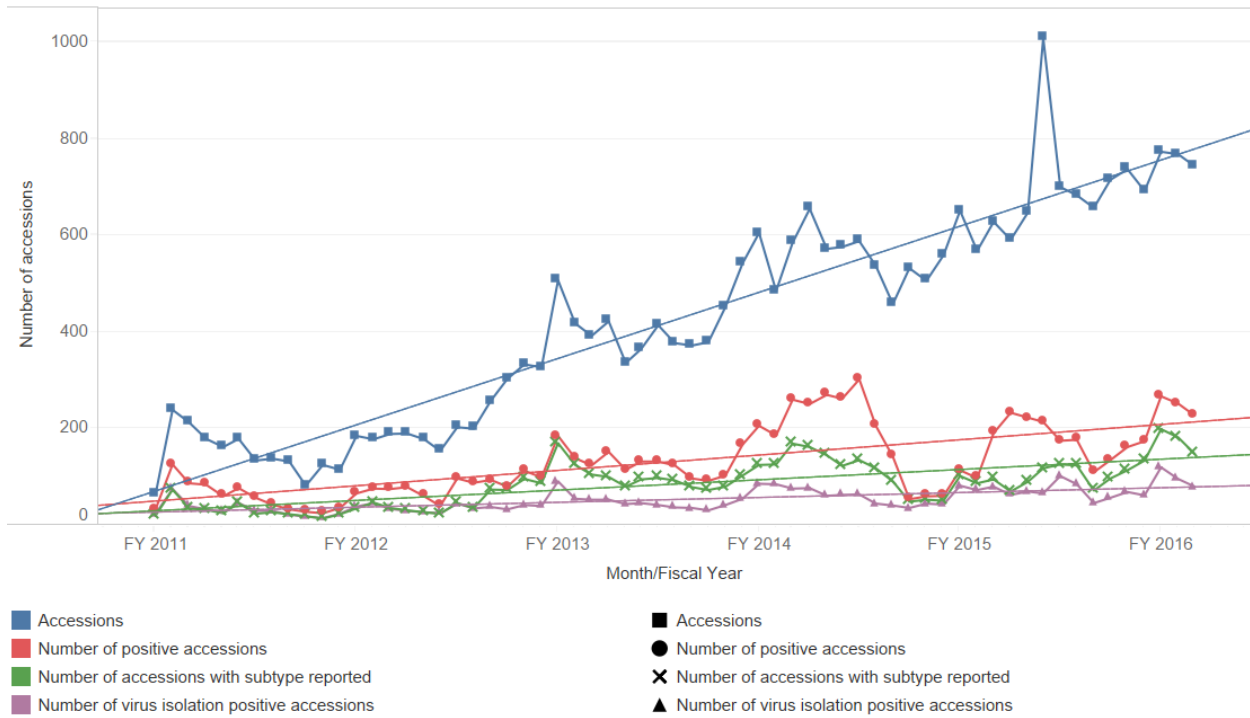


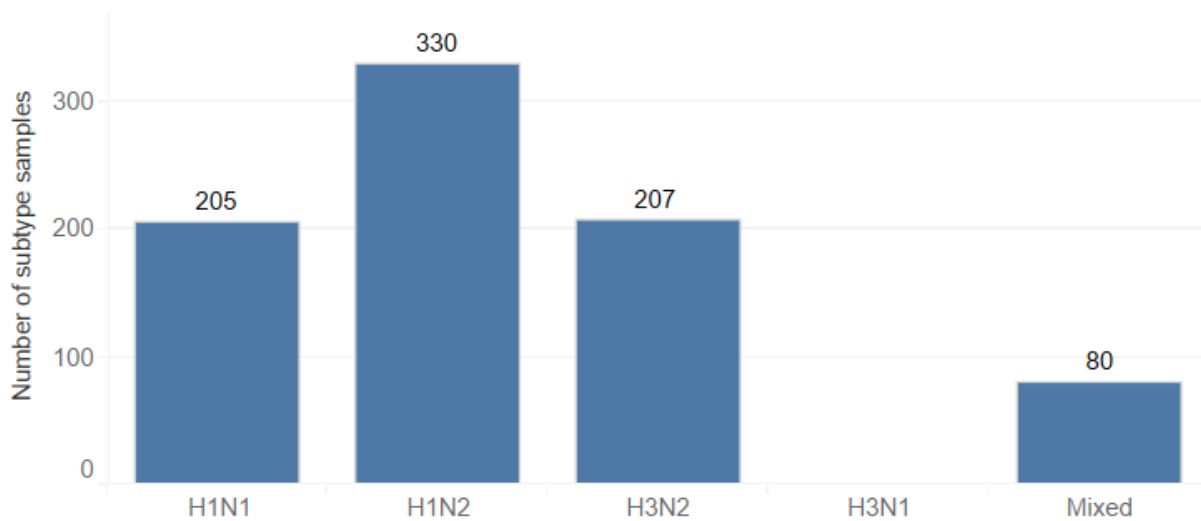
Figure 1. Surveillance for IAV in swine: Number of laboratory accessions and samples tested, FY 2011 – FY 2016 Q1

Influenza A Virus in Swine Surveillance Quarterly Report for FY 2016, Quarter 1



**Figure 2. Accessions submitted, subtyped accessions, positive accessions, and VI positive accessions over time with trend lines, FY 2011-FY2016 Q1**

Figure 3 shows the number of subtype detections in FY 2016 Q1. The total number of samples subtyped was 822, including 205 H1N1, 330 H1N2, 207 H3N2, 0 H3N1, and 80 mixed.



**Figure 3. FY 2016 first quarter subtype detections**

Figure 4 breaks down accessions by subtype rRT-PCR from FY 2011 to FY 2016 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.

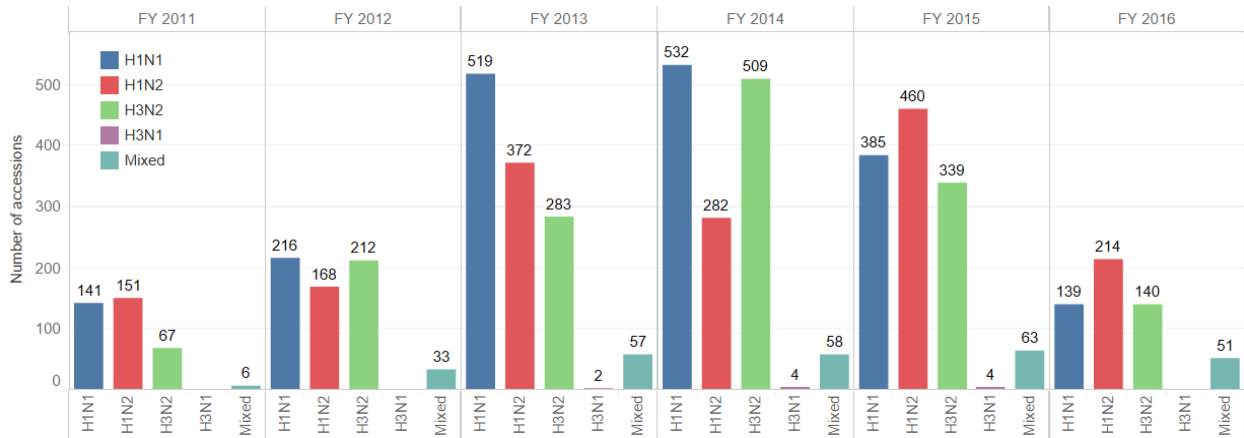


Figure 4. Number of subtypes, FY 2011 through FY 2016 Q1

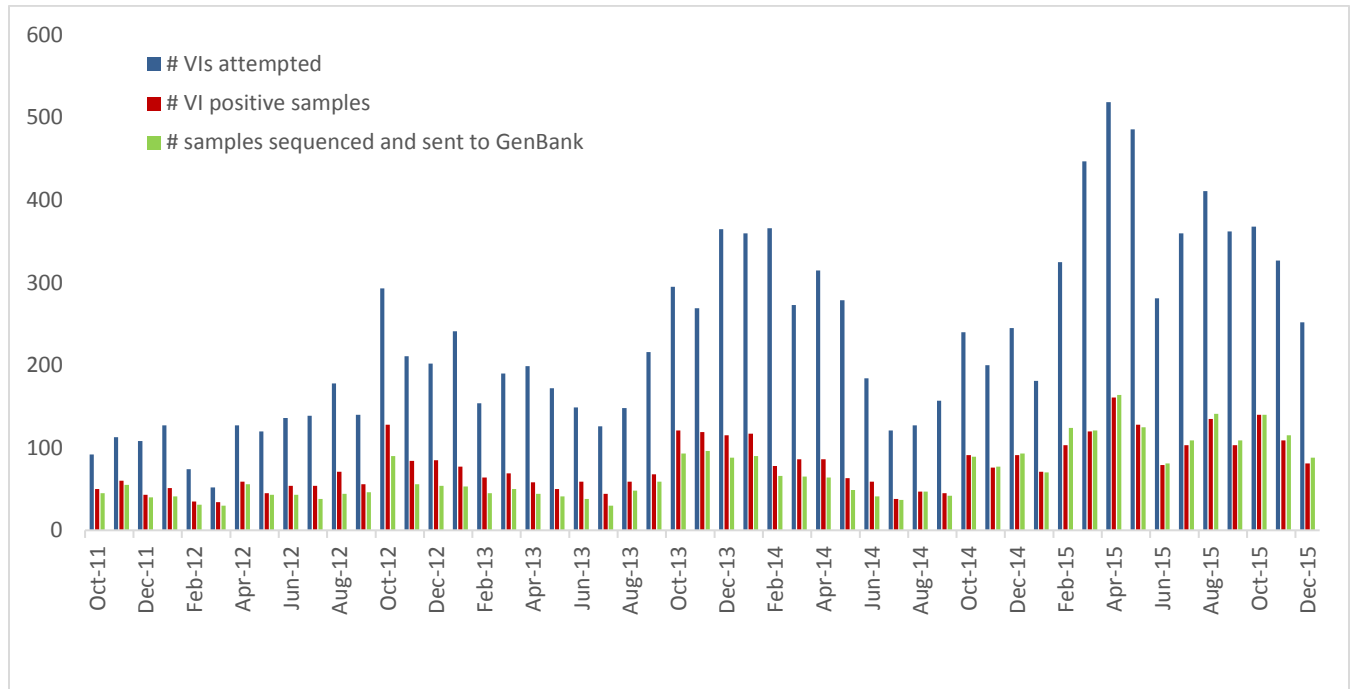


Figure 5. Number of virus isolations attempted, positive virus isolations, and GenBank submissions from FY 2012 through FY 2016 Q1

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 two occurrences of H1N1, three occurrences of H1N2, and one of H3N2. 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, Q1 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	48	10	23	0	12	6
Nursery	103	36	40	0	25	6
Grower/Finisher	92	39	21	0	23	12
Sow/Boar	6	2	3	0	1	0
Not Recorded/Unknown	280	52	127	0	79	27

**Table 2.** Number of positive accessions tested for IAV-S by specimen type and by viral subtype, Q1 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	165	85%	61	60	0	38	8	149
Nasal or Nasal Swab	99	68%	25	49	0	27	3	77
Oral Fluids	278	32%	58	109	0	78	41	101
Other Specimens	2	50%	0	0	0	0	0	16

\*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.

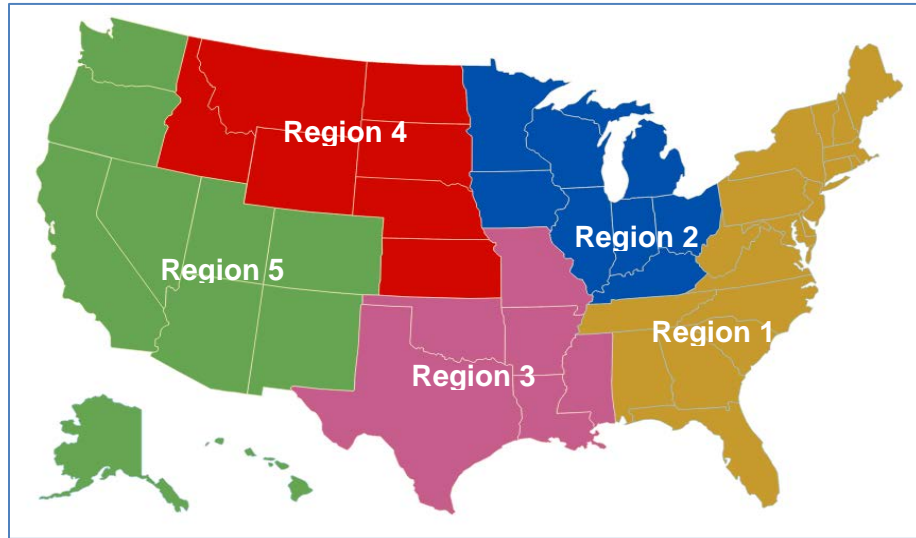


Figure 6. A map of the regions for national IAV-S surveillance

### Summary of Regional Data and Predominant Subtypes from ARS

Table 3. Summary of predominant subtypes in each region for FY 2014 Q2 through FY 2016 Q1

**Most Predominant HA/NA phylo-types overall:**

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

**Region 1 (Total HA/NA: 356)**

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

**Region 4 (Total HA/NA: 180)**

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

**Region 2 (Total HA/NA: 841)**

Most diversity of all regions  
 Gamma H1/Classical N1  
 Delta1 H1/2002-N2  
 IV-A H3/2002-N2  
 IV-B H3/2002-N2  
 Low frequency but consistent detections of additional clusters of H1 and H3 e.g. IV-E H3/2002-N2

**Region 3 (Total HA/NA: 107)**

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

**Region 5 (Total HA/NA: 4)**

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 2014 through Q1 FY 2016. Region 1 demonstrates H1N2 as the predominant subtype. Regions 2 and 3 demonstrate H1N2 as the predominant subtype. H3N2 and H1N1 were predominant in Region 4, and Region 5 saw H1N2 and mixed as the predominant subtypes.



**Figure 7. Percentage of subtyped accessions by region for Q1 FY 2014 through Q1 FY 2016 Q1**

In December 2015, USDA surveillance identified an H4N6 subtype in a commercial swine herd in Region 3. A small number of gilts exhibited clinical illness; a few aborted. NAHLN testing resulted in a positive Matrix PCR for influenza A, however the subtyping PCRs for H1 and H3 were negative. Whole genome sequencing at NVSL confirmed H4N6, a common influenza virus found in wild birds. H4N6 is not considered a highly pathogenic avian influenza (HPAI) or low pathogenic avian influenza (LPAI). Follow-up investigation found no within-herd transmission.

## 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<sup>1</sup> of 462 isolates with published sequences in GenBank was characterized by phylogenetic analysis in Q1 FY 2016. This analysis provides information on the genetic

<sup>1</sup> 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.



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.

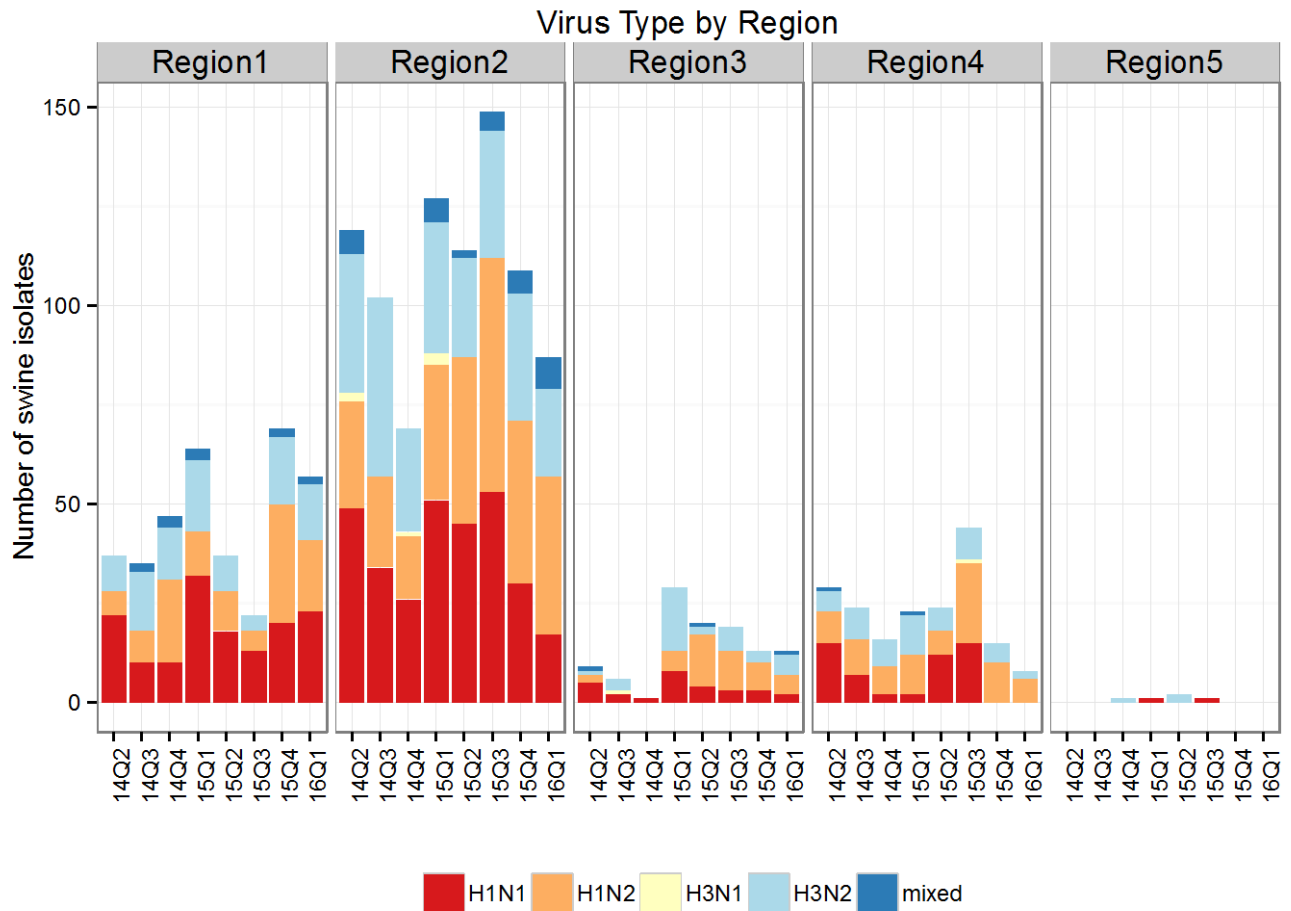


Figure 8. Virus type by region 2-year summary Q2 FY 2014 to Q1 FY 2016

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 were fewer alpha detections in Q1 of FY 2016 than in the previous quarter and there was a relative increase in delta H1 compared to delta 2 and gamma.

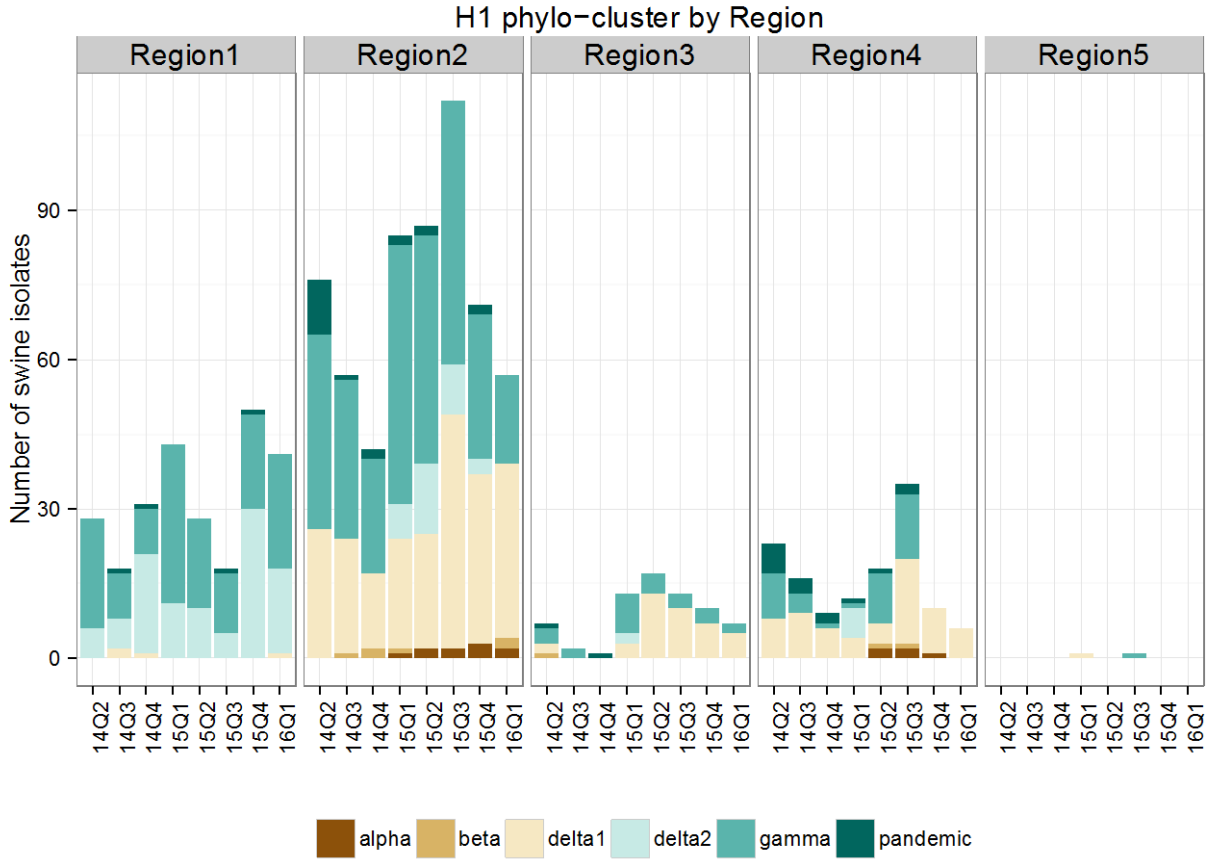


Figure 9. H1 phylo-cluster by region – 2-year summary Q2 FY 2014 to Q1 FY 2016

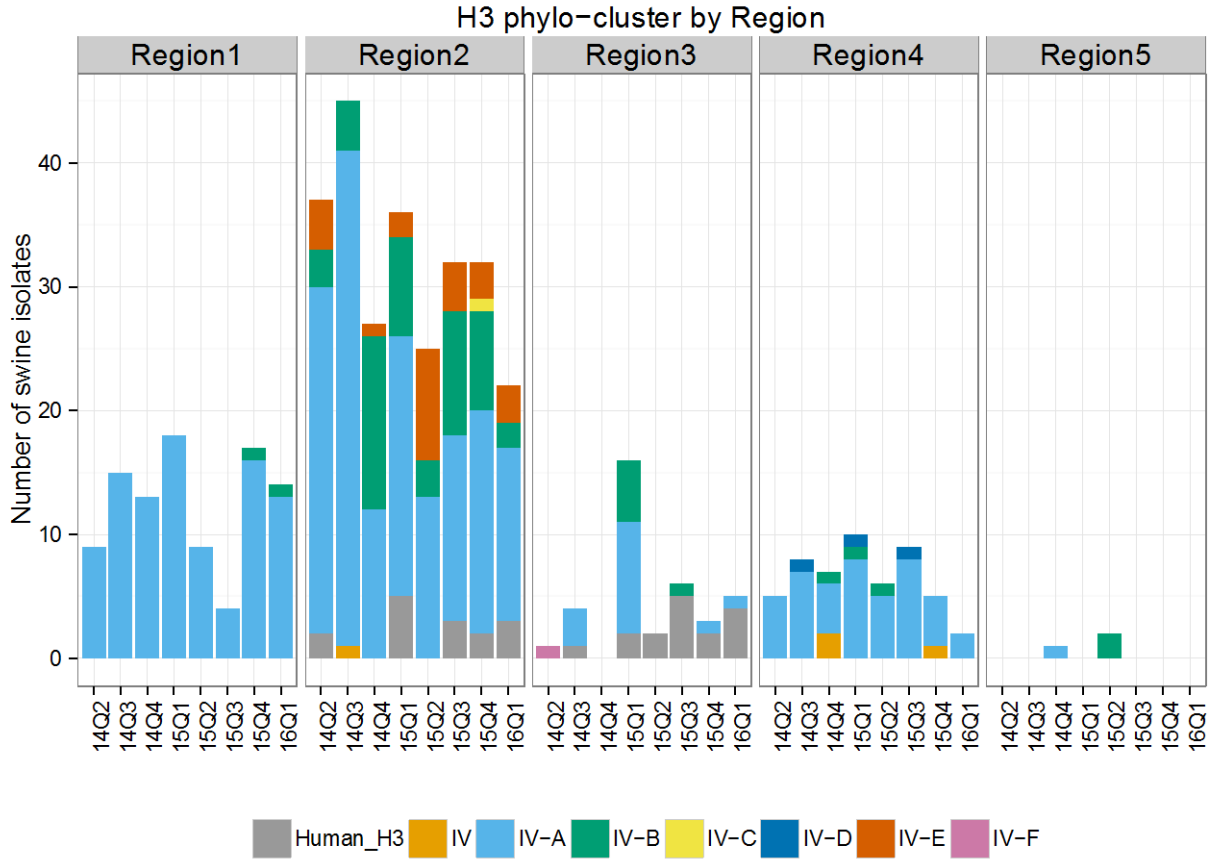


Figure 10. H3 phylo-cluster by region, 2-year summary Q2 FY 2014 to Q1 FY 2016

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 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 continued to be more frequently detected than the 1998-lineage. The 1998-lineage N2 is most frequently paired with the delta2 H1.

**National phylogenetic information M gene**

Only the H1N1pdm09 M gene was detected this quarter.

**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 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 3. Virus isolates received in repository**

Virus isolates in the repository	
<b>2016 YTD</b>	238
<b>2015</b>	883
<b>2014</b>	765
<b>2013</b>	820
<b>2012</b>	915
<b>TOTAL TO DATE</b>	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. 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	
<b>H3N2</b>	1,212
<b>H3N1</b>	9
<b>H1N1</b>	1,555
<b>H1N2</b>	1,320
<b>Mixed</b>	275
<b>TOTAL</b>	4,371

### **Conclusion**

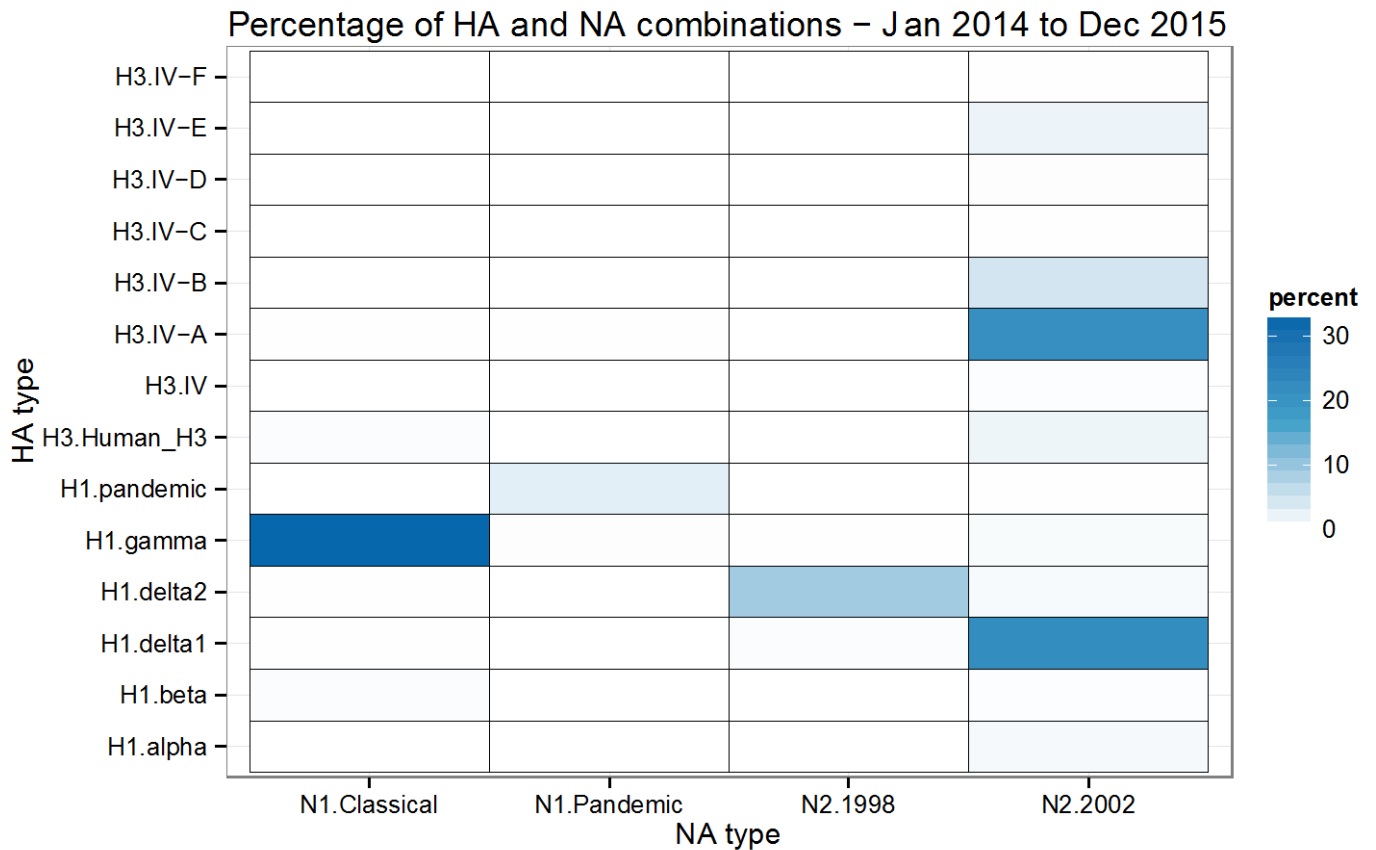
The IAV-S voluntary surveillance system continues to provide insight into the genetic makeup of circulating influenza virus in limited populations of commercial pigs. Genetic information and virus isolates are made publicly available for further research and possible vaccine strain selection and efficacy 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 2014 to December 2015. 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.

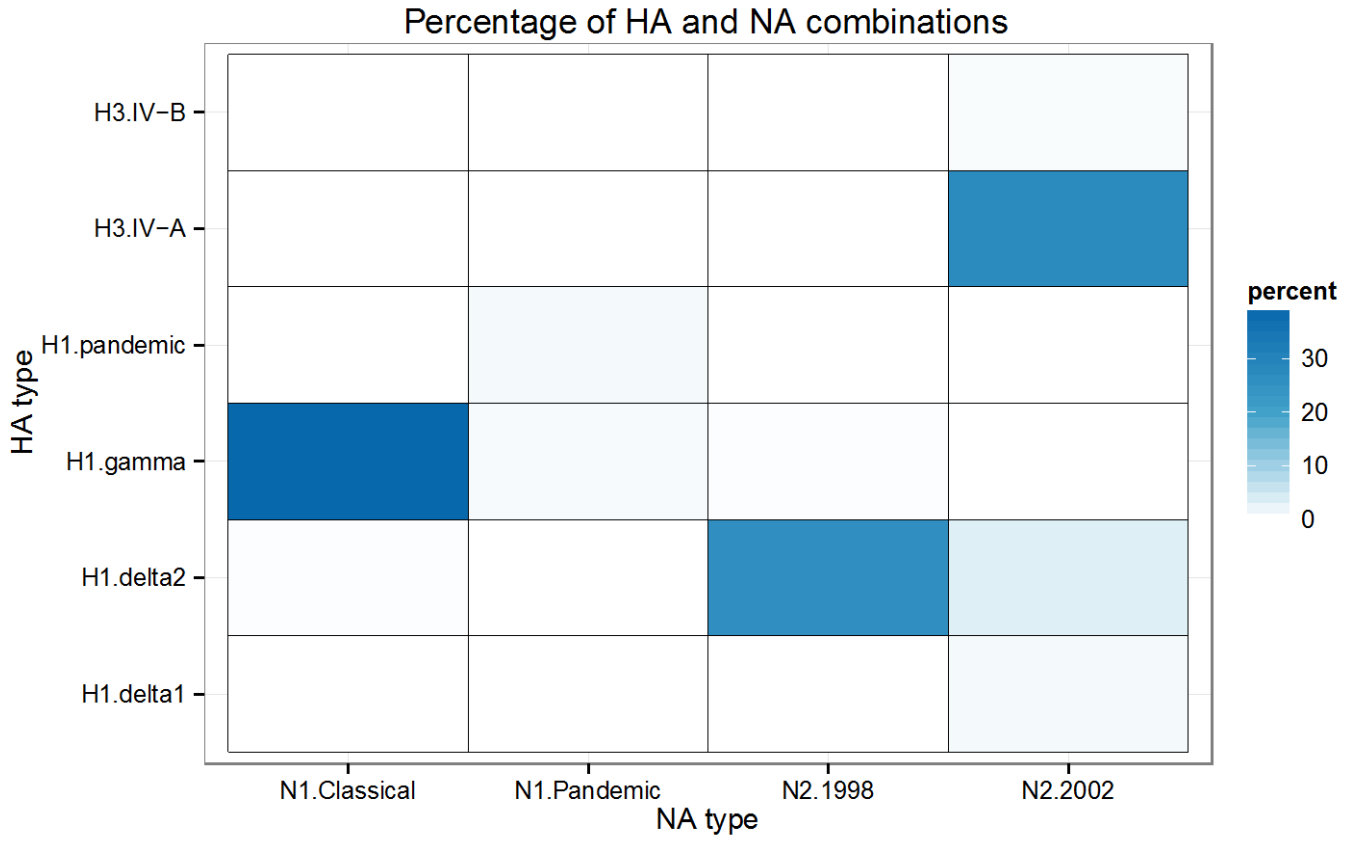


Total HA & NA combinations – 1,496



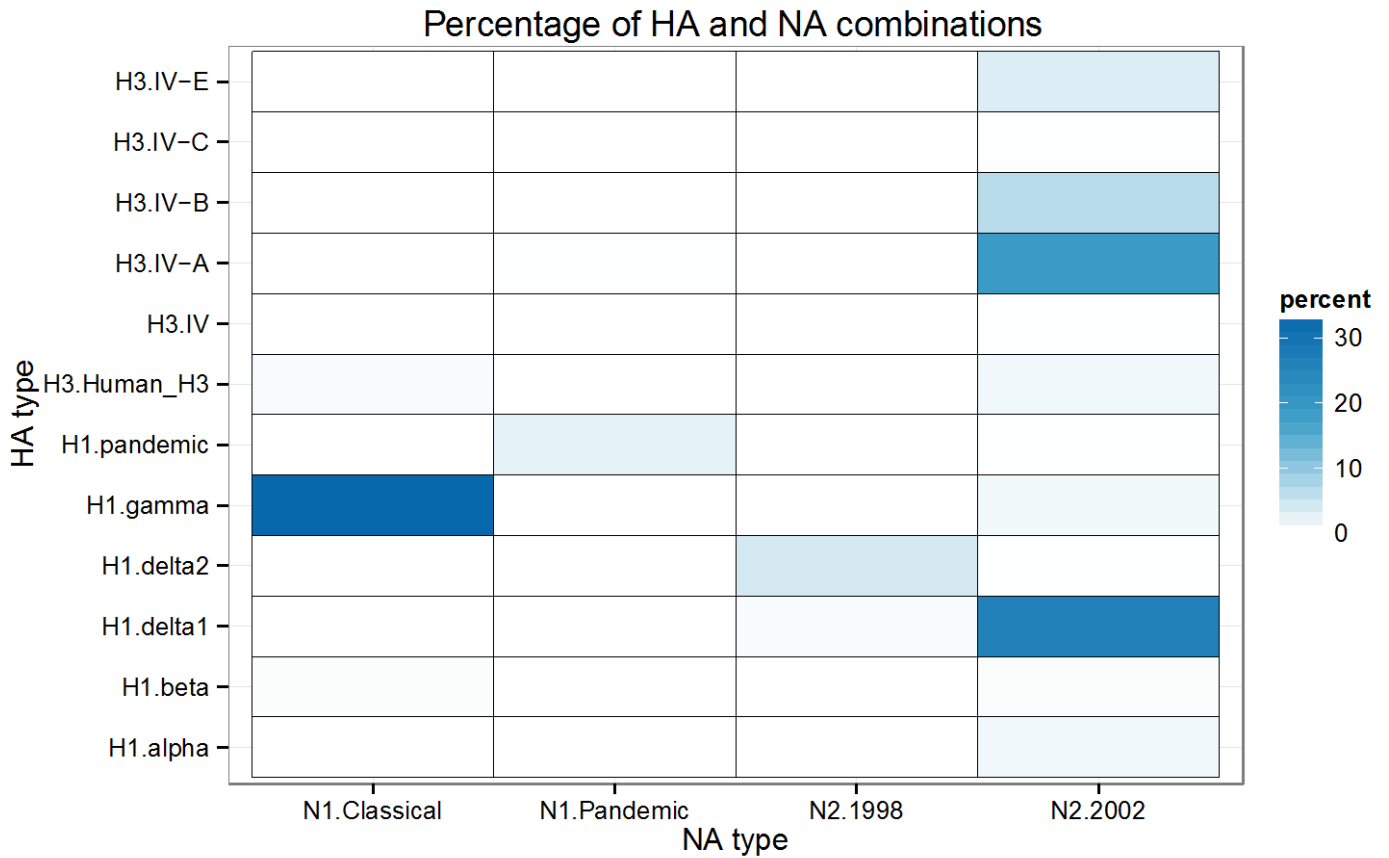


**Region 1: Total HA & NA combinations – 356**



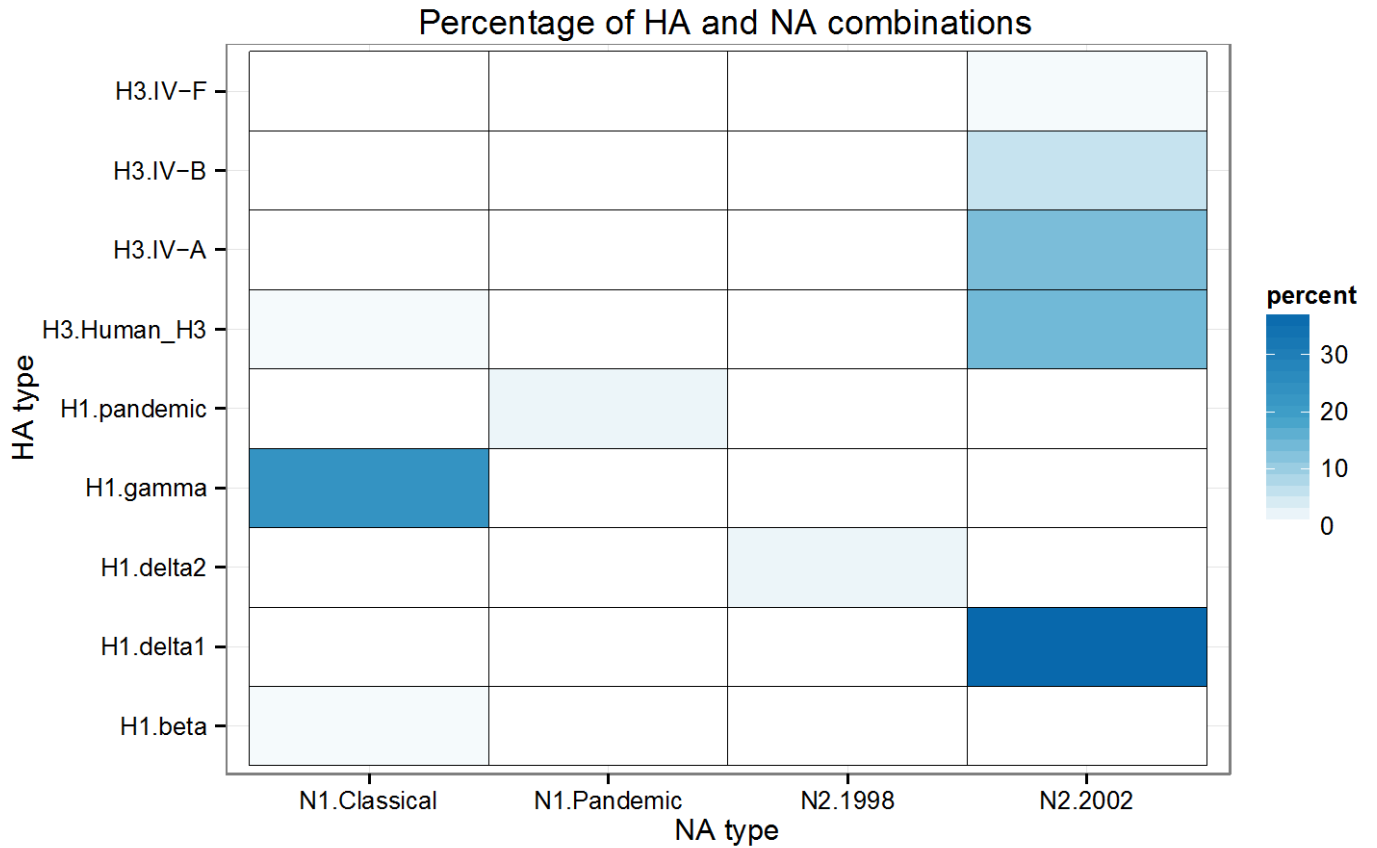


**Region 2: Total HA & NA combinations – 841**



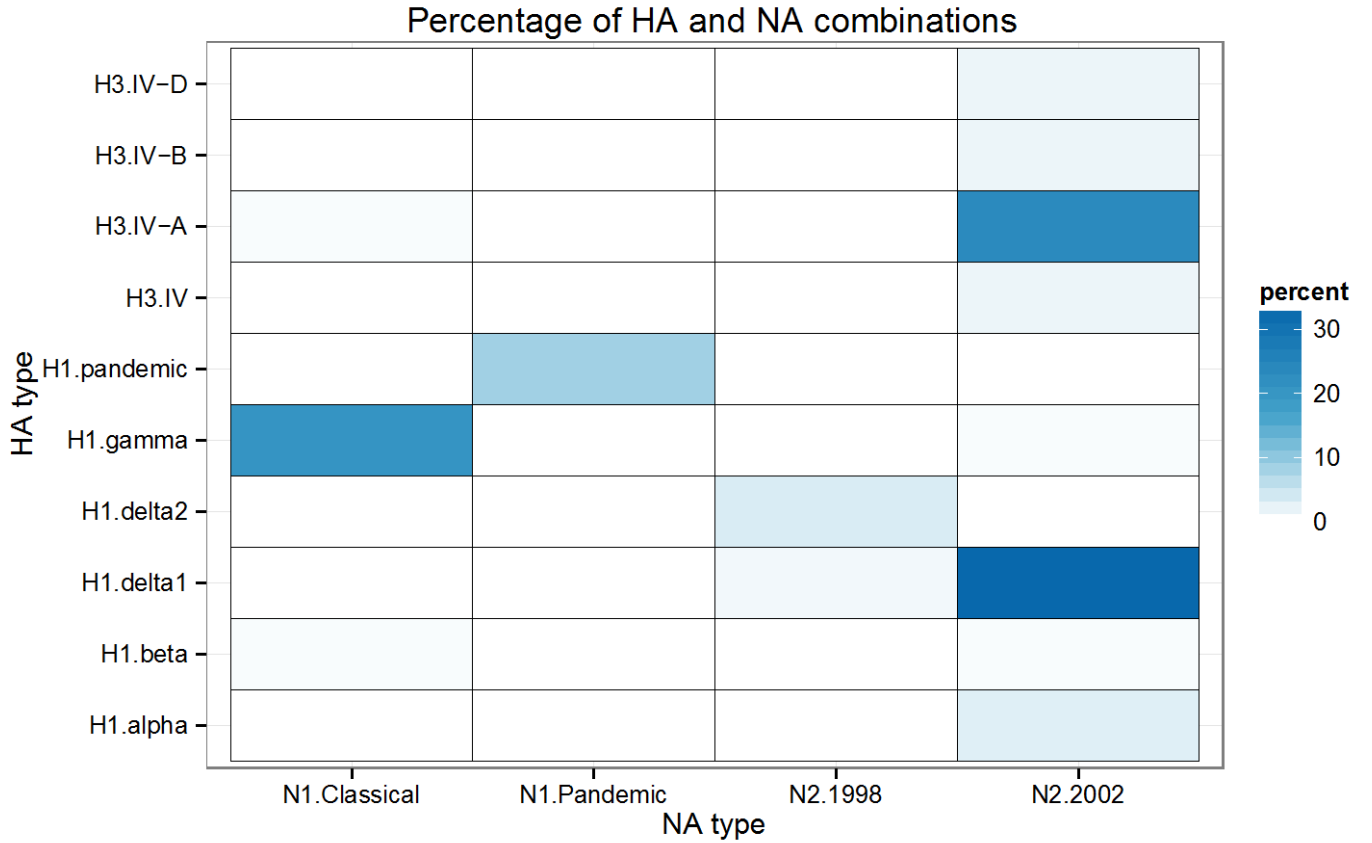


**Region 3: Total HA & NA combinations – 107**





**Region 4: Total HA & NA combinations – 180**





**Region 5: Total HA & NA combinations – 5**

