USDA

Influenza A Virus in Swine Surveillance

Fiscal Year 2015 Quarterly Report

Surveillance Summary for Third Quarter FY 2015: April 1 – June 30, 2015

Report Summary

- This report covers the third quarter (Q3) of Fiscal Year (FY) 2015, from April 1, 2015 – June 30, 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, 7,106 samples were submitted for IAV-S surveillance from 2,033 accessions.
- Submissions for FY 2015 are on track to exceed those in FY 2014.
- H1N1 and H1N2 subtypes are predominant; findings of H1N2 and H3N2 increased in Q3 from Q2.
- Regions 1 and 2 observed H1N1 as the predominant subtype, H1N2 was observed to be predominant in Region 3, H3N2 was predominant in Region 4, and H1N1 was predominant in Region 5, and 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 in FY 2015.
- 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.
- 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 August 18, 2015, is intended to provide 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 when the 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 fitting the case definition and may 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 5 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 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

External and internal reviews of the IAV-S surveillance program are ongoing. APHIS' Policy and Program Development Division is conducting the internal review. This review will assess program objectives, achievements, and outcomes generated by collecting information from program collaborators and stakeholders. The internal analysis is expected to provide information on the desired future of the program and identify cost factors and forward cost projections. External reviewers have been contracted to conduct that analysis. The external review is intended as a technical epidemiologic review to not only provide a critical assessment but also provide recommendations for process improvement and system efficiency.

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 NVSL for virus isolation (VI) and submission banking. ARS-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 for FY 2014 exceeded numbers from FY 2013. FY 2015 numbers of accessions and samples are on track to exceed FY 2014, and there was a spike in submissions in early to mid-2015 (Figure 1).
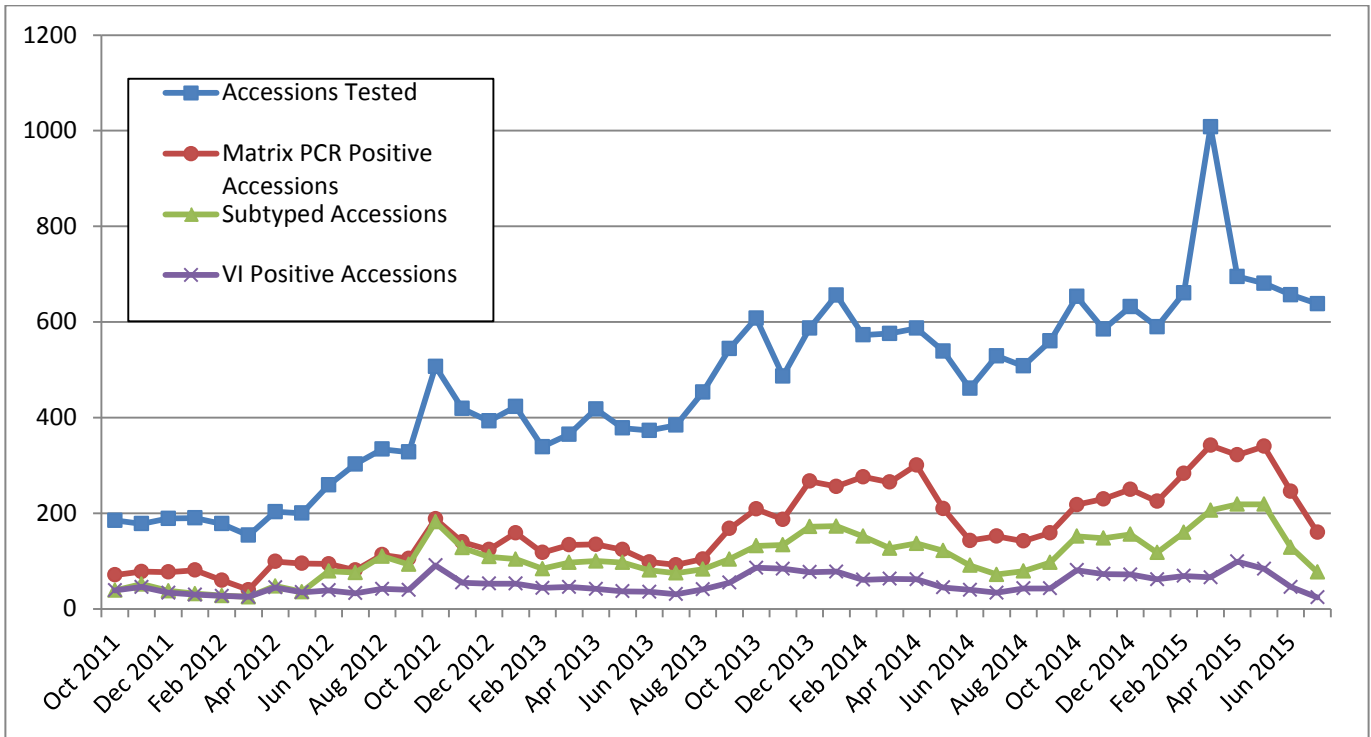
Figure 1. Accessions submitted, subtyped accessions, positive accessions, and VI positive accessions over time

For FY 2015's third quarter, 7,106 samples have been tested from 2,033 accessions (Figure 2) in addition to the 4,129 accessions and 12,769 samples from FY 15 Q1 and Q2.
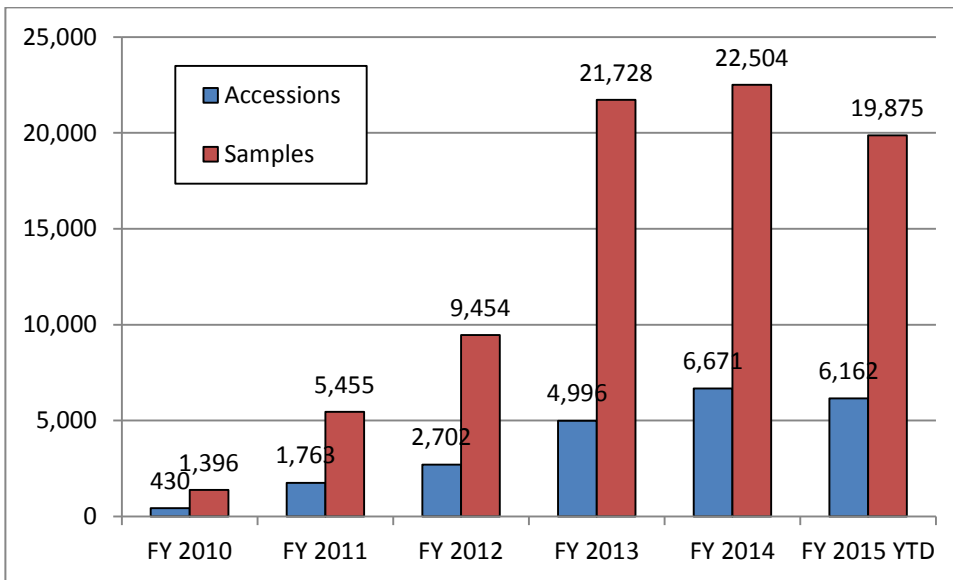

Figure 2. Surveillance for IAV in swine: Number of laboratory accessions and samples tested, FY 2010 – Q3 FY 2015.

Figure 3 shows the number of subtype instances in FY 2015 Q3. The total number subtyped was 873, including 256 H1N1; 297 H1N2; 250 H3N2; 1 H3N1; and 69 mixed.
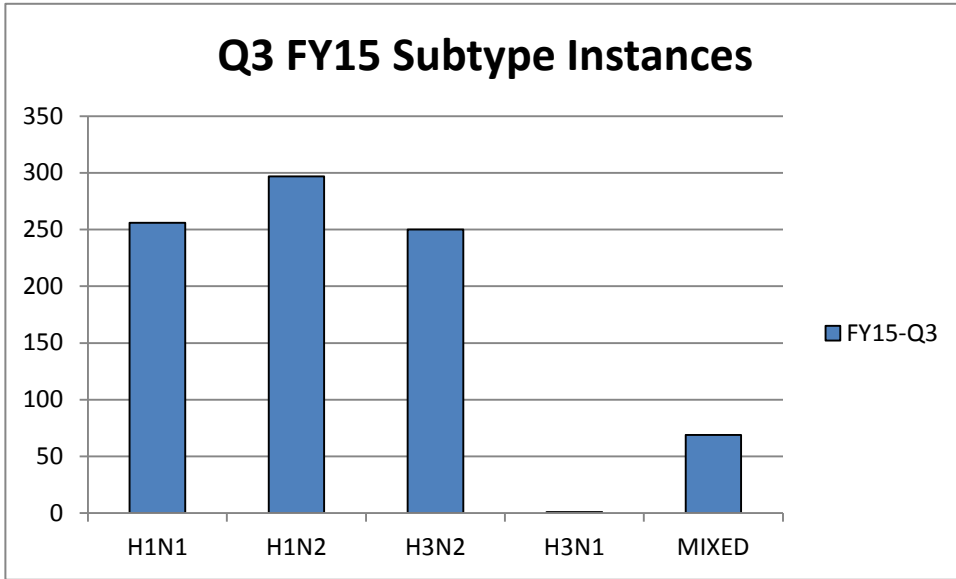
Q3 FY15 Subtype Instances

Figure 3. Q3 FY 2015 subtype instances.

Figure 4 breaks down accessions by subtype rRT-PCR from FY 2010 to Q3 FY 2015. H1N1 remains the major subtype over the course of the surveillance. However, in Q3, H1N2 predominated with 297 subtype instances detected out of 873 total subtypes. H1N1 and H3N2 detections remained steady. More detailed ARS-NADC information can be found later in this report on page 6. 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.
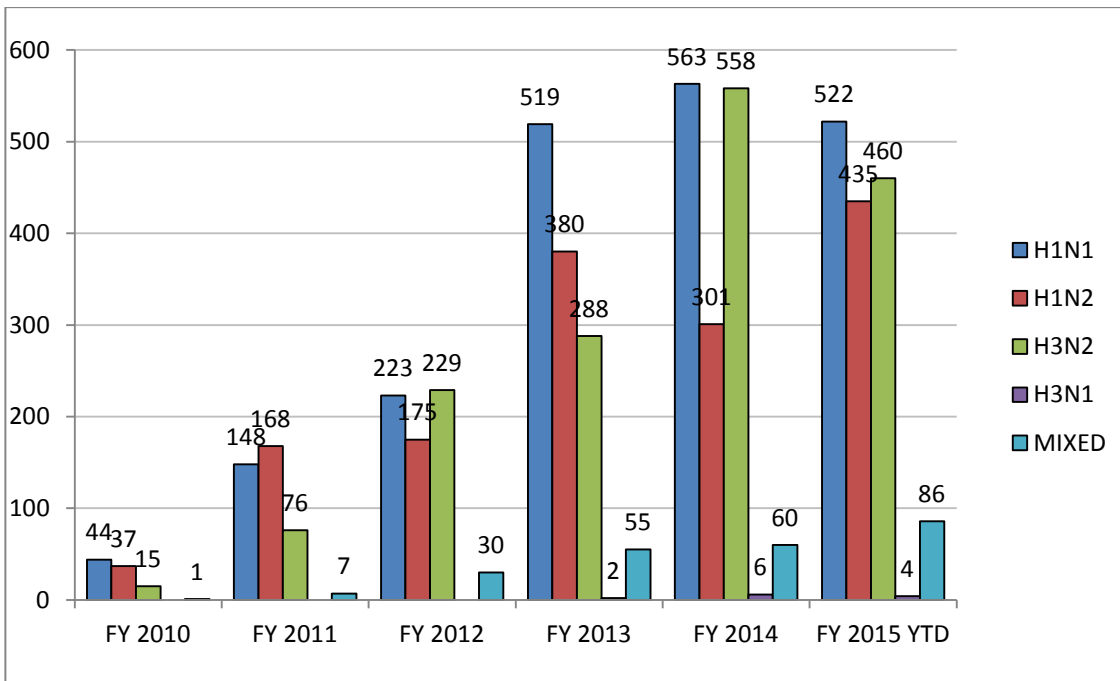

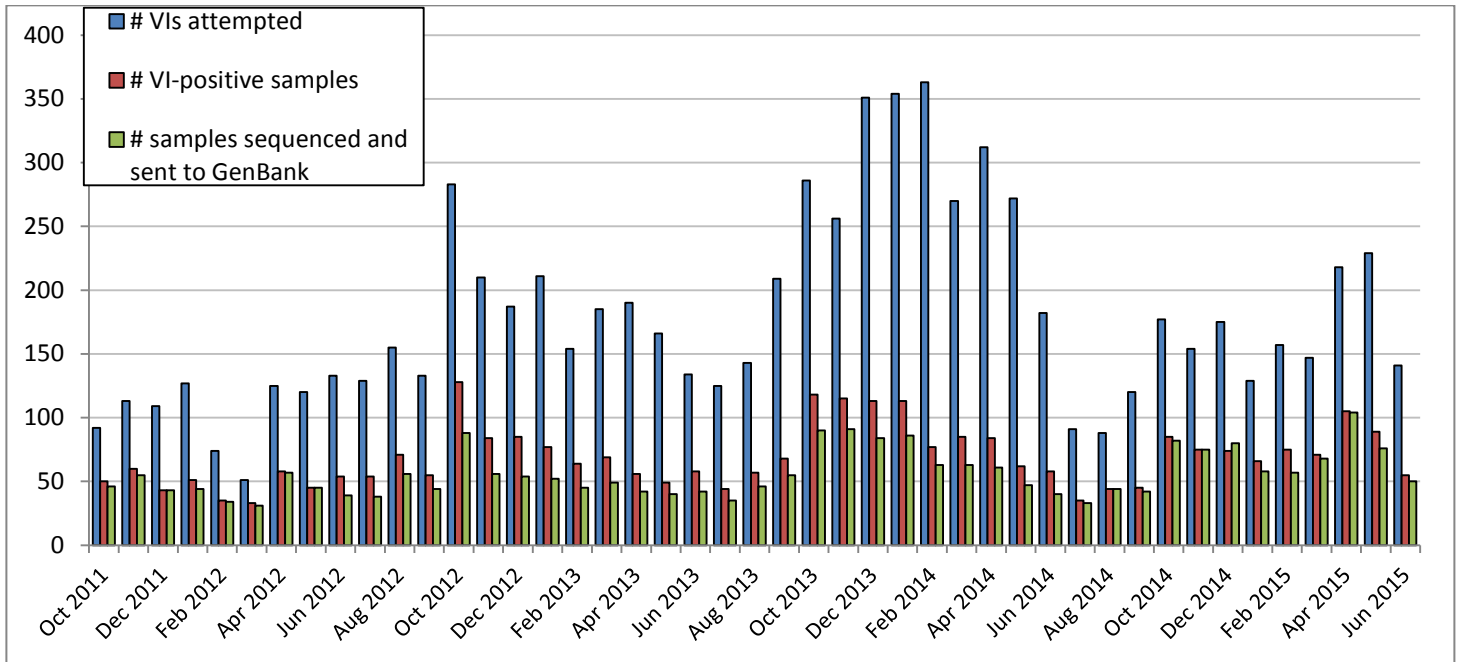**Figure 4. Number of subtypes, FY 2010 through Q3 FY 2015.**

Figure 5. Number of virus isolations attempted, positive virus isolations, and GenBank submissions from FY 2012 through Q3 FY 2015.

When accessions were evaluated by age-class for the third quarter, the following observations were noted. H3N2 was the most common subtype among Suckling. H1N1 was the common subtype among Nursery. H1N1 and H1N2 were the most common subtypes among Grower/Finishers. Sow/Boar had limited testing, with H3N2 being the predominant subtype. 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, followed by nasal/nasal swab, and then lung/lung swab (Table 2).

Table 1. Number of positive accessions tested for IAV-S by age class and by subtype in Q3 FY 2015

Age Class	Number of accessions with subtype reported*	H1N1	H1N2	H3N2	H3N1	Mixed
Suckling	79	13	26	28	…	12
Nursery	101	43	23	22	…	13
Grower/Finisher	112	37	37	29	…	9
Sow/Boar	11	…	3	5	1	2
Not recorded/ Unknown	271	78	105	73	…	15

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

Table 2. Number of positive accessions tested for IAV by specimen type and by subtype in Q3 FY 2015.

Specimen Type	Number of accessions with subtype reported*	Percent of accessions with positive virus isolation	Percent of accessions with sequence submitted to GenBank	H1N1	H1N2	H3N2	H3N1	Mixed
Lung or lung swab	102	74%	66%	37	39	21	…	5
Nasal or nasal swab	128	56%	59%	30	60	35	1	2
Oral fluids	348	24%	21%	105	96	105	…	42
Other**	…	…	…	…	…	…	…	…

*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, and therefore no sampling strategies can be applied to the regions.
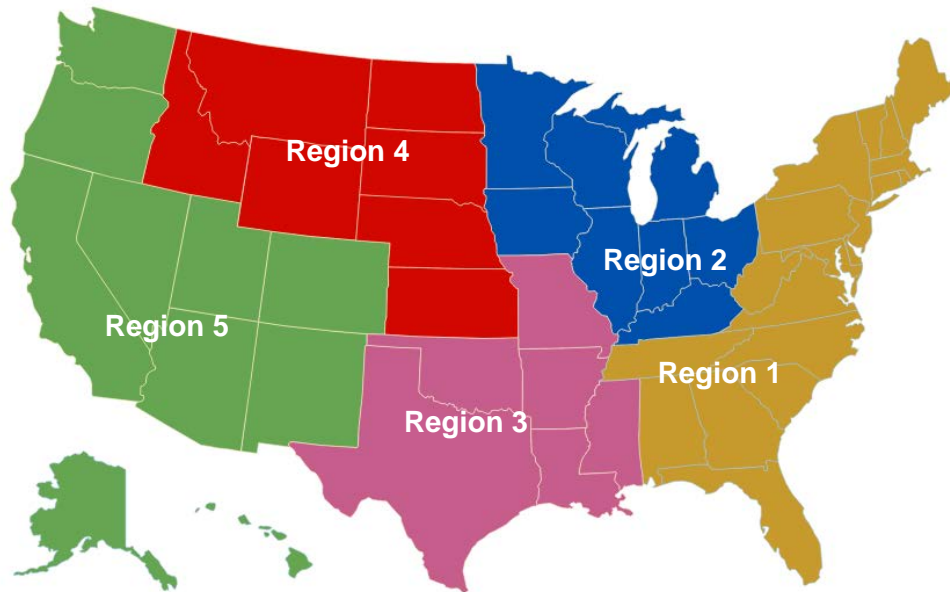

Figure 6. A map of the regions for national IAV-S surveillance

Figure 7 shows the distribution of rRT-PCR subtyped accessions among the five regions. Regions 1 and 2 demonstrate H1N1 as the predominant subtype. Region 3 demonstrates H1N2 as the predominant subtype. H3N2 was predominant in Region 4, and Region 5 saw H1N1 as the predominant subtype. H3N1 continues to be in the minority of subtypes for all regions.
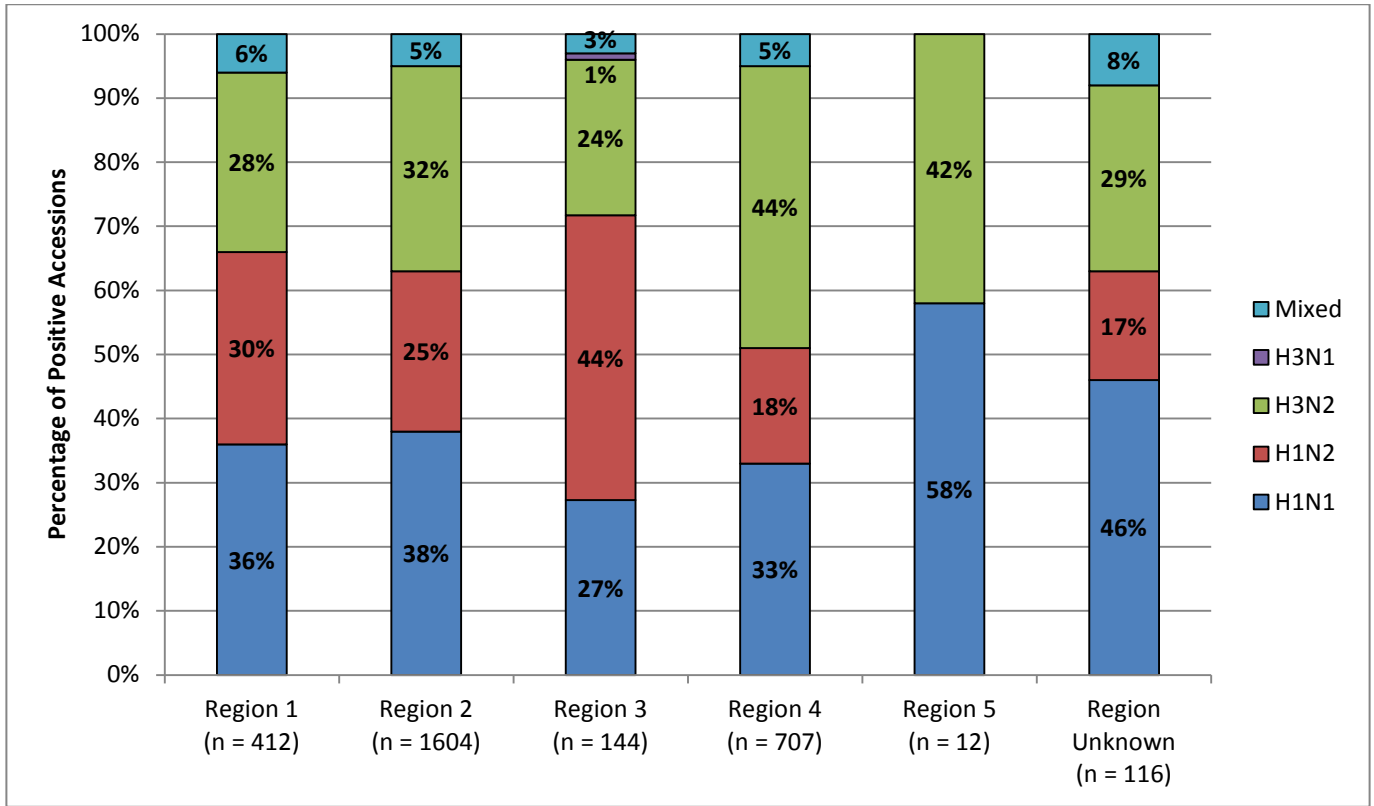

Figure 7. Percentage of subtyped accessions by region for FY 2014 through Q3 FY 2015.

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 485 isolates with published sequences in GenBank was characterized by phylogenetic analysis in Q3 FY 2015. 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.
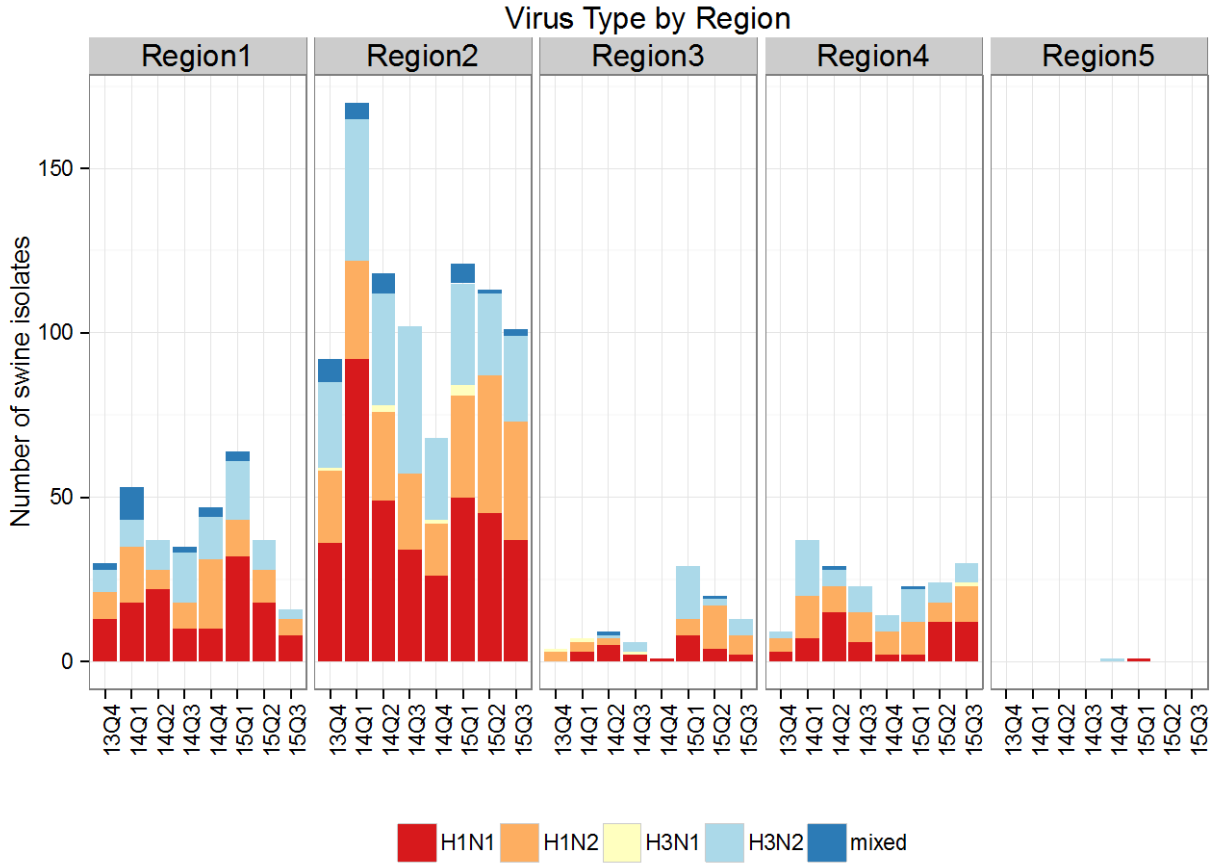
Figure 8. Virus type by region 2-year summary Q4 FY 2013 to Q3 FY 2015

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 and 5 in FY 2015.

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.
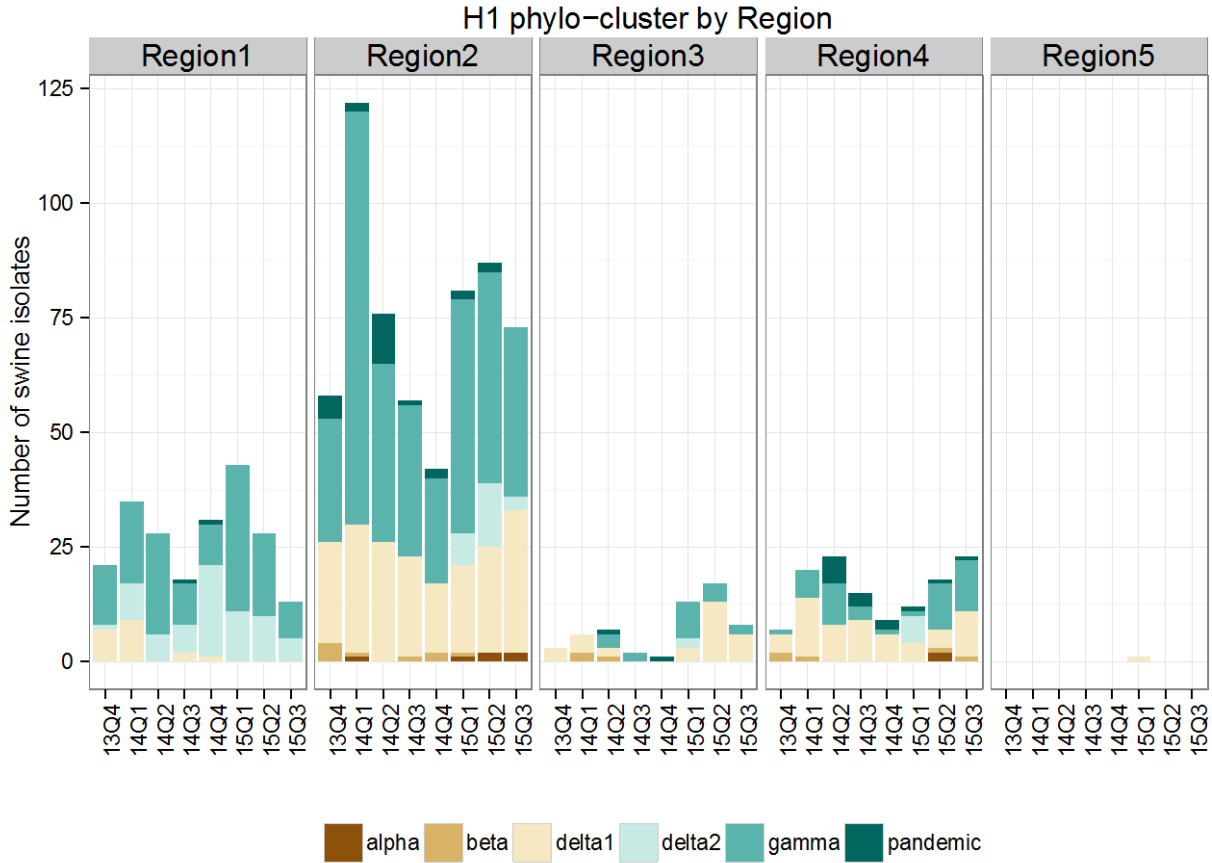
Figure 9. H1 phylo-cluster by region – 2-year summary Q4 FY 2013 to Q3 FY 2015

In the H1 subtypes (see Figure 9), there was one H1N1pdm09 virus detected in May 2015. The number of delta-2 H1 viruses experienced a decrease in the third quarter of 2015 and there were less delta H1 detections than in previous quarters, save FY2015 Q3 Region 4. There were a few alpha H1 detections and gamma H1 remained the predominant HA clade overall.
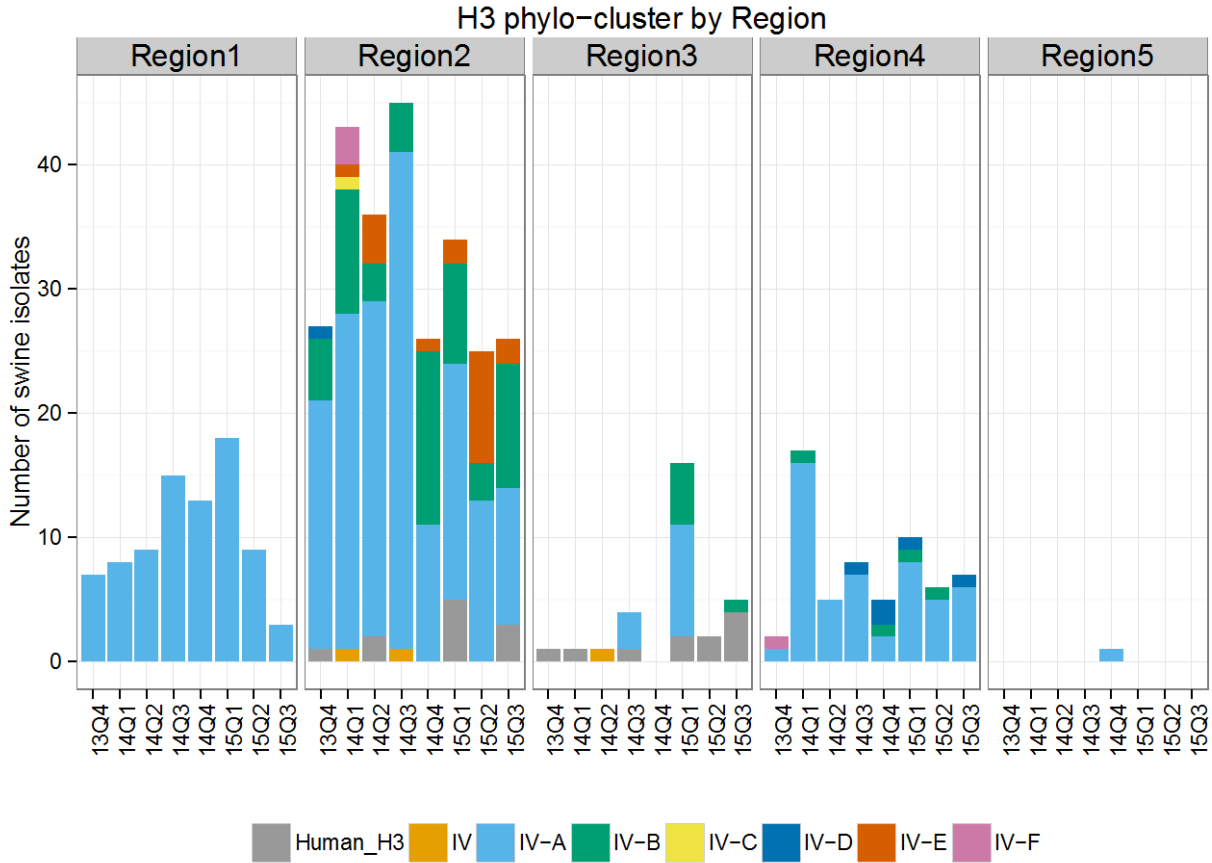
Figure 10. H3 phylo-cluster by region, 2-year summary Q4 FY 2013 to Q3 FY 2015

The number of H3 detections increased in Regions 2, 3, and 4 in FY 2015 Q3 from Q2, but decreased in Region 1 (Figure 10). The Cluster IV-A viruses continued to be the dominant cluster. New human-like H3 detections were identified in Regions 2 and 3. There was a decrease in IV-E detections in the third quarter in Region 2 when compared to the second quarter.

National phylogenetic NA gene information
Both the N1 and N2 subtypes are found in circulating swine viruses. Detections of H1N1pdm09 N1 in January were consistent with detection of pdm09 H1. Decreased detections of pdm09 N1 were noted between July and November 2014; this is consistent with the decline in pdm09 H1 that was observed. Classical N1 continued to be the predominant N1 clade. Starting in July 2014, there was a relative increase in detections of 1998-lineage N2 paired with delta-2 H1, although the 2002-lineage of N2 continued to be more frequently detected than 1998-lineage.

National phylogenetic information M gene
Only the H1N1pdm09 M gene was detected during the third quarter of FY 2015.

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. For the third quarter of FY 2015, the NVSL Diagnostic Virology Laboratory provided 55 isolates to five institutions: one governmental, one academic, two pharmaceutical, and one international. NVLS received 288 isolates into the repository, increasing the 2015 YTD isolates received to 720 (Table 3). Table 4 reports the number of isolates available in the repository by subtype.

Table 3. Virus isolates received in repository

Virus isolates in the repository	
2015 YTD	720
2014	765
2013	820
2012	915
TOTAL TO DATE	3,220

Table 4. Number of subtyped isolates available through the repository

Subtyped isolates available through repository	
H3N2	1,124
H3N1	9
H1N1	1,459
H1N2	1,157
Mixed	255
TOTAL	4,004

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, governmental entities, academic institutions, and other scientific community partners for research and vaccine strain selection and efficacy testing. NVSL and ARS staff are consulted by these parties as subject matter experts in IAV-S when necessary.

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 July 2013 to June 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. A color legend is provided on the right side of the chart.
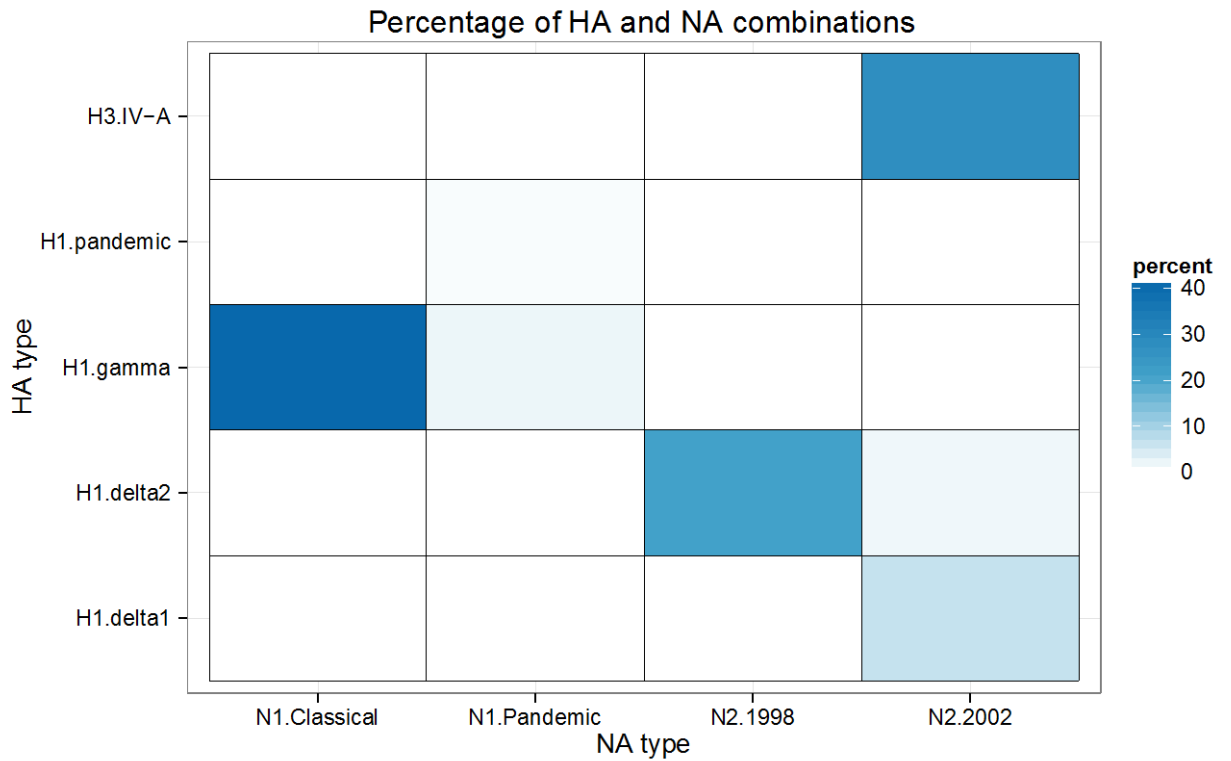

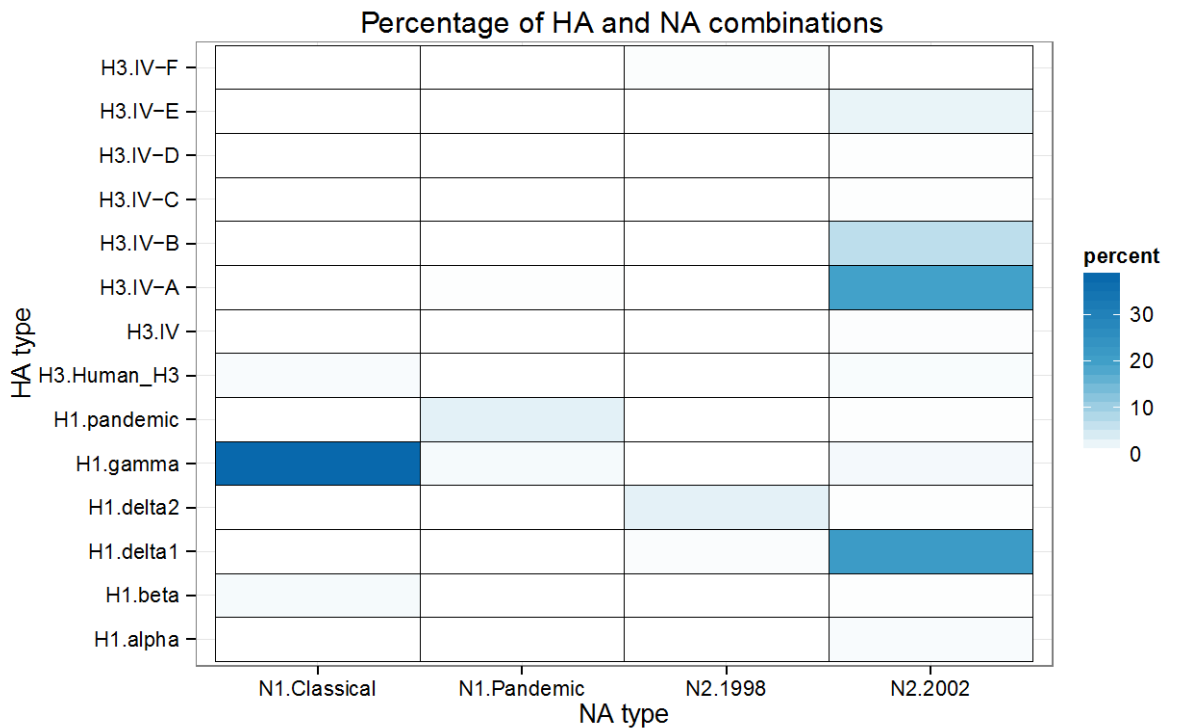Percentage of HA and NA combinations − Jul 2013 to Jun 2015
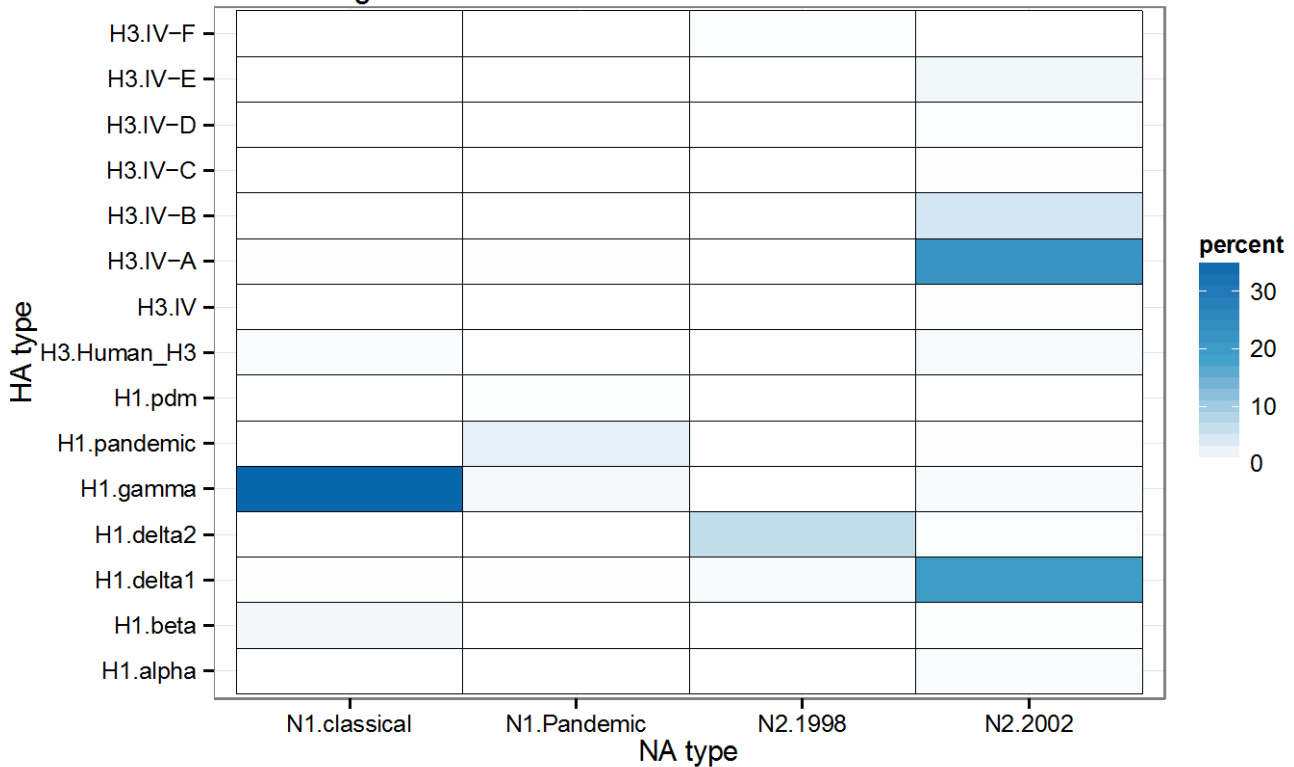
Total HA & NA combinations across the U.S. − 1,441
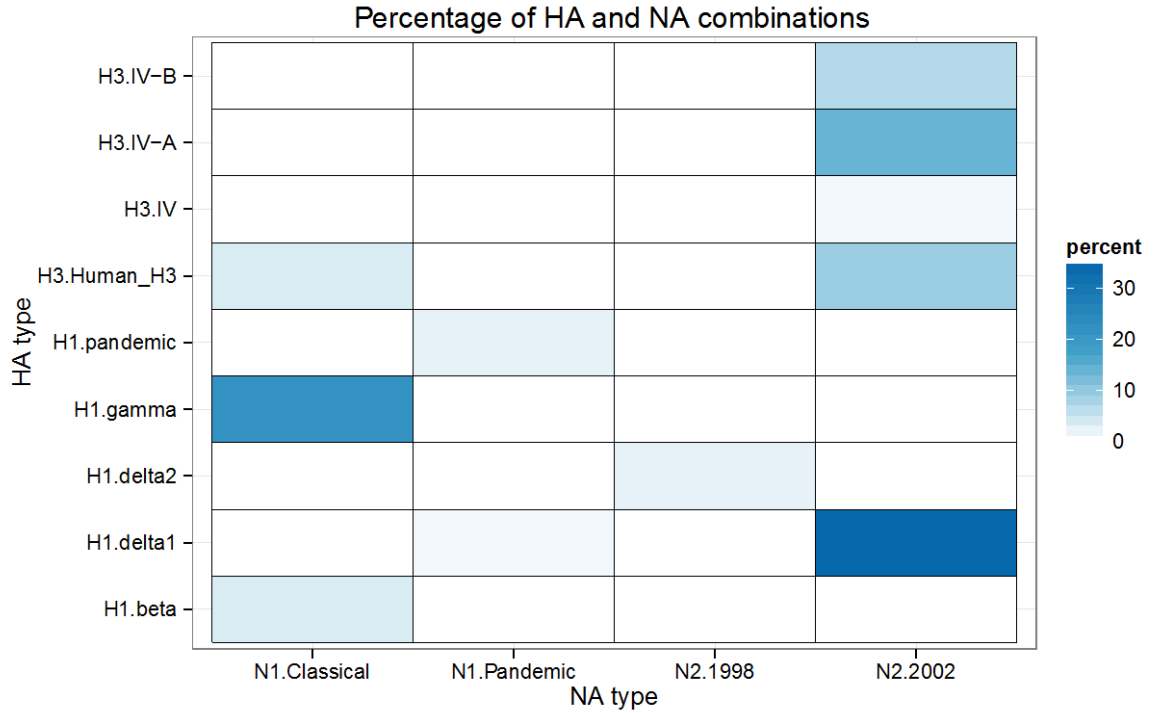
Region 1: Total HA & NA combinations – 299

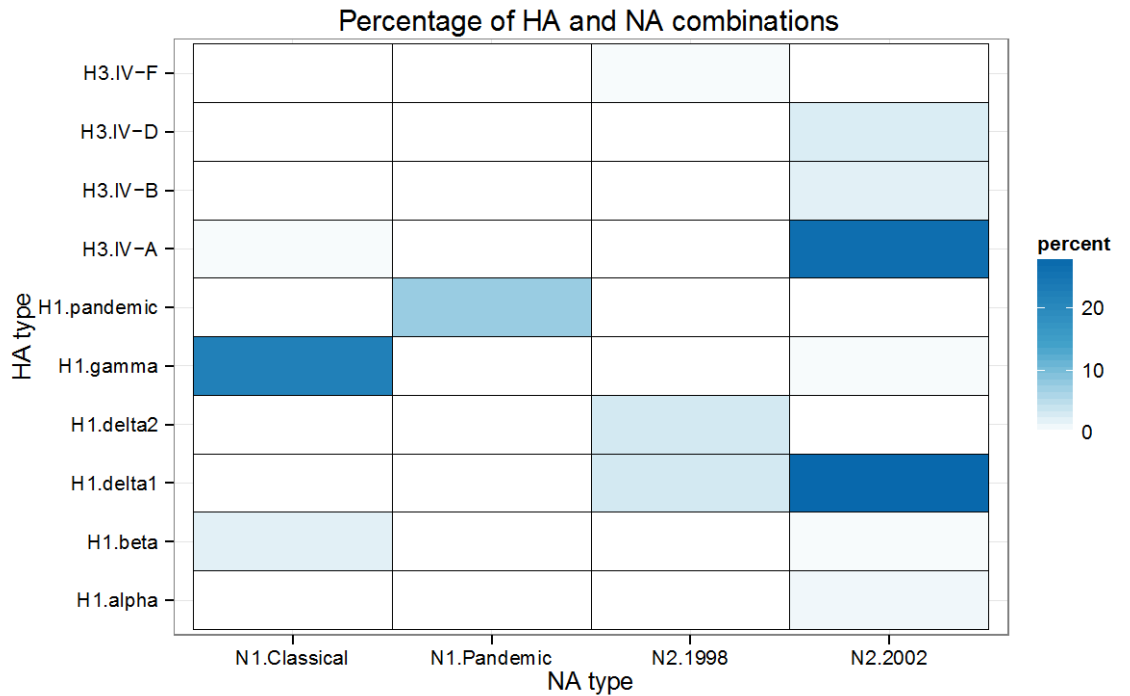**Region 2:** Total HA & NA combinations – 857

Region 3: Total HA & NA combinations – 87

Region 4: Total HA & NA combinations – 187

Region 5: Total HA & NA combinations – 2