Influenza A Virus in Swine Surveillance

Fiscal Year 2015 Quarterly Report

Surveillance Summary for Fourth Quarter FY 2015:
July 1 – September 30, 2015

Report Summary

- This report covers the fourth quarter (Q4) of Fiscal Year (FY) 2015, from July 1, 2015 – September 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, 7,614 samples were submitted for IAV-S surveillance from 2,128 accessions.
- H1N2 and H3N2 subtypes remain predominant
- No changes were noted from the third quarter in the predominant subtype by region: Regions 1 and 2 observed H1N1 as the predominant subtype; H1N2 was predominant in Region 3; H3N2 was predominant in Region 4; and H1N1 was predominant in Region 5. 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 February 10, 2016, 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 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 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 2015's fourth quarter, 7,614 samples have been tested from 2,128 accessions (Figure 1) in addition to the 6,176 accessions and 19,866 samples tested during FY 15 Q1 – Q3. Matrix PCR positive accessions continue to rise over time, but Matrix PCR positive accessions, subtyped accessions, and VI positive accessions flattened in the fourth quarter of FY 2015 (Figure 2). A spike in accessions tested in March of 2015 was noted, but due to the anonymity of submissions, follow-up investigations did not occur.
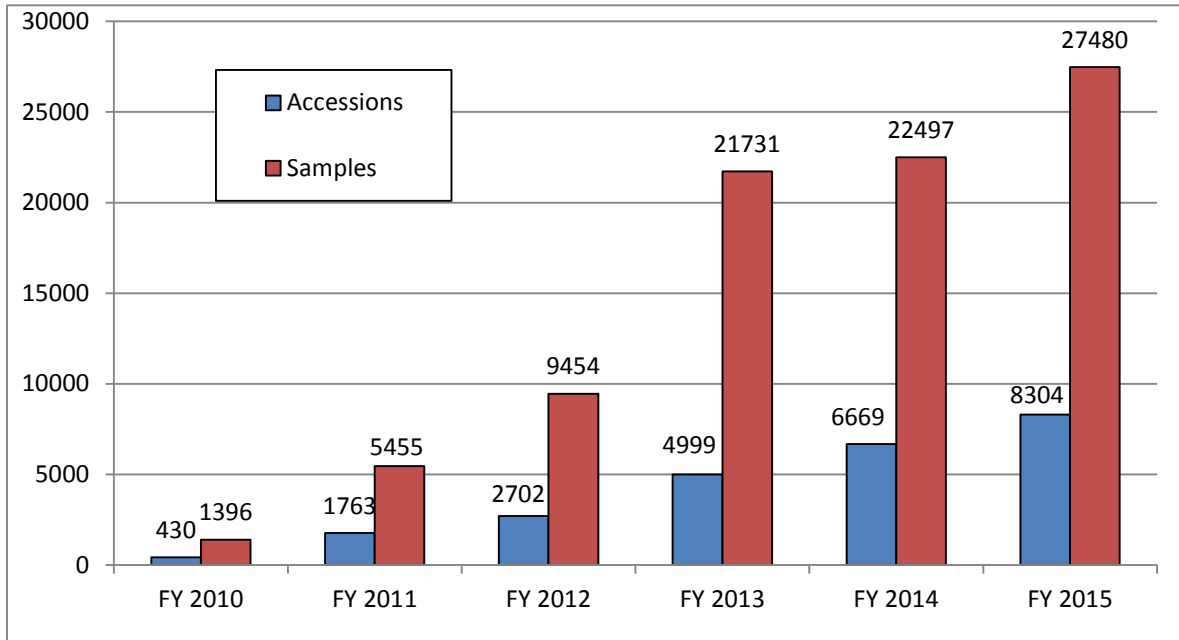

Figure 1. Surveillance for IAV in swine: Number of laboratory accessions and samples tested, FY 2010 – FY 2015
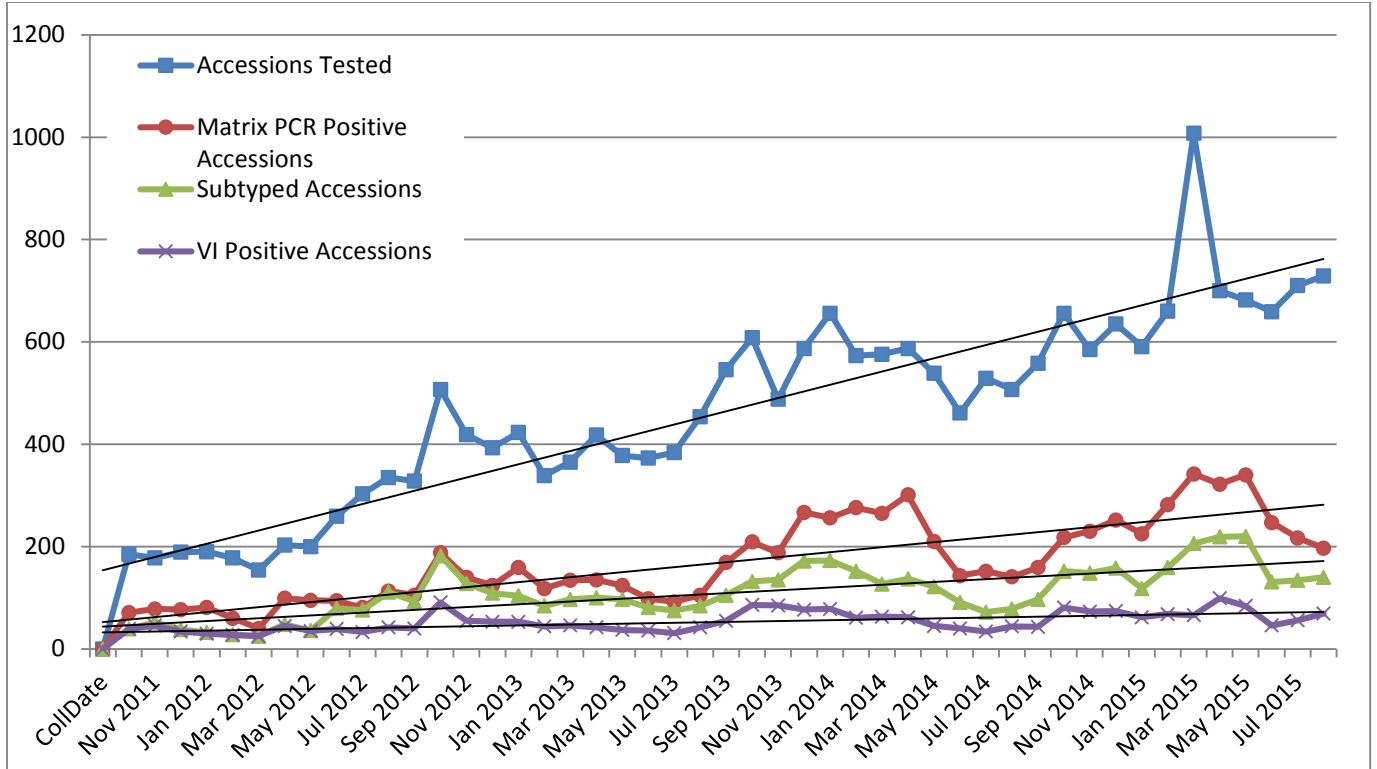
Figure 2. Accessions submitted, subtyped accessions, positive accessions, and VI positive accessions over time with trend lines, October 2011-September 2015

Figure 3 shows the number of subtype detections in FY 2015 Q4. The total number of samples subtyped was 642, including 122 H1N1, 246 H1N2, 246 H3N2, 0 H3N1, and 28 mixed.
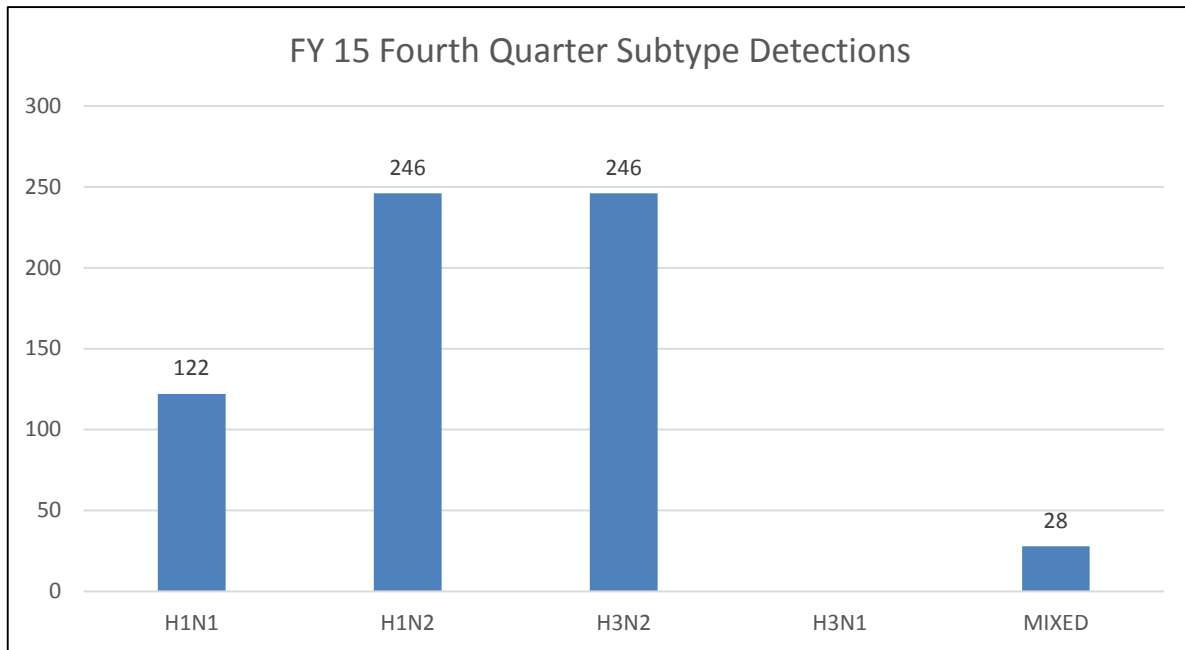

Figure 3. FY 2015 Fourth Quarter subtype detections

Figure 4 breaks down accessions by subtype rRT-PCR from FY 2010 to FY 2015. H1N1 remains the major subtype over the course of the surveillance; however, H1N2 and H3N2 detections have increased substantially since 2012. 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.
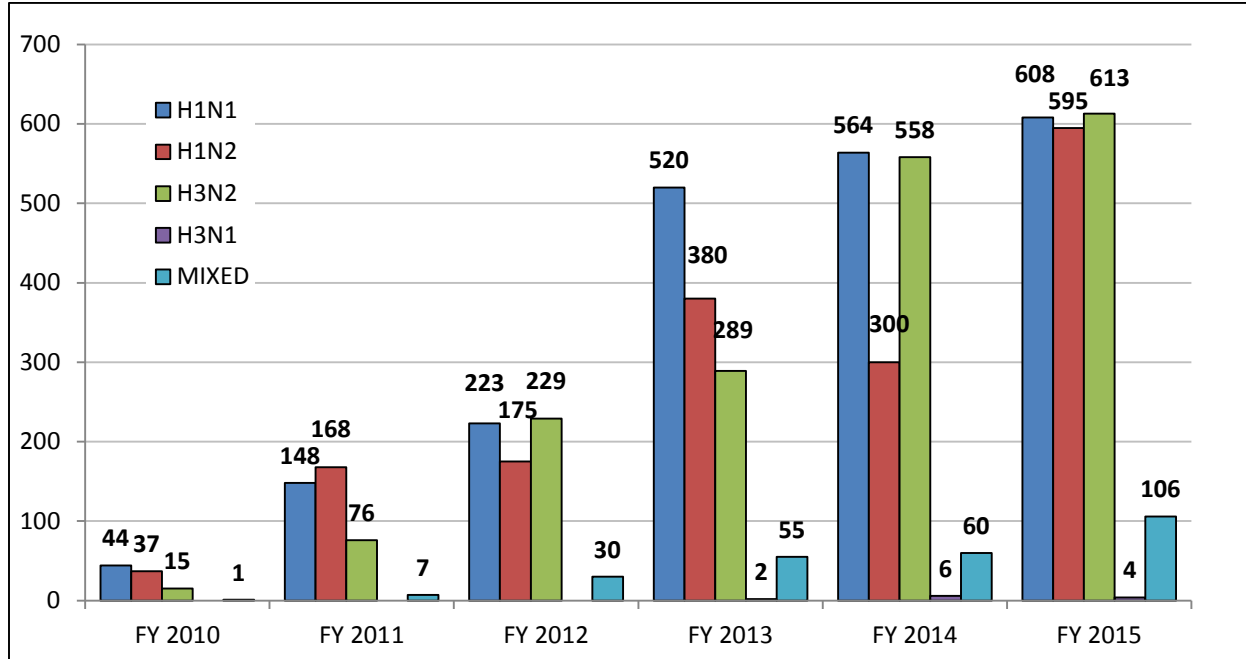

Figure 4. Number of subtypes, FY 2010 through FY 2015
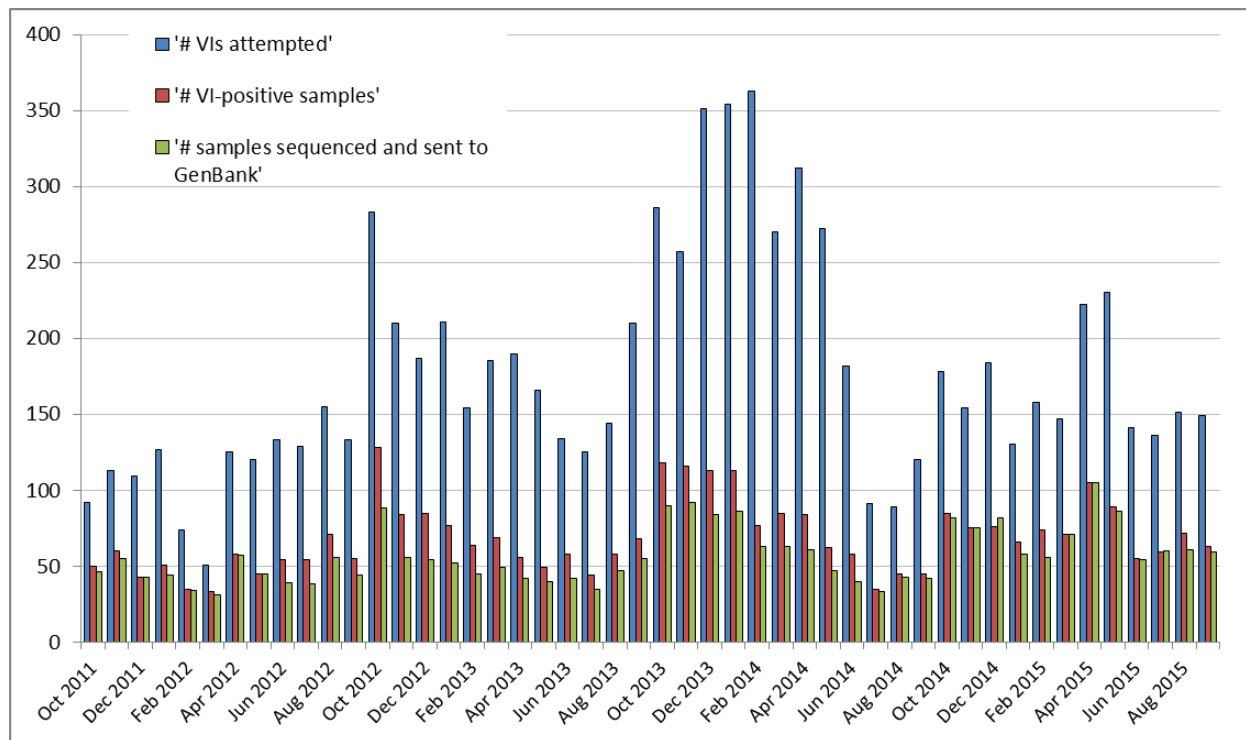

Figure 5. Number of virus isolations attempted, positive virus isolations, and GenBank submissions from FY 2012 through FY 2015

When accessions were evaluated by age-class for the fourth quarter, the following observations were noted. H3N2 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 H3N2 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, FY 2015 Q4

Age Class	Number of accessions with subtype reported	H1N1	H1N2	H3N2	H3N1	Mixed
Suckling	34	4	12	18	…	…
Nursery	83	17	27	35	…	4
Grower/Finisher	60	24	16	19	…	1
Sow/Boar	3	2	…	1	…	…
Not recorded/ Unknown	235	38	104	79	…	14

Table 2. Number of positive accessions tested for IAV-S by specimen type and by viral subtype, FY 2015 Q4

Specimen Type	Number of Accessions with subtype reported*	Percent of subtyped accessions with positive virus isolation	Percent of subtyped accessions with sequence submitted to GenBank	H1N1	H1N2	H3N2	H3N1	Mixed
Lung or Lung Swab	105	78%	80%	32	50	19	…	4
Nasal or nasal swab	86	57%	48%	15	51	20	…	…
Oral Fluids	235	24%	22%	40	63	117		15
Other**	1	0%	100%	…	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.
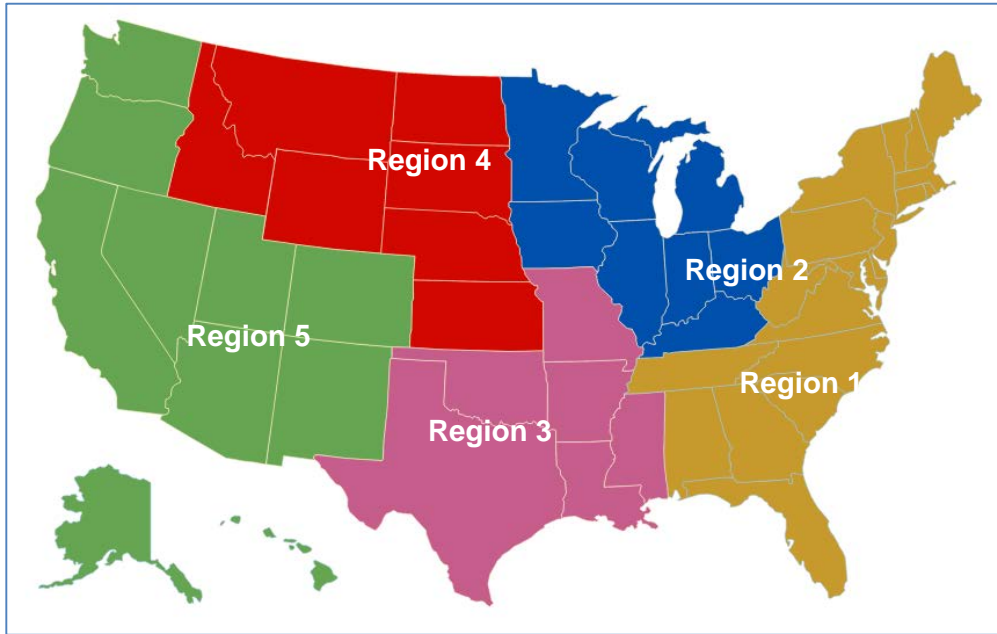
Figure 6. A map of the regions for national IAV-S surveillance

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.
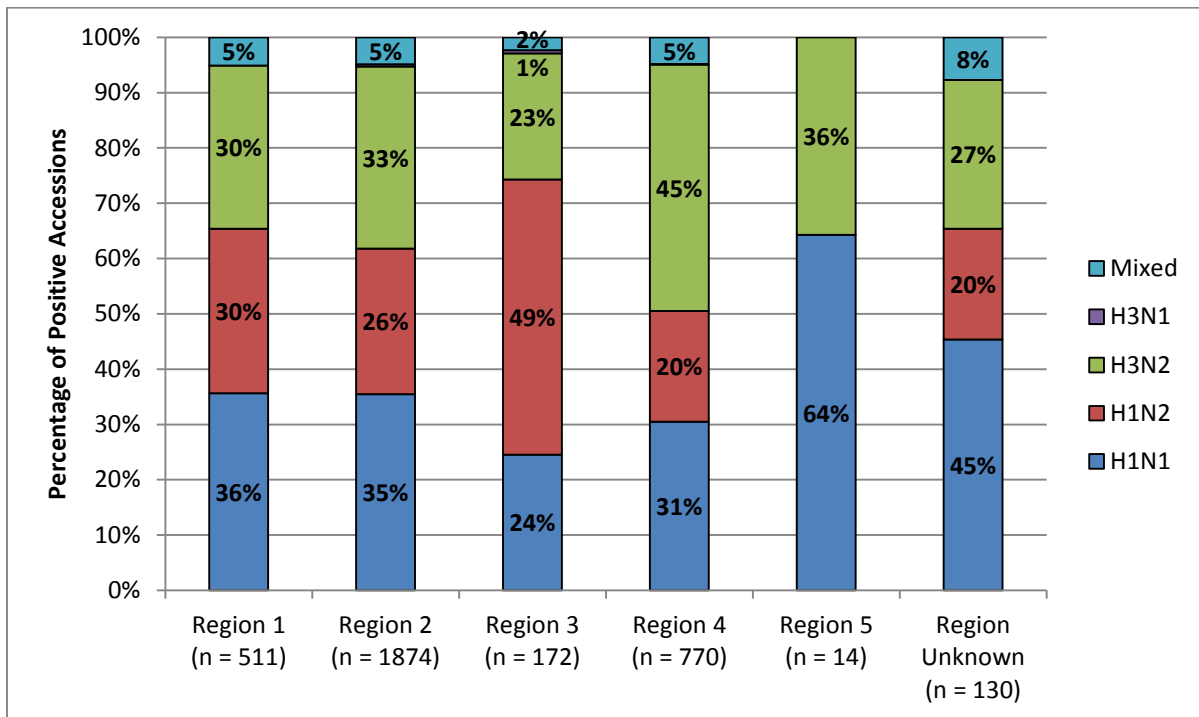

Figure 7. Percentage of subtyped accessions by region for FY 2014 through FY 2015

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 594 isolates with published sequences in GenBank was characterized by phylogenetic analysis in Q4 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.
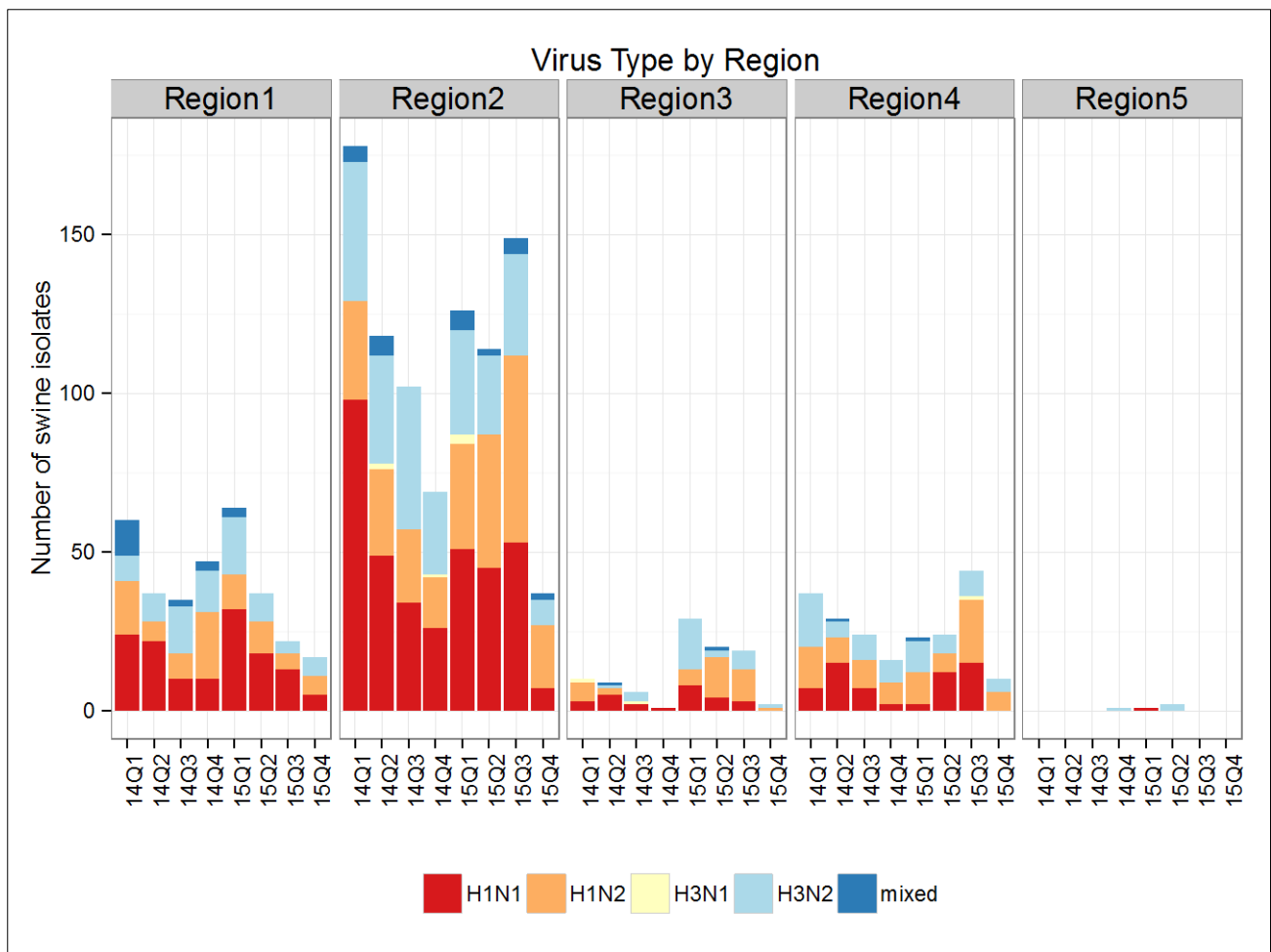

Figure 8. Virus type by region 2-year summary Q1 FY 2014 to Q4 FY 2015

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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 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.

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, except for FY 2015 Q3 Region 4. There was a relative increase in delta1 H1 detections, with decreases in delta2 and gamma for the fourth quarter.
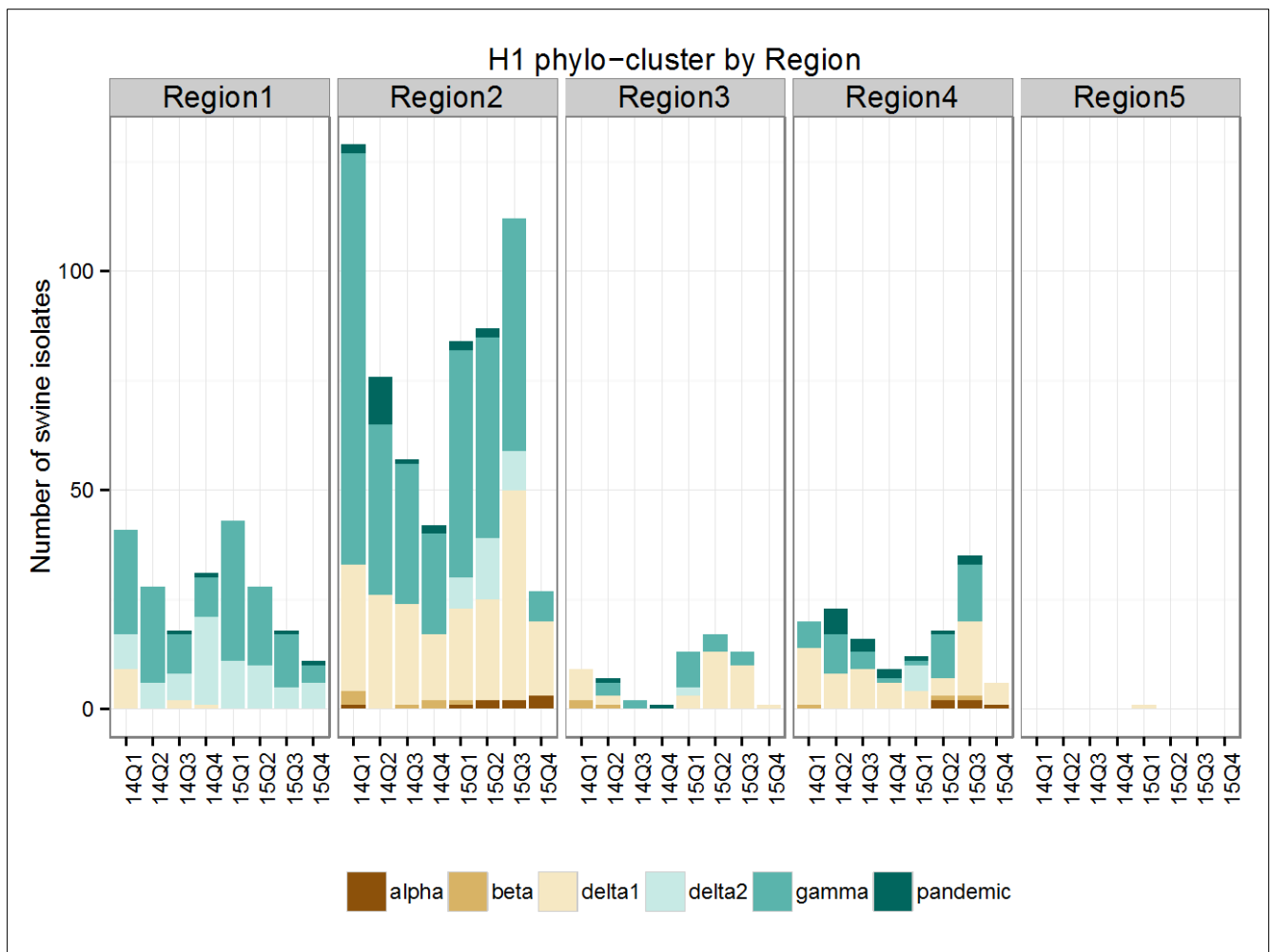

Figure 9. H1 phylo-cluster by region – 2-year summary Q1 FY 2014 to Q4 FY 2015
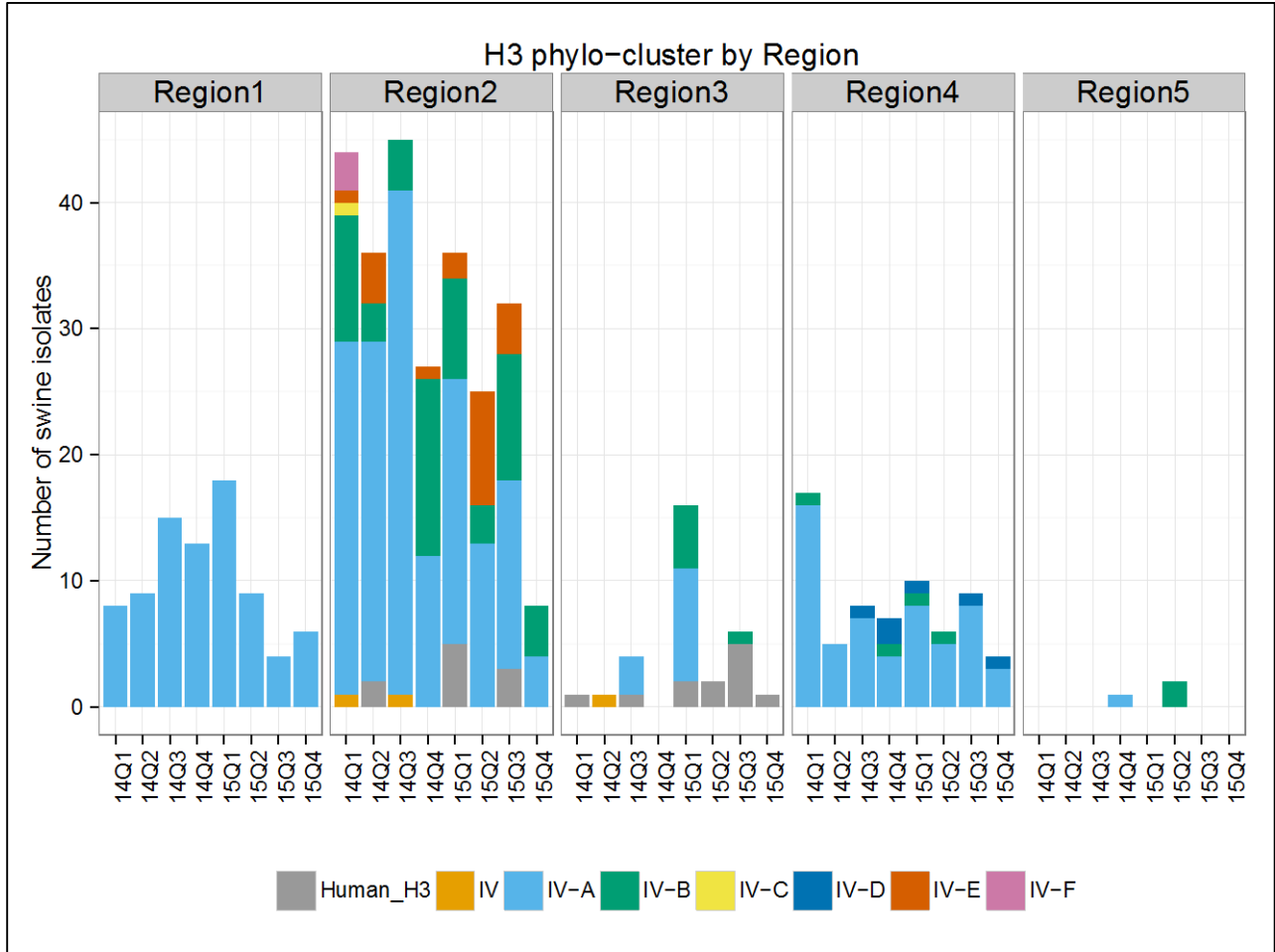
Figure 10. H3 phylo-cluster by region, 2-year summary Q4 FY 2013 to Q3 FY 2015

There was one human-like H3 detection in Region 3(Figure 10). The Cluster IV-A viruses continued to be the dominant cluster and Cluster IV-B detections remained similar to the previous quarter. There was a one detection of Cluster IV-D in Region 4.

National phylogenetic NA gene information
Both the N1 and N2 subtypes are found in circulating swine viruses. Classical N1 continued to be the dominant cluster with few pdmN1s consistent with pdmH1 detections. 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 during the fourth 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 fourth quarter of FY 2015, the NVSL Diagnostic Virology Laboratory provided 58 isolates to four institutions: one governmental, two academic, and one pharmaceutical. NVLS received 163 isolates into the repository, increasing the total FY 2015 isolates received to 883 (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	883
2014	765
2013	820
2012	915
TOTAL TO DATE	3,220

Table 4. Number of subtyped isolates available through repository

Subtyped isolates available through repository	
H3N2	1,158
H3N1	9
H1N1	1,488
H1N2	1,219
TOTAL	3,874

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.

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.
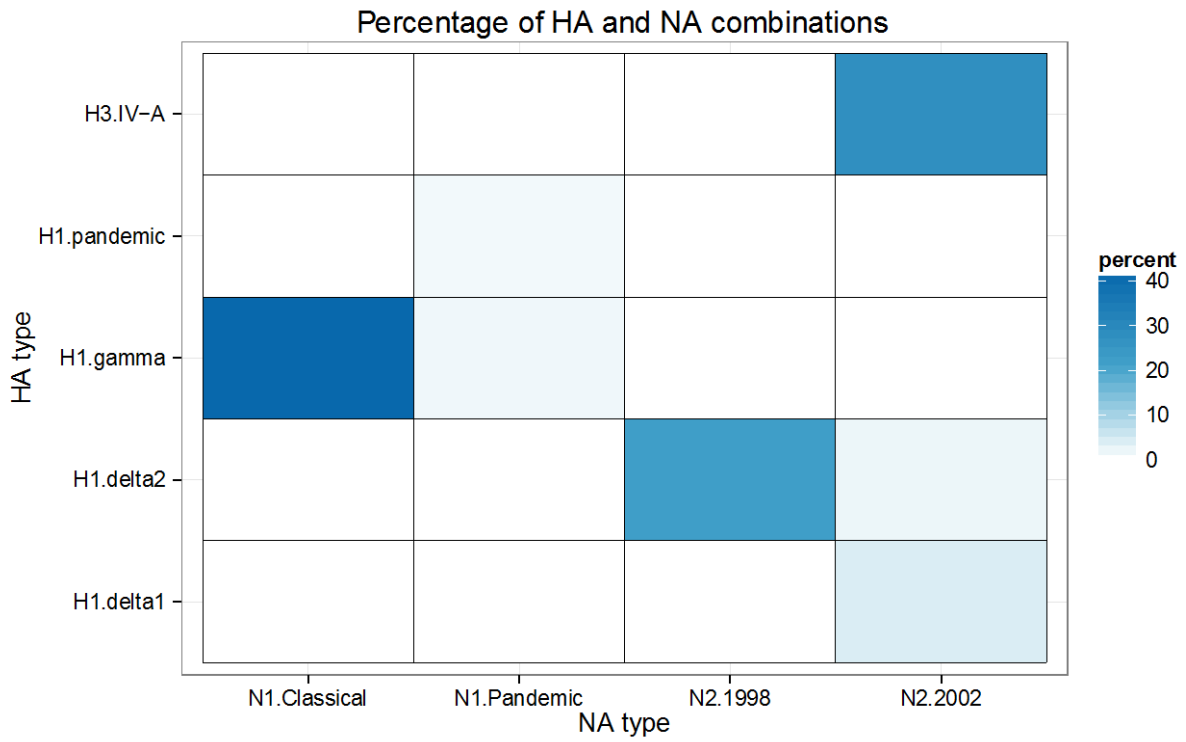

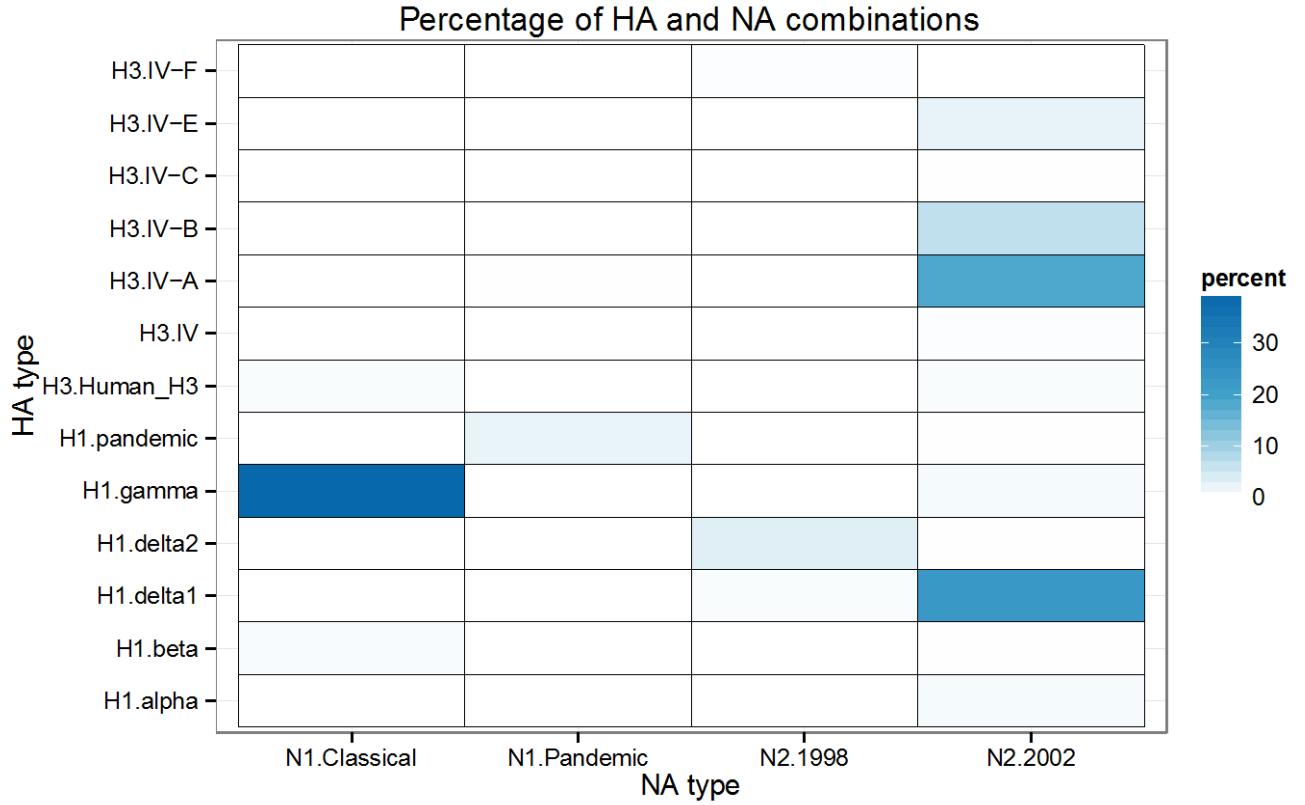Percentage of HA and NA combinations − Oct 2013 to Sept 2015
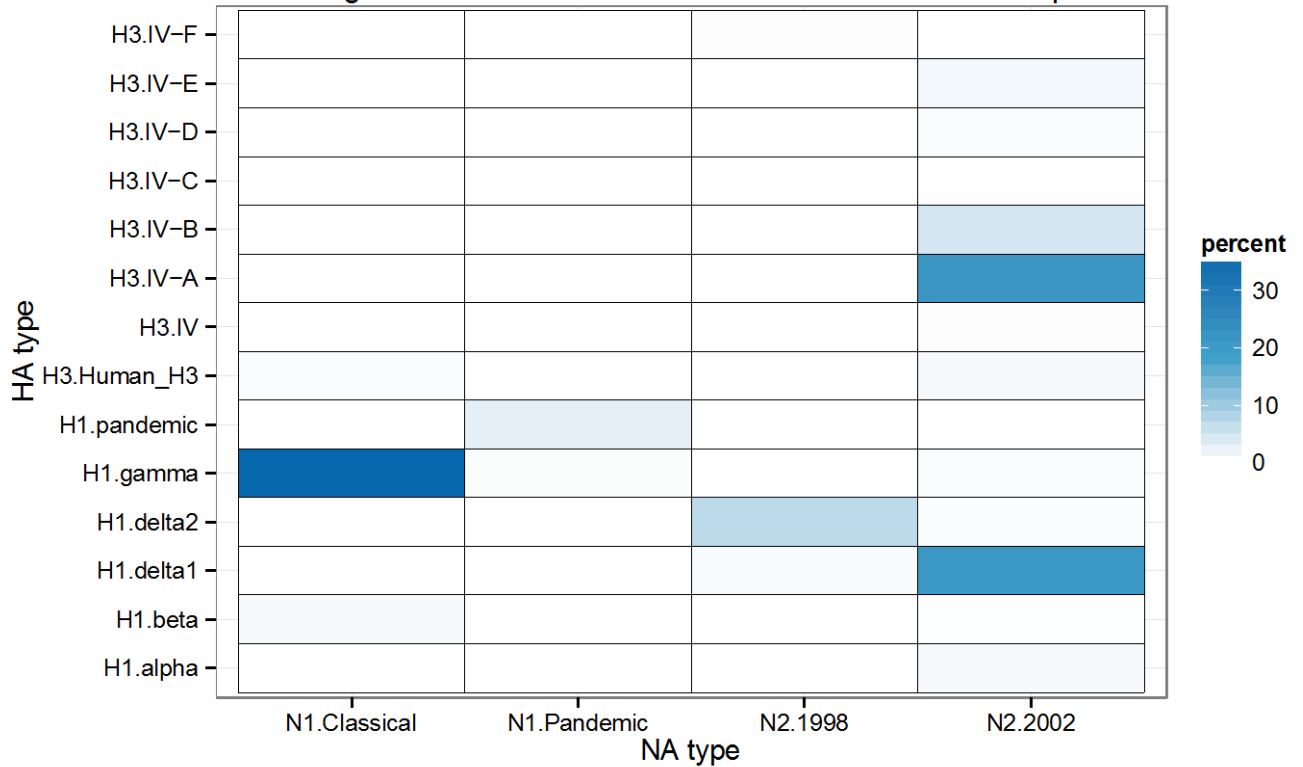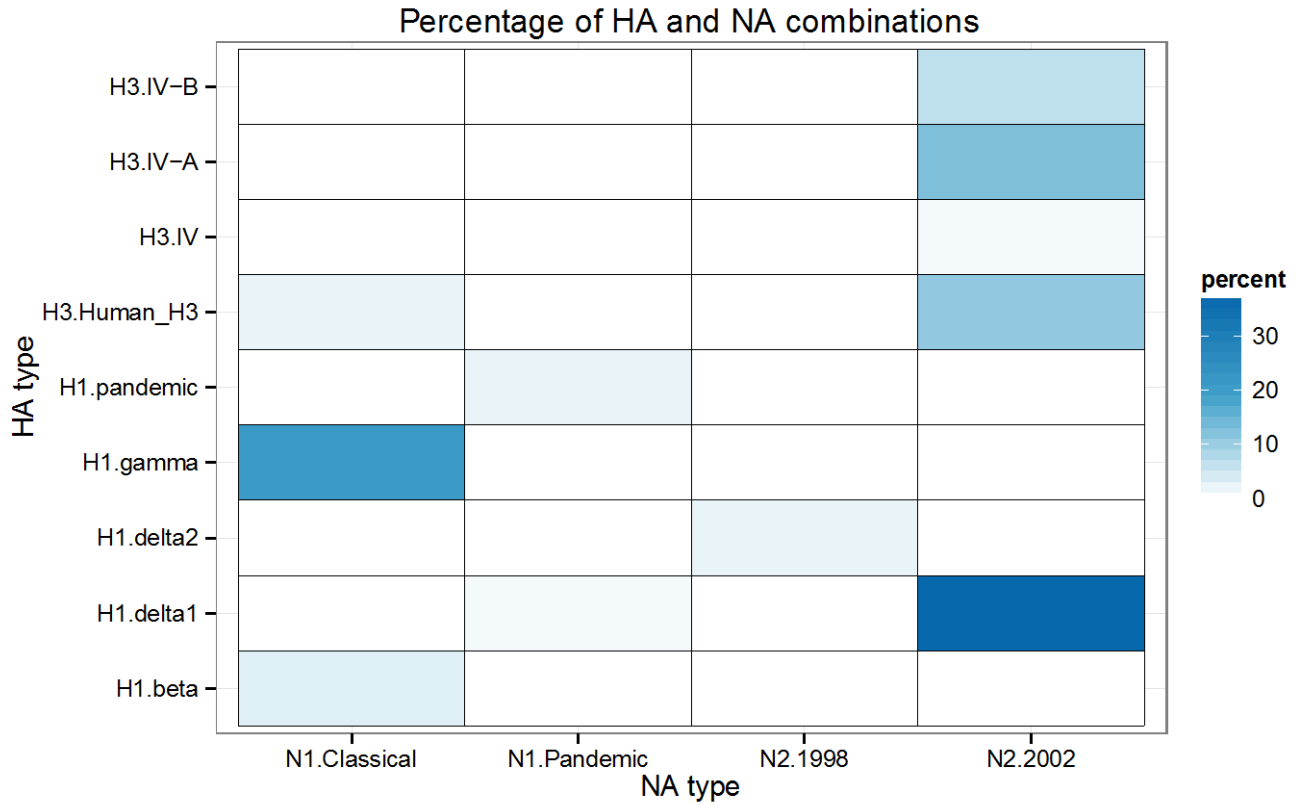
Region 1: Total HA & NA combinations – 300

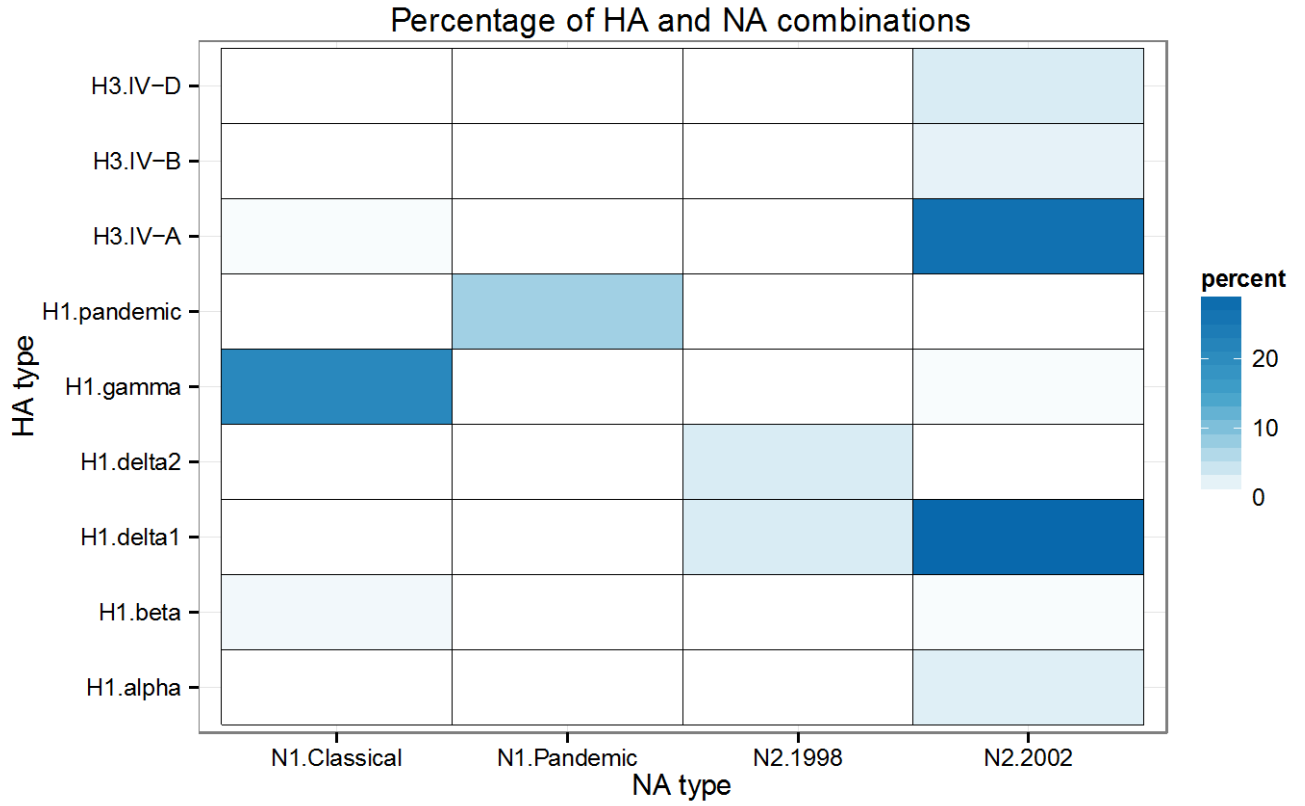### Percentage of HA and NA combinations

Region 2: Total HA & NA combinations – 866

Percentage of HA and NA combinations

Region 3: Total HA & NA combinations – 94

Percentage of HA and NA combinations

Region 4: Total HA & NA combinations – 205

Percentage of HA and NA combinations

Region 5: Total HA & NA combinations – 4

Percentage of HA and NA combinations