



United States Department of Agriculture

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Inspection  
Service

Veterinary  
Services

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# Influenza A Virus in Swine Surveillance

## Quarterly Report for Fiscal Year 2015

### Surveillance Summary for First Quarter FY 2015

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#### Report Highlights

- This report covers the first quarter (Q1) of fiscal year (FY) 2015, from October 1, 2014 – December 31, 2014.
- 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, 5,460 samples were submitted for influenza A virus in swine (IAV-S) surveillance from 1,844 accessions.
- Submissions for FY 2015 are on track to exceed those in FY 2014.
- During 2014, the H1N1 and H3N2 subtypes predominated and both are prominent in Q1.
- Regions 1 and 2 observed H1N1 as the predominant subtype and H3N2 was predominant in Regions 3, 4, and 5 (see map in regional section).
- There were three detections of H3N1 in Q1, all in Region 2, compared to six detections in FY 2014.
- Limited accessions from a region can skew data and lead to misinterpretation. Care should be taken when reviewing results from Regions 3 and 5 in FY 2015 Q1 which realized fewer accessions.
- 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 prevalence, but it can help identify IAV-S trends.

#### Introduction

This report summarizes the results from the USDA-APHIS-VS IAV-S surveillance program for Q1 of FY 2015. The report, based on data received as of May 15, 2015, is intended to provide swine producers, practitioners, diagnosticians, and the public with a brief status update of national surveillance for IAV-S. The APHIS-USDA 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 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. 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 reverse transcriptase polymerase chain reaction (RT-PCR). The subtype result is based on the RT-PCR based subtyping assays. Virus isolation and sequencing are only attempted on RT-PCR positives meeting criteria listed below. Phylogenetic analyses are based on successful sequencing results, with sequences deposited into the public sequence database GenBank.

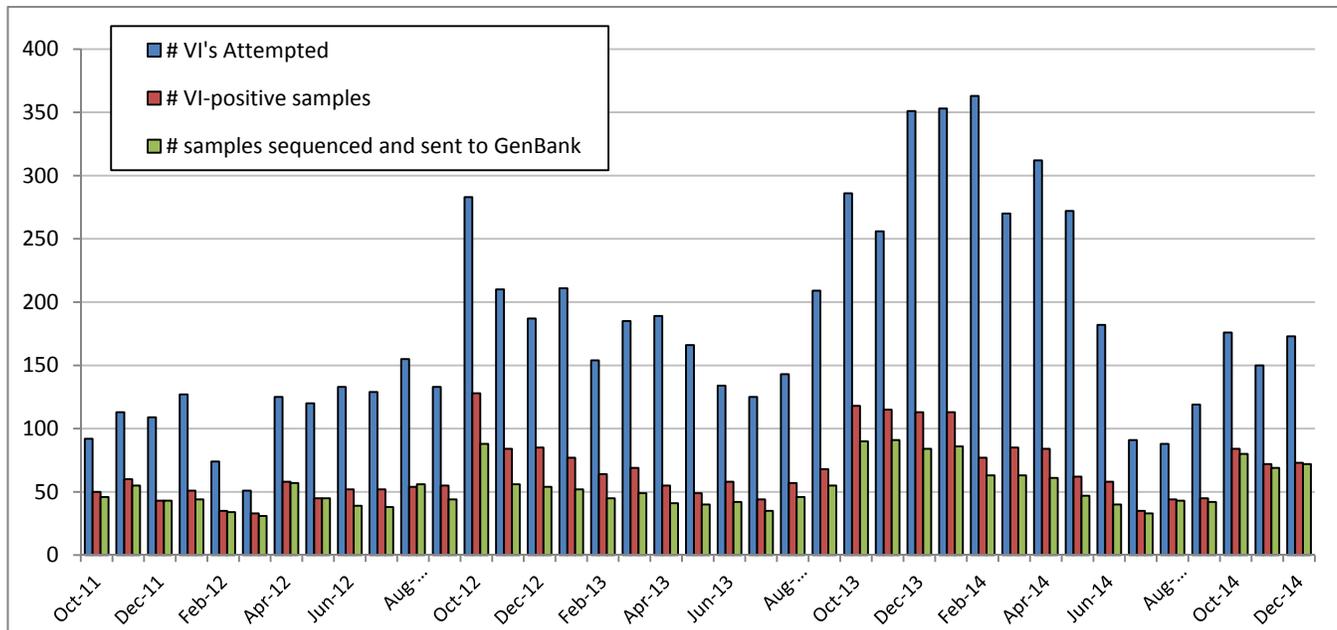
## **Program Updates**

### **Modifications to the testing algorithm**

The National Animal Health Laboratory Network (NAHLN) technical working group has reviewed and approved modifications to the program’s testing algorithm. The following changes were implemented in June 2014:

1. Oral fluid samples with a cycle threshold (CT) value of 30 or more and nasal swab samples with a CT value of 35 or more will not undergo further testing under the USDA program. This policy does not prevent further testing at the owner’s expense.
2. While 10 remains the maximum number of samples per accession, only 5 of the 10 can be oral fluid samples.

As a result of implementing modification 1 above, the percentage of virus isolation (VI) positive samples from submitted samples has increased from approximately 31 percent to approximately 46 percent since the summer of 2014. Figure 1 below illustrates the total number of VIs attempted (blue bar) and the respective number of those attempts that yielded a positive VI (red bar) along with the respective number of positive VIs whose genetic sequence was submitted to GenBank (green bar).



**Figure 1. Number of virus isolations attempted, positive virus isolations, and GenBank submissions from FY 2012 through Q1 FY 2015.**

### Adjustments to the NVSL virus repository

In February 2015, the National Veterinary Services Laboratories (NVSL) began limiting the number of viruses it propagates from samples received in NAHLN laboratories and other sources. Instead, NVSL now catalogues and stores the virus isolates in the virus repository until it receives a request for the isolate. As a result, there may be a 2-4 week delay in providing an isolate not already expanded. Each month NVSL randomly selects 16 geographically representative samples to sequence the entire genome. These viruses, along with any viruses identified by research, academic, or public health partners, will be propagated and available upon request.

### IAV-S surveillance program review

The IAV-S surveillance program is currently undergoing internal and external reviews. 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. The external analysis is being performed under contract and is intended as a technical epidemiologic review to not only provide a critical review 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 and vaccine seed stock products and updating diagnostic assays.

## 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, of which selective samples undergo further analysis by NVSL for virus isolation 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 for FY 2014 exceeded numbers from FY 2013. FY 2015 numbers of accessions and samples are on track to match or exceed 2014. For FY 2015's first quarter, 5,460 samples have been tested from 1,844 accessions (Figure 2).

Figure 3 breaks down accessions by subtype RT-PCR from FY 2010 to Q1 FY 2015. H3N2 increased in predominance and was relatively equivalent with H1N1 in FY 2014, although H1N1 has been the major subtype over the course of the system. The H1N2 subtype maintains a steady presence, although at lower levels than the other subtypes. In FY 2014, the H3N1 subtype appeared in the surveillance data. H3N1s are uncommon in U.S. swine herds, but have been detected on rare occasions. The ARS-NADC performed phylogenetic analyses of the H3N1s and found that two of the viruses carried a novel human seasonal hemagglutinin (HA) gene from contemporary human viruses distinct from the current swine H3 viruses. Further investigation found that this human seasonal HA gene was incorporated into H3N2 viruses as well. Potential spread in swine of H3N1 or H3N2 that possess the human-like H3 could significantly impact swine herds due to poor herd immunity. Preliminary findings by ARS-NADC indicate the H3N1 with the human-like H3 gene virus is fully virulent and causes typical influenza disease in swine. More detailed ARS-NADC information for all subtypes can be found in this report in the Regional Phylogenetic Analysis section.

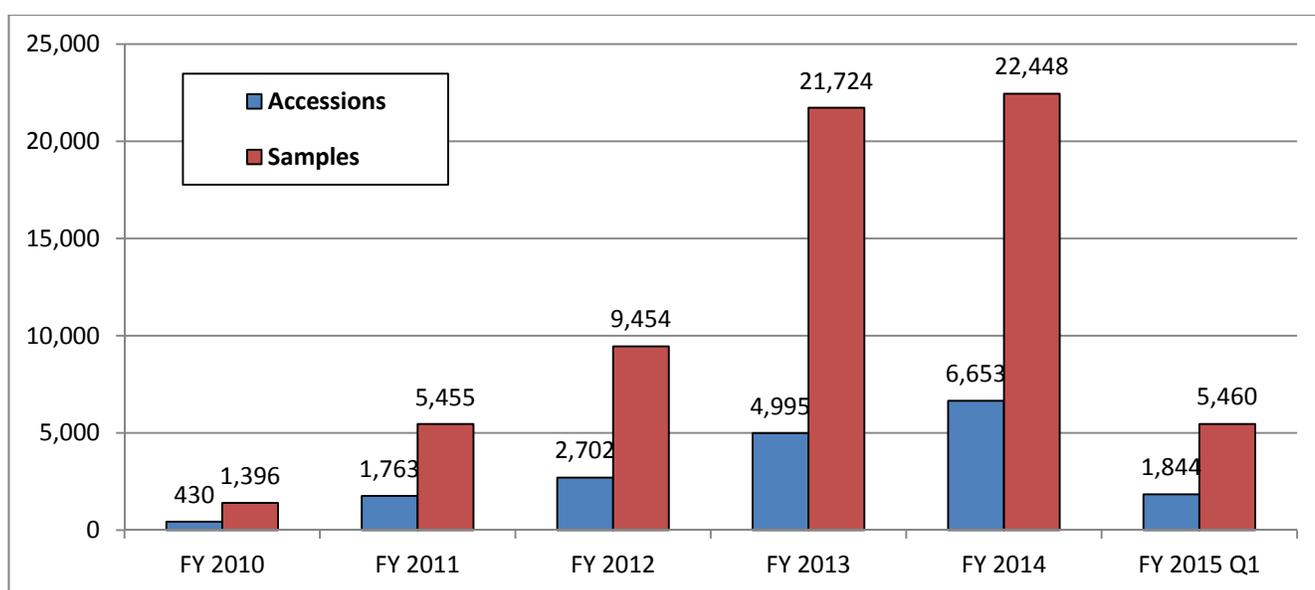
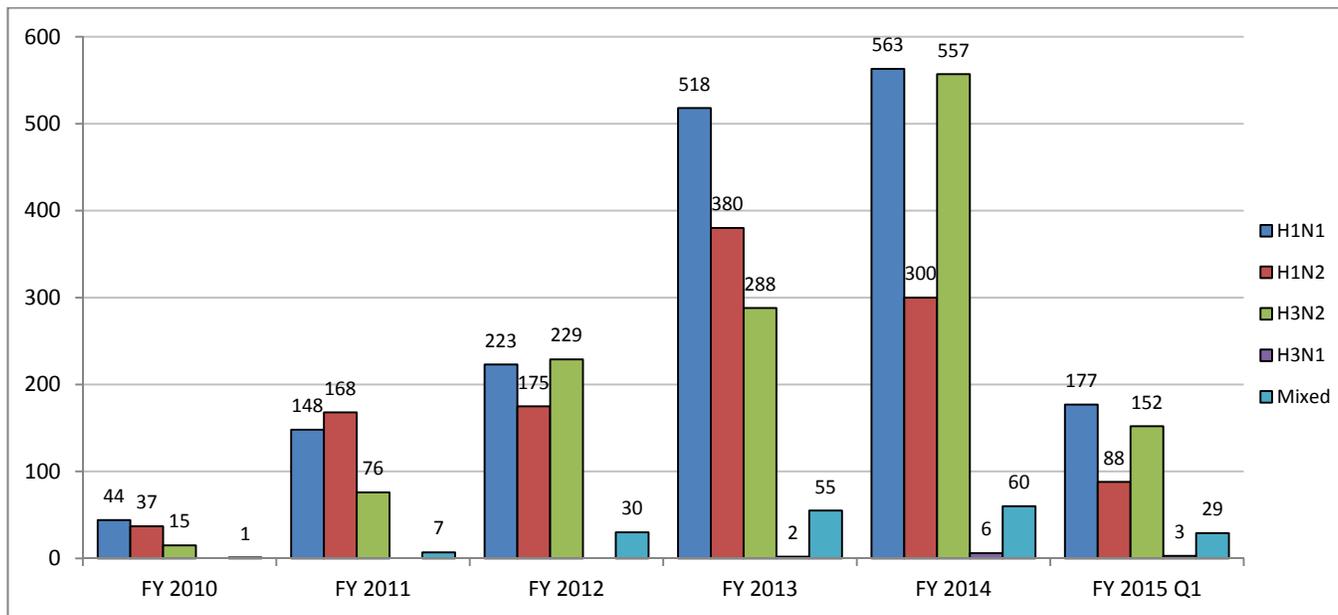


Figure 2. Number of laboratory accessions and samples tested, FY 2010 through Q1 FY 2015.



**Figure 3. Number of subtypes by FY, from 2010 through Q1 FY 2015**

When accessions were evaluated by age-class for the first quarter, H1N2 and H3N2 were found more often in suckling and nursery; H1N1, H1N2, and H3N2 were common for Grower/Finishers; and Sow/Boar had limited testing with H1N1 being the predominant subtype (Table 1). Similar trends were seen in FY 2014 (Table 2). When looking at specimen type submitted, oral fluids were the predominant sample type, followed by lung/lung swab, then nasal/nasal swab (Table 3).

**Table 1. Number of positive accessions tested for IAV-S by age class and by subtype in Q1 FY 2015**

Age Class	Number of accessions with subtype reported*	H1N1	H1N2	H3N2	H3N1	Mixed
Suckling	13	2	5	6	...	...
Nursery	57	22	5	27	1	2
Grower/Finisher	95	39	23	26	2	5
Sow/Boar	7	5	...	1	...	1
Not recorded/ Unknown	279	111	55	92	...	21

\*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-S by age class and by subtype in FY 2014**

Age Class	Number of accessions with subtype reported*	H1N1	H1N2	H3N2	H3N1	Mixed
Suckling	114	36	9	63	...	6
Nursery	443	159	94	170	3	17
Grower/Finisher	551	219	119	194	1	18
Sow/Boar	16	11	2	3	...	...
Not recorded/ Unknown	377	144	80	132	2	19

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

**Table 3. Number of positive accessions tested for IAV by specimen type and by subtype in FY 2014**

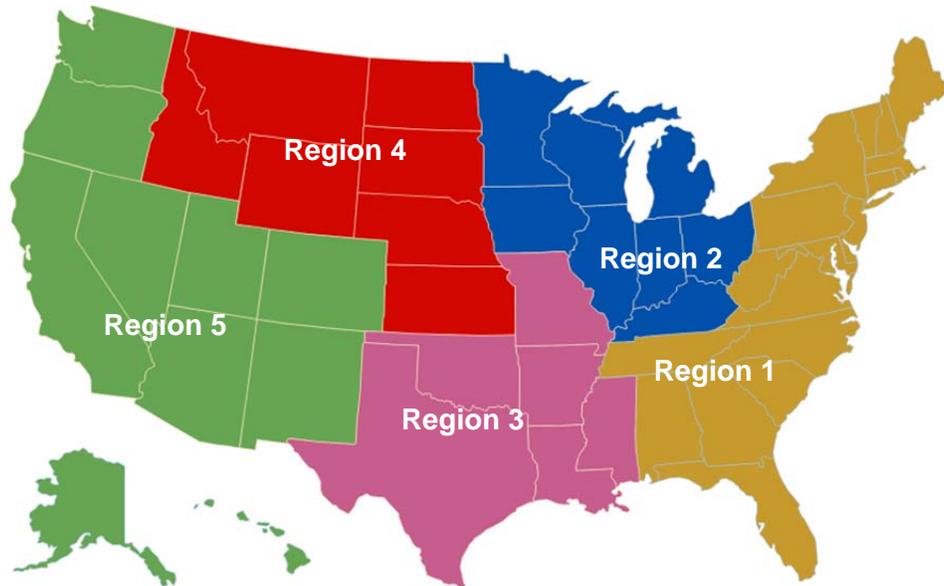
Specimen Type	Number of accessions with subtype reported*	H1N1	H1N2	H3N2	H3N1	Mixed
Lung or lung swab	440	187	111	123	4	15
Nasal or nasal swab	216	79	42	83	1	11
Oral fluids	835	297	150	352	1	35
Other**	7	3	1	3	...	...

\*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 (see Figure 4) 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 4. A map of the regions for national IAV-S surveillance**

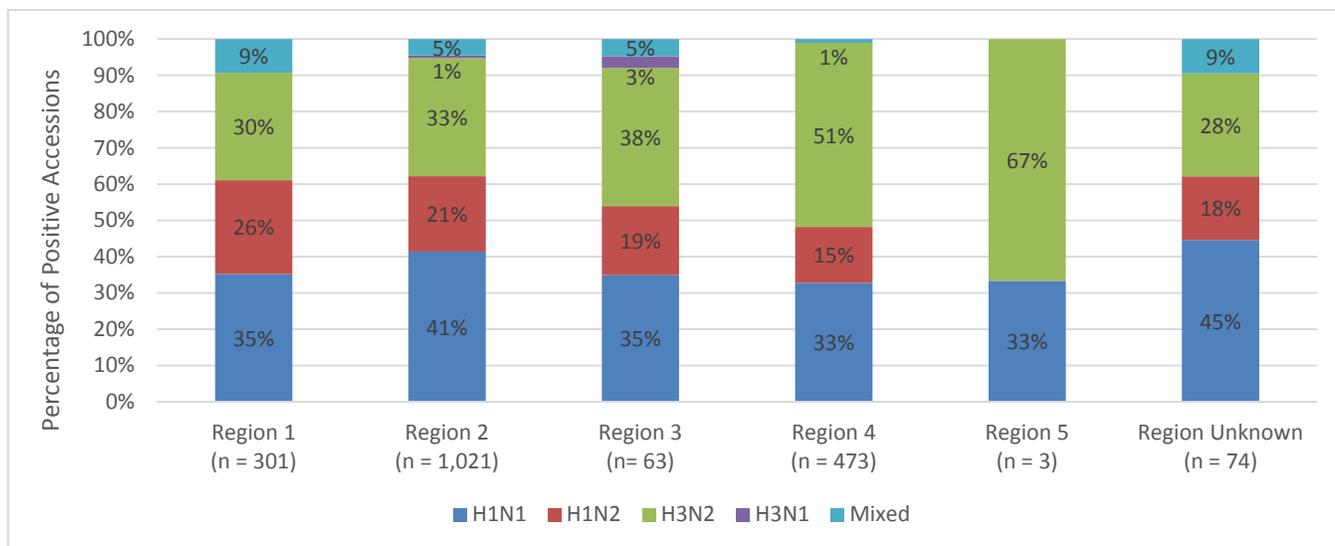
**Region 1:** Alabama, Connecticut, Delaware, Florida, Georgia, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, North Carolina, Pennsylvania, Puerto Rico, Rhode Island, South Carolina, Tennessee, Vermont, Virginia, Virgin Islands, West Virginia.

**Region 2:** Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, Ohio, Wisconsin.

**Region 3:** Arkansas, Louisiana, Mississippi, Missouri, Oklahoma, Texas.

**Region 4:** Idaho, Kansas, Montana, Nebraska, North Dakota, South Dakota, Wyoming.

**Region 5:** Alaska, Arizona, California, Colorado, Hawaii, Nevada, New Mexico, Oregon, Utah, Washington.



**Figure 5. Percentage of subtyped accessions by region, FY 2014 through Q1 FY 2015**

Figure 5 shows the distribution of RT-PCR subtyped samples among the five regions. Regions 1 and 2 demonstrate H1N1 as the predominant subtype while H3N2 was the predominant subtype in Regions 3, 4, and 5. H1N2 and H3N1 continue to be in the minority of subtypes for all regions.

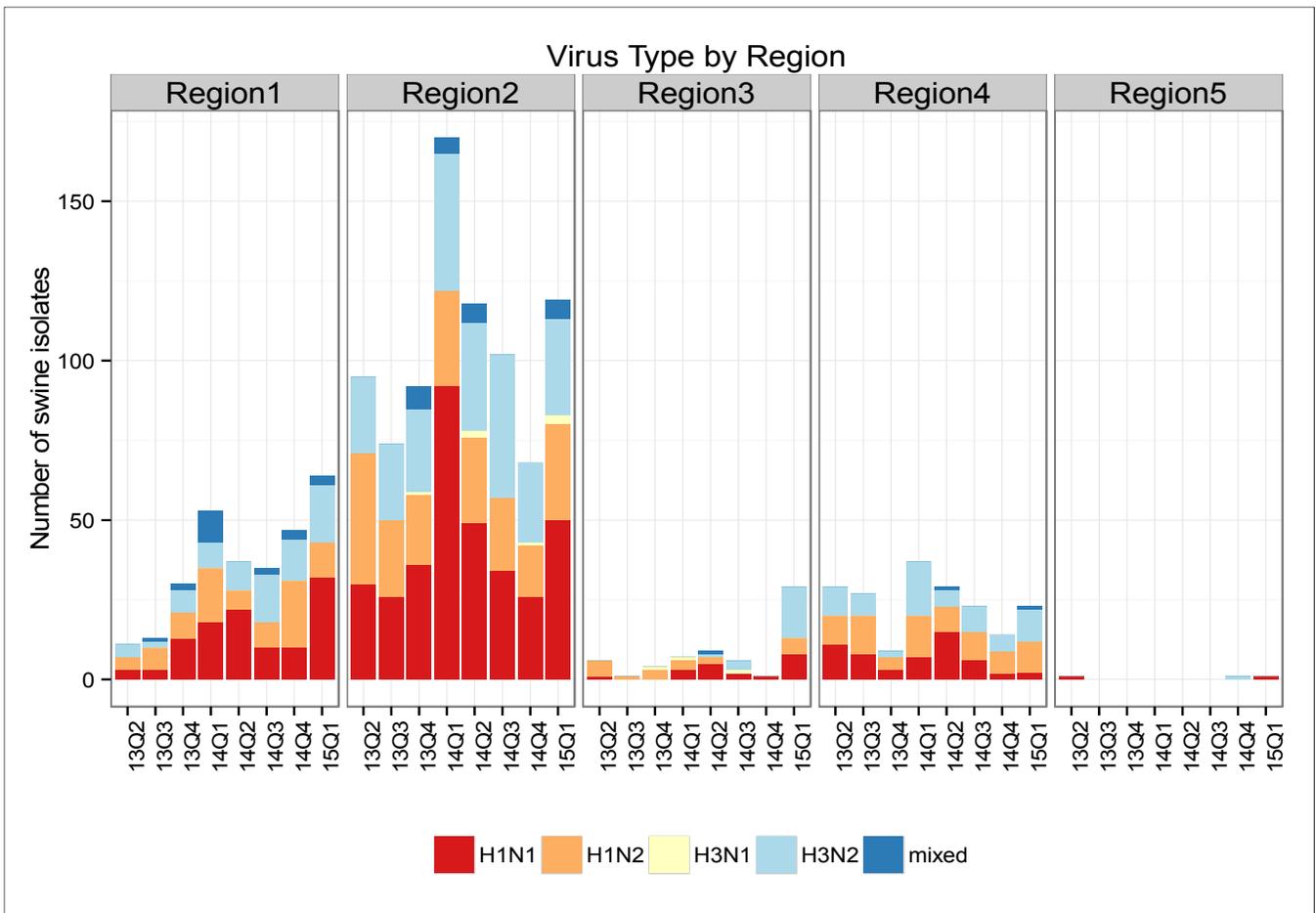
## Regional Phylogenetic Analysis

### Phylogenetic analysis of sequences from the IAV-S surveillance system

Phylogenetic analysis of gene sequences of the influenza A virus in swine is being conducted to further examine genetic changes that occur in HA, NA, and M genes of this rapidly changing virus. Through collaboration with ARS-NADC, a dataset<sup>1</sup> of 741 isolates with published sequences in GenBank was characterized by phylogenetic analysis in Q1 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 a 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.

<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.

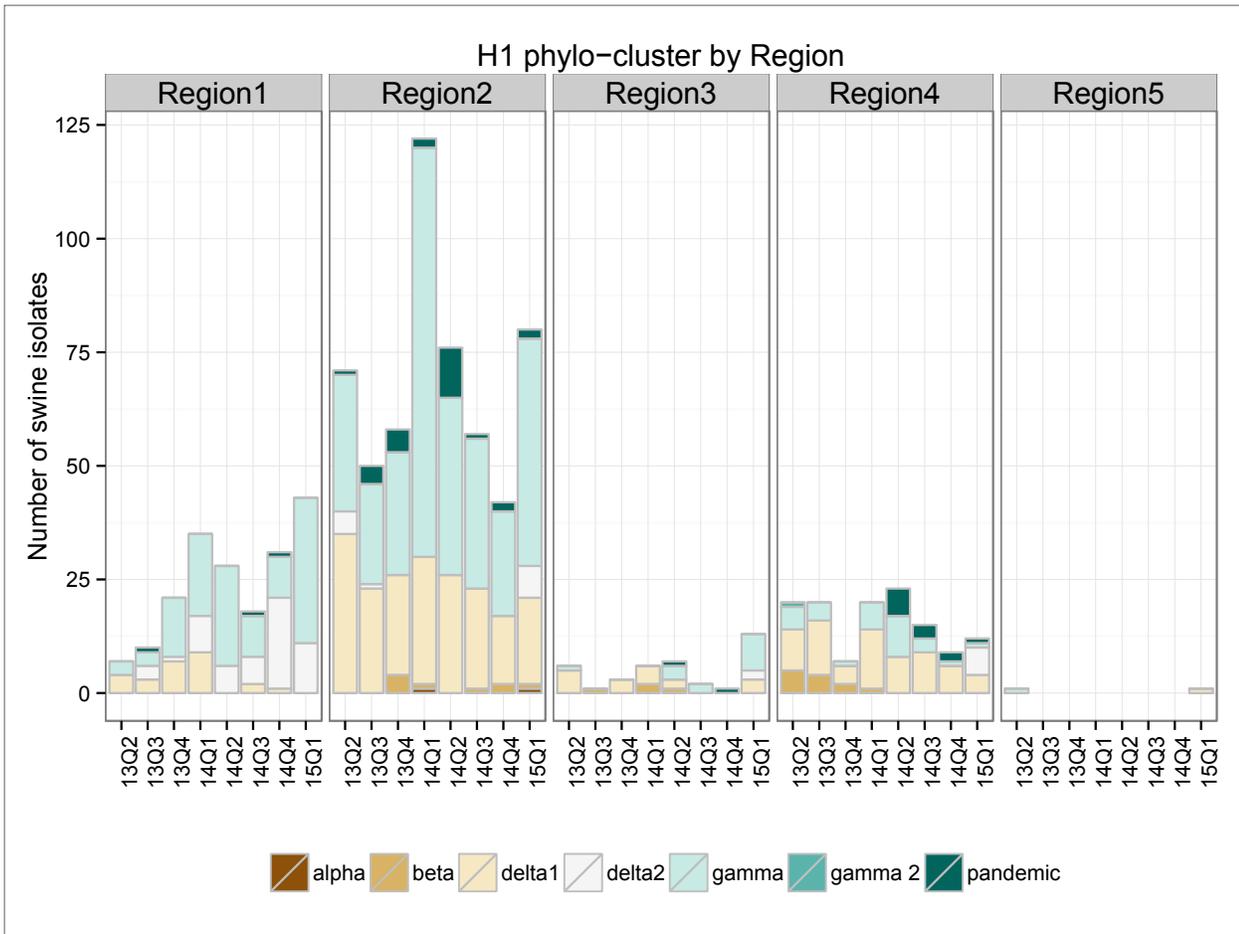


**Figure 6. Virus type by region, 2-year summary Q2 2013 to Q1 FY 2015**

Figure 6 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. H3N1 was first detected in the fourth quarter of FY 2013 (July 1 – September 30, 2013) in Region 2 and in the third quarter of FY 2014 (April 1 – June 30, 2014) in Region 3. Note that 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 Q1.

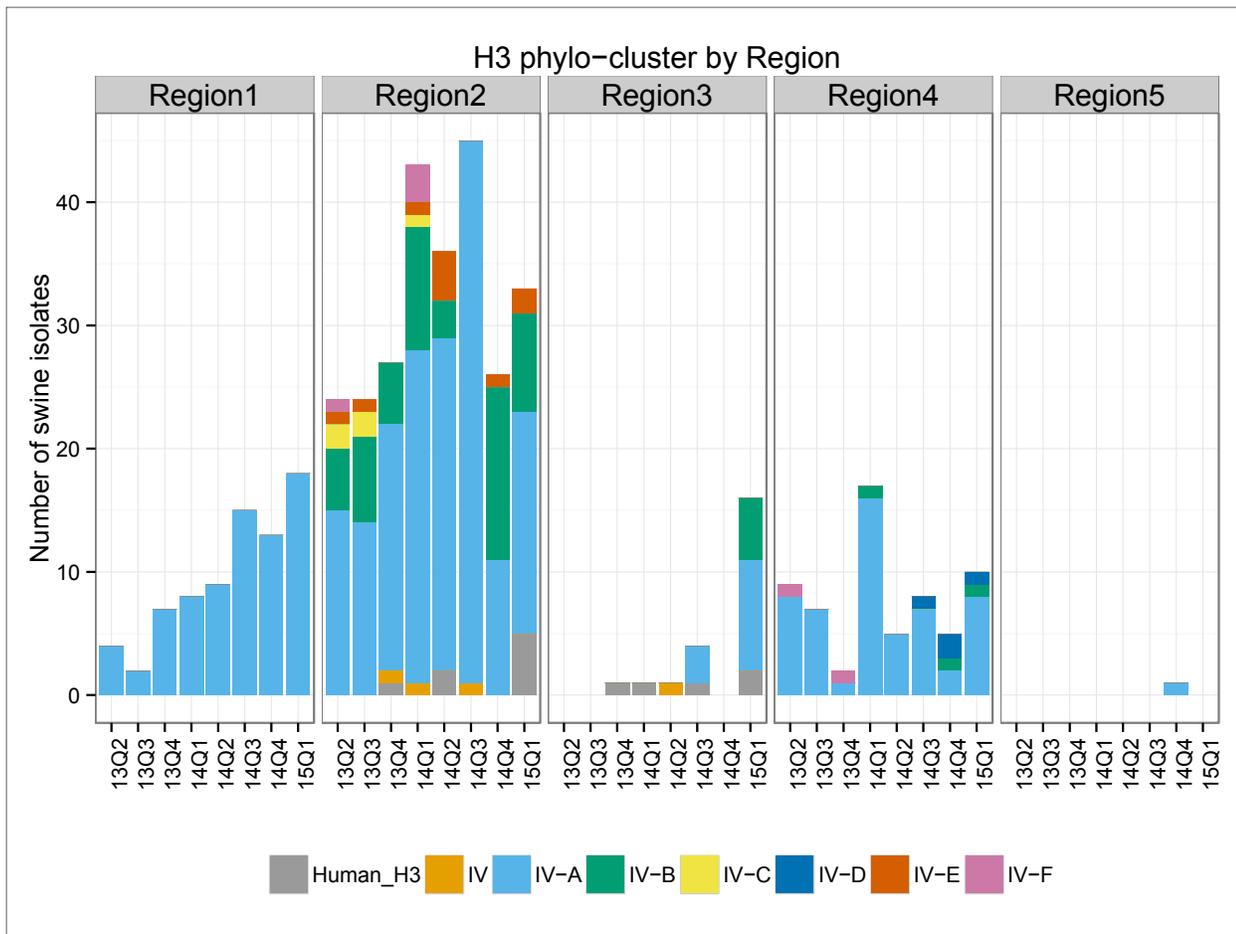
**National phylogenetic HA gene information**

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 were 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 7. H1 phylo-cluster by region: 2-year summary Q2 2013 to Q1 FY 2015**

In the H1 subtypes (see Figure 7), few H1N1pdm09 viruses were detected during Q1 2015. The number of delta-2 H1 viruses continued a relative increase in Q1 2015, and the number of delta-1 H1 viruses detected between September and December reflected a relative decrease in occurrence. The gamma H1 remained the predominant H1 clade overall.



**Figure 8. H3 phylo-cluster by region: 2-year summary Q2 2013 to Q1 FY 2015**

Cluster IV-A H3N2 viruses continued to be the dominant cluster (see Figure 8) and there were increased detections of Cluster IV-B viruses from July to December of 2014. New human-like H3 detections were identified from October through December 2014.

**National phylogenetic NA gene information**

Both the N1 and N2 subtypes are found in circulating swine viruses. A single H1N1pdm09 N1 virus was seen with a gamma 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 dominant 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 was more frequently detected than 1998 lineage.

**National phylogenetic information M gene**

Only the H1N1pdm09 M gene was detected between September and December 2014.

**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 first quarter of FY 2015, the NVSL Diagnostic Virology Laboratory provided 16 isolates to two

institutions, one governmental and one a pharmaceutical company. Since 2010, 329 isolates have been sent out to 21 different requesters; 5 were from academia (1 from Europe), 9 were governmental groups (3 international), and 7 were pharmaceutical. Tables 4 and 5 show the isolates received over time and the subtypes available.

**Table 4. Virus isolates received in repository**

Virus isolates in the repository	
Q1 2015	234
2014	765
2013	820
2012	915
TOTAL	>3,000

**Table 5. Number of subtyped isolates available through the repository\***

Subtyped isolates available through repository	
H3N2	1,012
H3N1	8
H1N1	1,309
H1N2	1,107
Mixed	154
TOTAL	3,590

\*Data from approximately 60 subtyped viruses pending from participating laboratories

### **Objective 3. Select Proper Isolates for Development of Relevant Diagnostic Reagents, Updating Diagnostic Assays, and Vaccine Seed Stock Products**

Under Objective 3, 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. These parties consult NVSL and ARS staff for subject matter expertise in IAV-S when necessary.

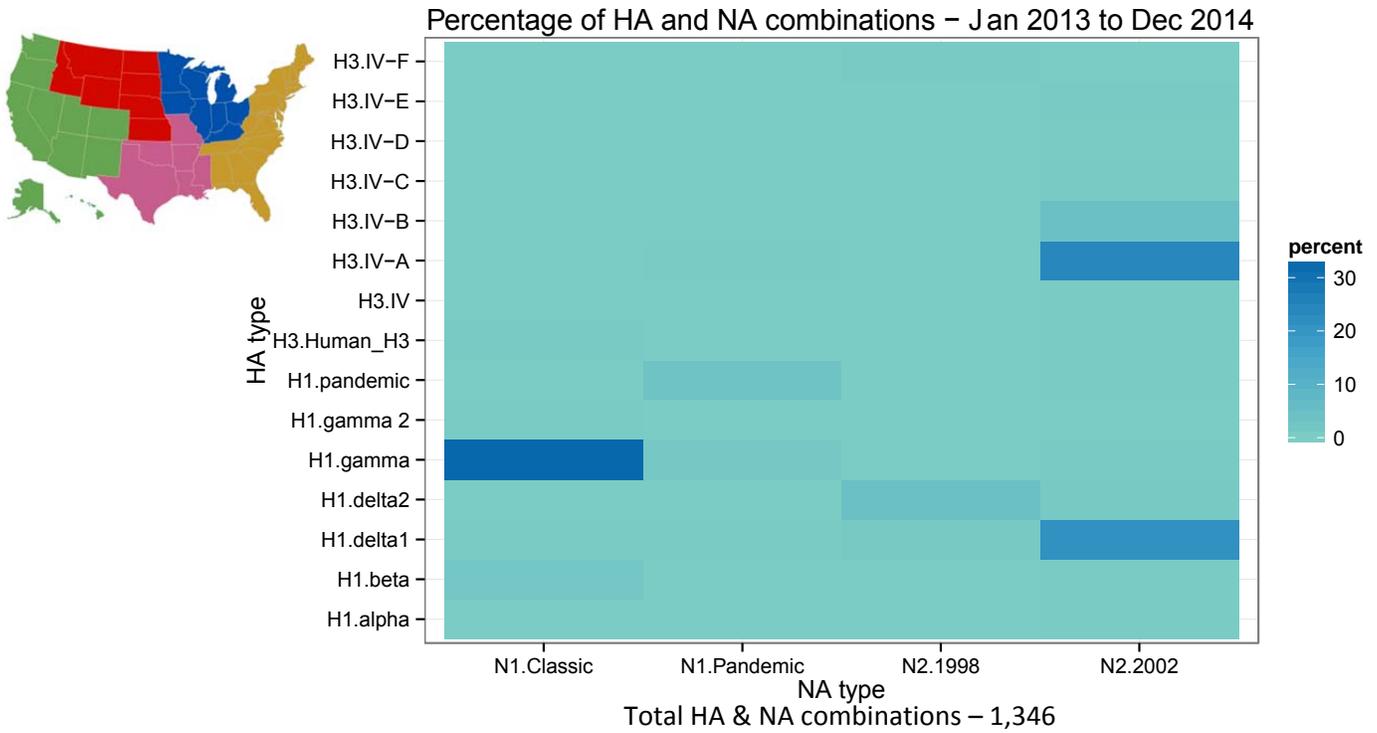
### **Conclusion**

The IAV-S voluntary surveillance system continues to provide insight into the genetic makeup of circulating influenza virus in populations of commercial pigs. Genetic information and virus isolates are made publicly available for further research and 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.

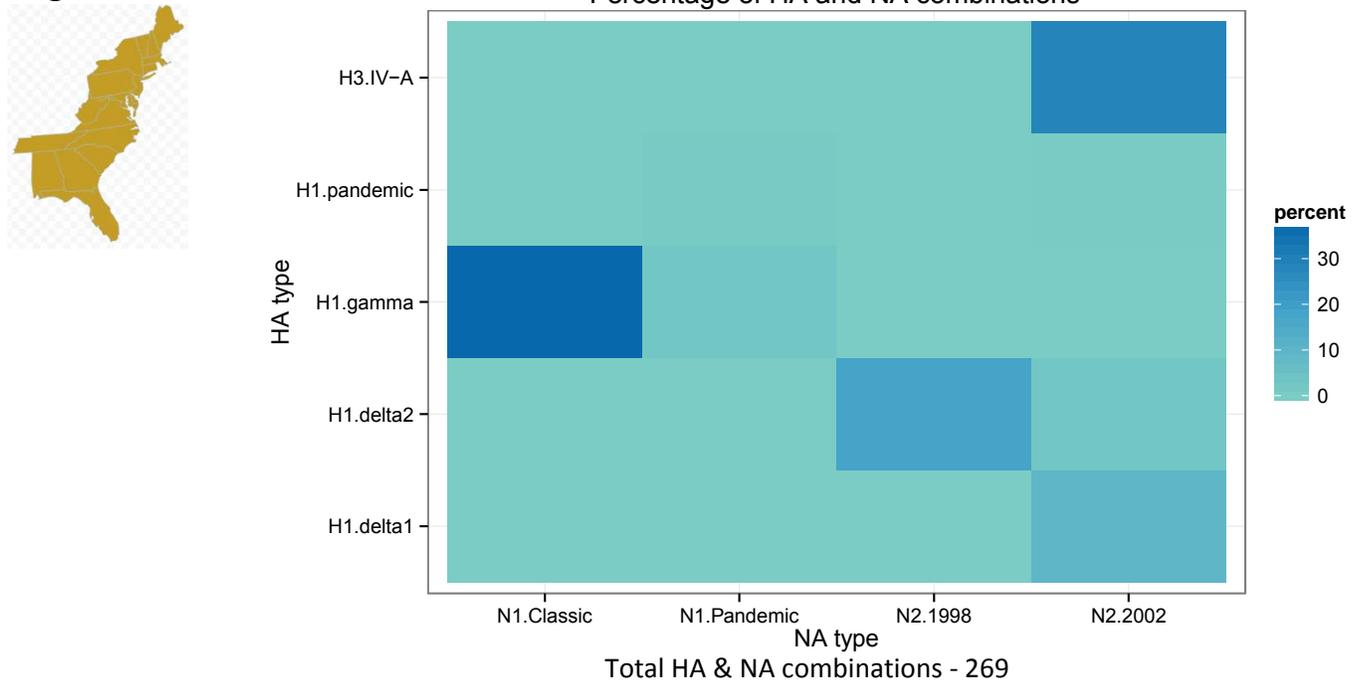
The internal and external reviews of IAV-S surveillance currently underway will help USDA evaluate the system and provide recommendations for process improvement and system efficiency.

# Appendix 1: Regional Charts of HA and NA Combinations by Percentage, CY 2013 and 2014

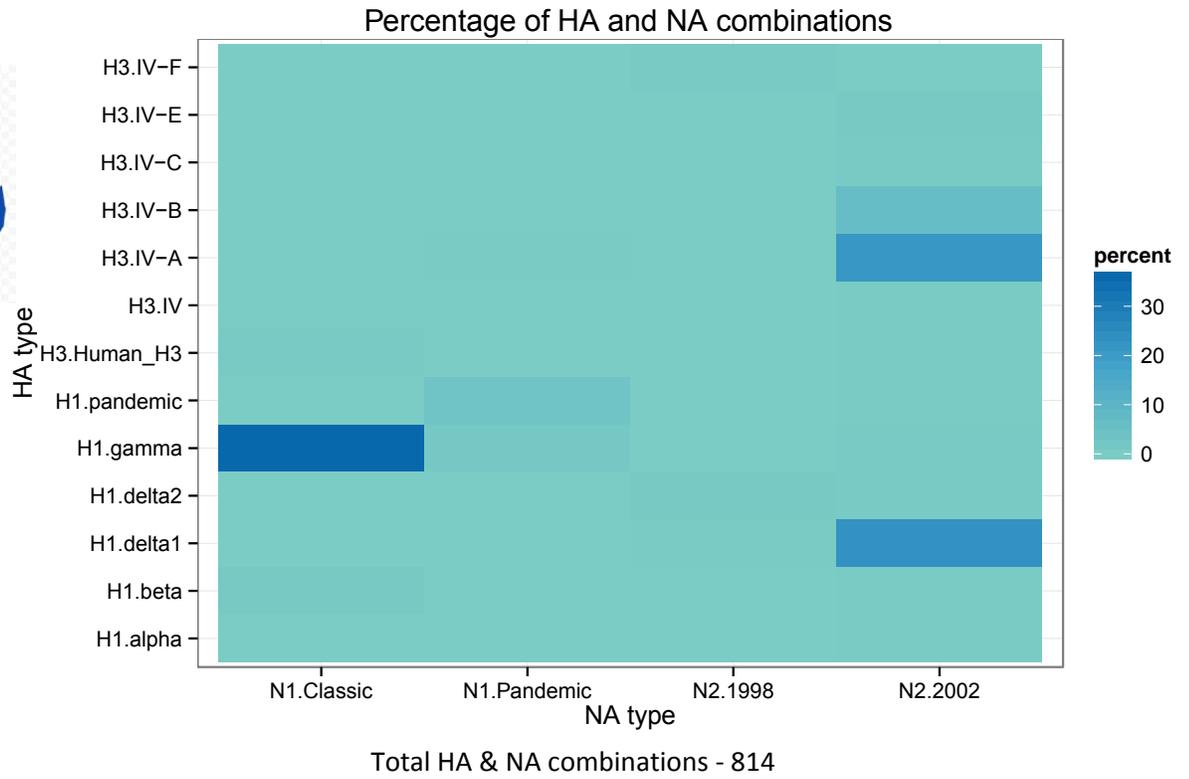
Charts are presented below on the percentages of combinations of HA and NA on national and regional scales, based on ARS-NADC phylogenetic analyses. The results are reported from January 2013 to December 2014.



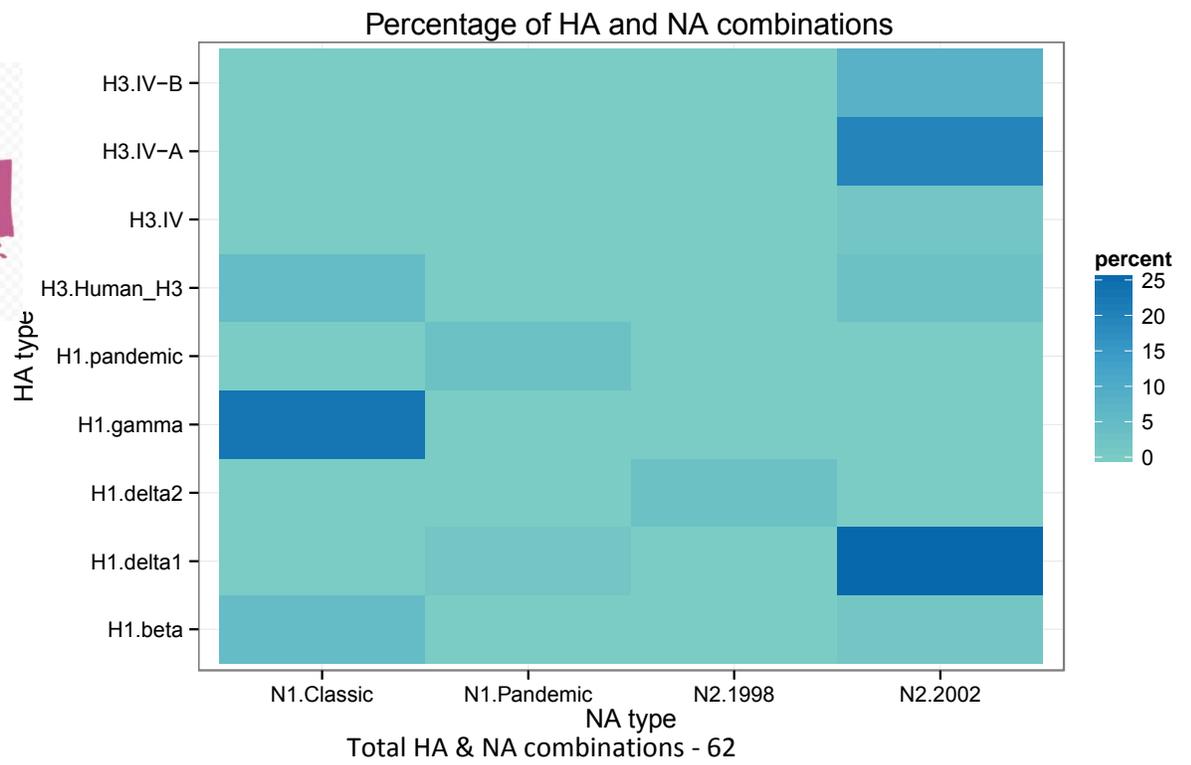
## Region 1



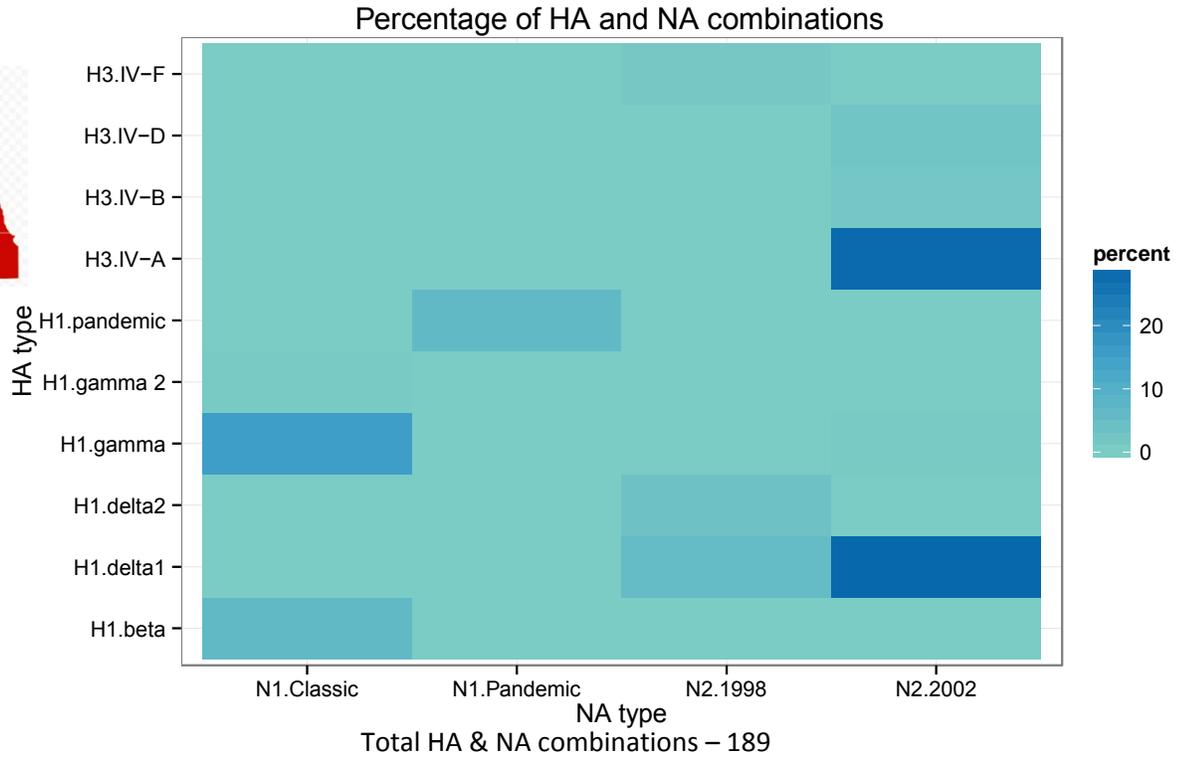
## Region 2



## Region 3



**Region 4**



**Region 5**

