Animal and
Plant Health
Inspection
Service

Veterinary
Services

July 2017

Influenza A Virus in Swine Surveillance

Fiscal Year 2017 Quarterly Report

Surveillance Summary for Third Quarter FY 2017:
April 1 – June 30, 2017

Report Summary[1]

- This report covers the third quarter (Q3) of fiscal year (FY) 2017, from April 1, 2017– June 30, 2017.
- Where relevant, the report also includes previous years' data for historical perspective.
- The report provides data from both national and regional levels.
- In FY 2017 Q3, 1,904 samples were submitted for IAV-S surveillance from 776 accessions.
- H1N1 was the predominant subtype.
- Over the past 8 quarters, H1N2 predominated in Regions 1, 2, 3, 4 and 5. When Regions are recorded as "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, 4, 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 July 27, 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 laboratories. Reporting months are based on the month the 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

---

[1] In November 2016, VS modernized the process that prepares and stages laboratory results data for reporting. Consequently, 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.

The IAV-S surveillance program is voluntary and, as a result, the accessions and samples submitted represent a subset of the swine population. Submitted samples 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

The IAV-S program has undergone many changes since 2008 when discussions with the pork industry and the Centers for Disease Control and Prevention (CDC) about this IAV-S surveillance began. In May 2009 with the human pandemic H1N1, the focus of the surveillance shifted to survey for the appearance of the human pandemic virus in swine, and submissions into the new surveillance system were bleak. Industry concerns about influenza virus is pigs did not abate, however, and by October 2010, with the addition of an “anonymous” submission option, submissions picked up. At the same time, the surveillance focus shifted back to looking at all influenza viruses circulating in U.S. swine, tracking changes in those viruses, and making virus isolates available for researchers and biologic development. Oral fluids became a National Animal Health Laboratory Network (NAHLN)-approved sample type in 2012. In June 2014, USDA established cycle threshold (CT) cutoff values to be met in order for USDA to reimburse further testing. This change was intended to increase the likelihood of getting virus isolates from submissions. It was successful to some degree; however, in June 2016, USDA collaborated with stakeholders to make additional modifications to the program. Changes included lowering CT cutoff values for samples to be eligible for further testing that is reimbursable by USDA, shifting M gene sequencing to the National Veterinary Services Laboratories (NVSL), and shifting cost of IAV-S screen testing to the producer. These recent changes have again improved the program and have resulted in a higher percentage of virus isolation attempts yielding a virus for the surveillance system.

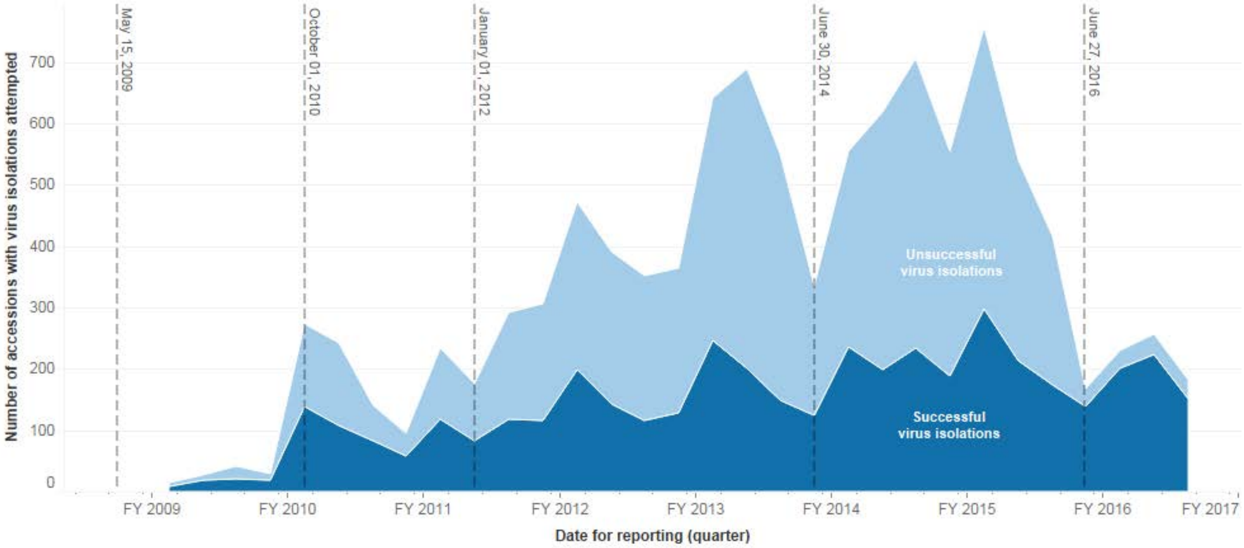


Figure 1. IAV-S surveillance system timeline with dates of modifications and their impact on unsuccessful versus successful virus isolations

The focus of this surveillance system remains on acquiring viruses, and USDA continues to encourage submissions. NAHLN has put several submission options in place to ensure unusual viruses or viruses identified by methods other than those approved for NAHLN testing can be submitted into the program. An updated version of the IAV-S NAHLN testing guidelines and instruction sheet can be found at: https://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/downloads/appendix_c_testing_guidelines.pdf

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 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, updated 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 through the submission of diagnostic laboratory samples to the surveillance system, collection of the viruses that are isolated from the samples, and analysis of the HA and NA sequences that are generated at the NAHLN laboratories. Each month selected viruses undergo whole genomic sequencing by NVSL. Phylogenic analysis of the genetic sequences is provided through an interagency agreement with the USDA's Agricultural Research Service (ARS) National Animal Disease Center (NADC).

National Surveillance Data Summary

From FY 2011 through FY 2016, the total number of accessions and samples submitted rose over time. Changes initiated in FY 2016 have resulted in decreased laboratory accessions and samples, but have resulted in a higher percentage of accessions that result in a virus that can be sequenced and analyzed. For FY 2017's third quarter, 1,904 samples were tested from 776 accessions (Figure 2) for a fiscal year-to-date total of 6,209 samples and 2,727 accessions. Figure 3 shows the overall increasing trends in total accessions, PCR-positive accessions, subtyped accessions, and VI positive accessions.

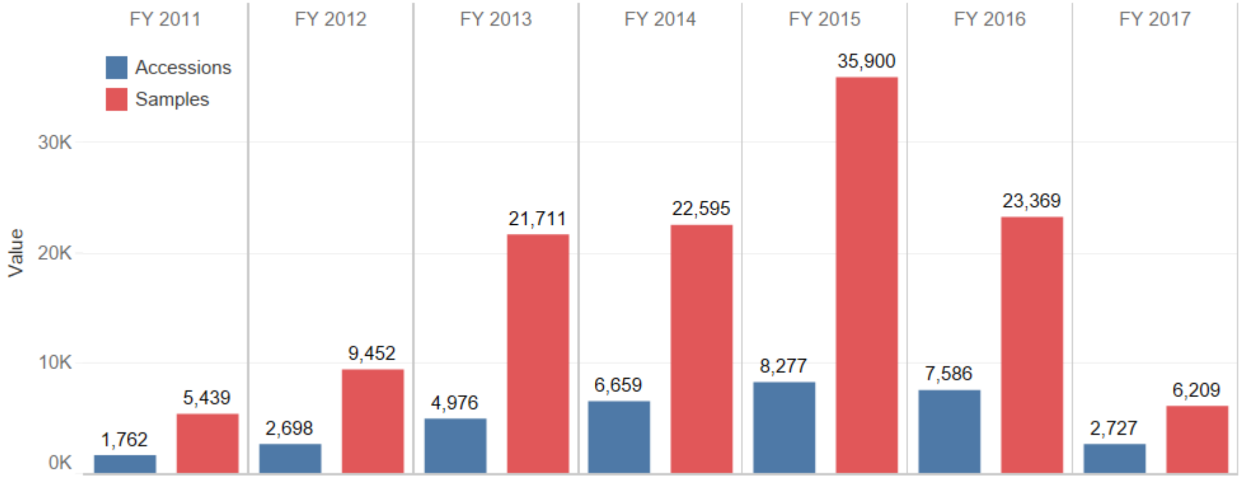


Figure 2. Number of IAV-S laboratory accessions and samples tested in swine, FY 2011 through FY 2017 Q3

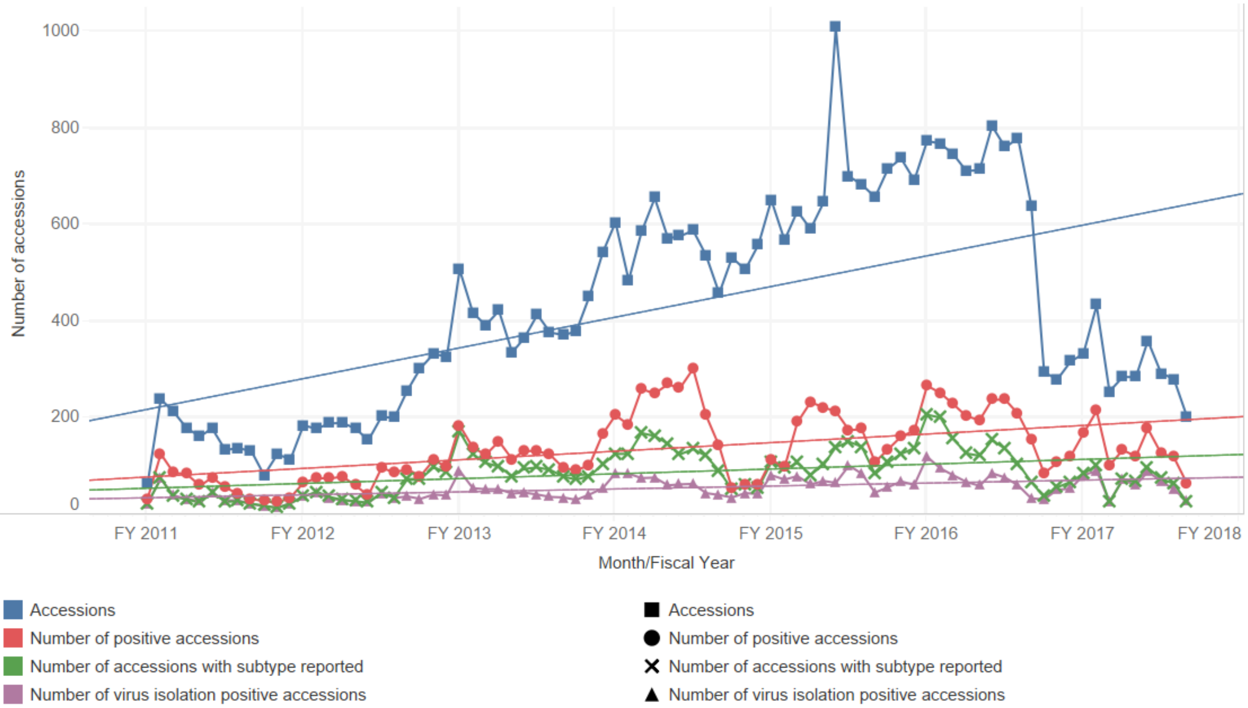


Figure 3. Accessions submitted, subtyped accessions, positive accessions, and VI positive accessions over time with trend lines, FY 2011 through FY 2017 Q3

Figure 4 shows the number of subtype detections in FY 2017 Q3. The total number of samples subtyped was 165, including 65 H1N1, 51 H1N2, 40 H3N2, 0 H3N1, and 9 mixed.

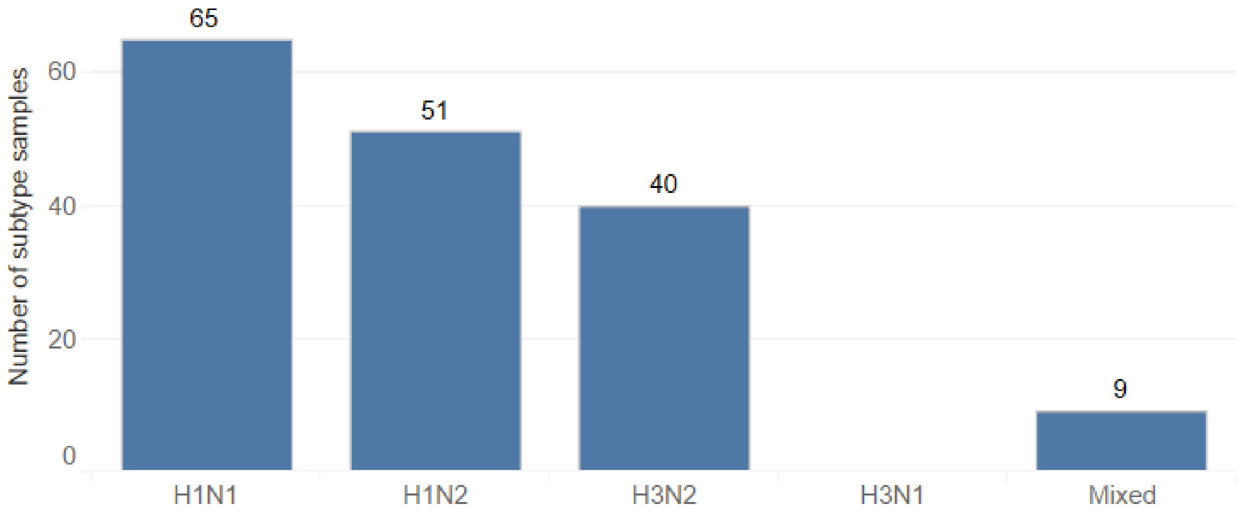


Figure 4. Number of subtype detections in FY 2017 Q3

Figure 5 breaks down accessions by rRT-PCR subtype from FY 2011 to FY 2017 Q3. 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 through the first, second, and third quarters of FY 2017.

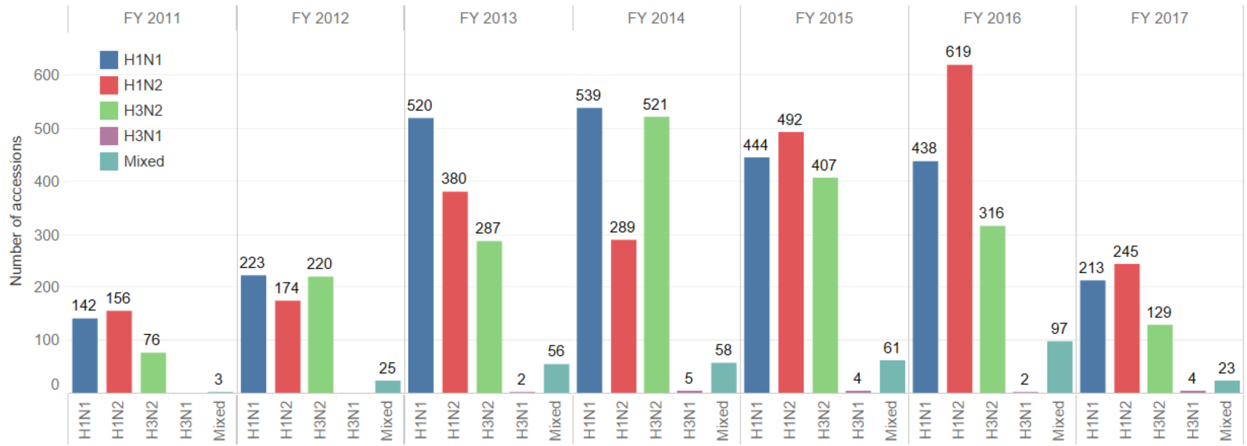


Figure 5. Number of subtypes, FY 2011 through FY 2017 Q3

Figure 6 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.

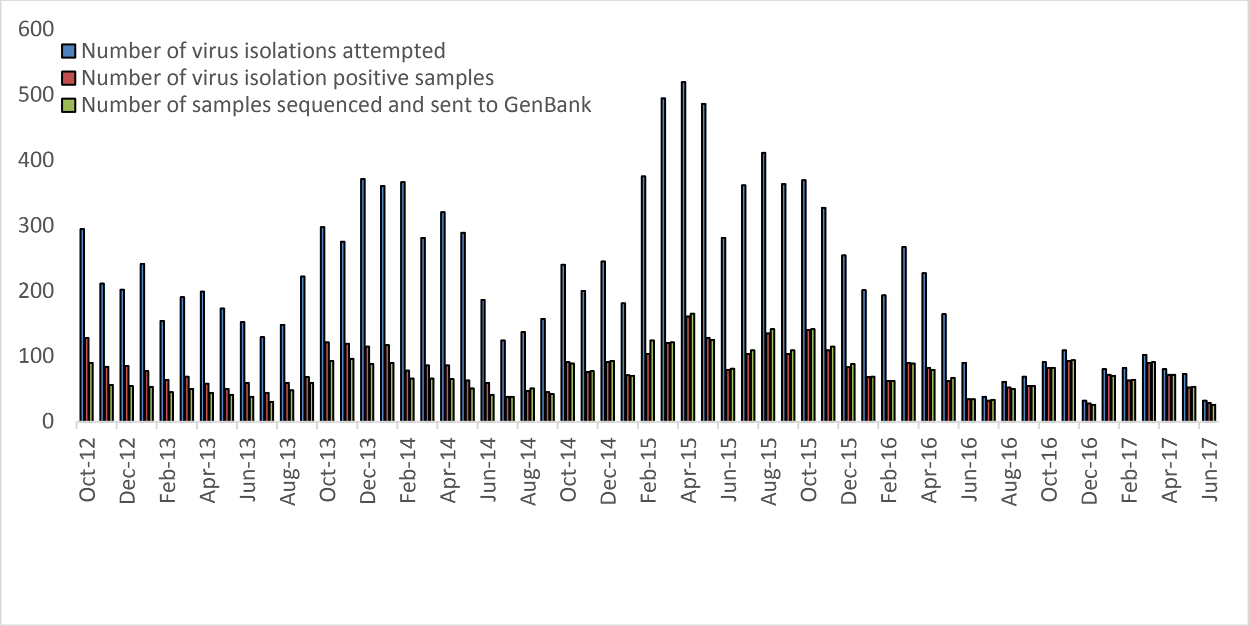


Figure 6. Number of virus isolations attempted, positive virus isolations, and GenBank submissions from FY 2013 through FY 2017 Q3

When accessions were evaluated by age-class for the third quarter, the following observations were noted. H1N2 was the most common subtype among Suckling. H1N1 was the most common subtype among Grower/Finishers and Nursery. Sow/Boar had limited testing, with two occurrences of H1N1, one occurrence of H1N2, zero of H3N1, three of H3N2, and zero mixed. Among accessions for which the age class was unknown or not recorded, H1N1 was the predominant subtype (Table 1). 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, Q3 FY 2017.

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	59	16	20	0	19	4
Nursery	46	18	15	0	11	2
Grower/Finisher	36	22	10	0	3	1
Sow/Boar	6	2	1	0	3	0
Not Recorded/Unknown	18	6	5	0	4	0

Table 2. Number of positive accessions tested for IAV-S by specimen type and by viral subtype, Q3 FY 2017.

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	138	93%	58	46	0	29	5	128
Nasal or Nasal Swab	23	96%	4	5	0	10	1	19
Oral Fluids	2	50%	0	0	0	1	1	2
Other Specimens	2	0%	2	0	0	0	0	2

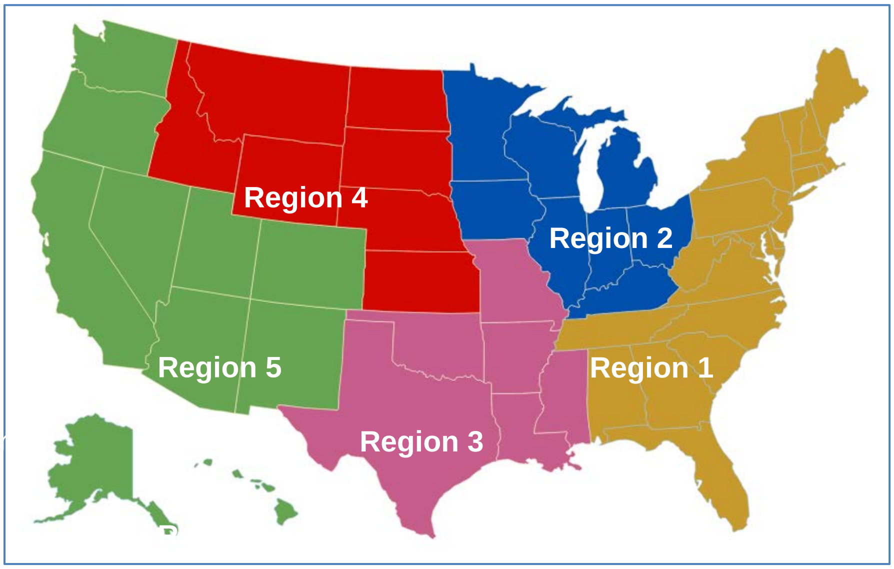
*Accessions may include samples with multiple specimen types. In these cases, individual accessions are counted in more than one specimen type category.

**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 2015 Q4 through FY 2017 Q3

Most Predominant HA/NA phylo-types overall:

H1N1 (Gamma H1/Classical N1)
H1N2 (Delta2 H1/1998-N2)
H3N2 (hu-like H3/2002-N2)
H1N2 (Delta1a H1/2002-N2)
H3N2 (IV-A H3/2002-N2)
H1N2 (Delta1b H1/2002-N2)

Region 1 (Total HA/NA: 382)

Gamma H1/Classical N1
IV-A H3/2002-N2
Delta2 H1/1998-N2
Low frequency but consistent detections of IV-B H3/2002-N2, pdm H1/pdm N1, delta2 H1/2002-N2, alpha H1/2002-N2

Region 2 (Total HA/NA: 1,050)

Most diversity of all regions
Gamma H1/Classical N1
Delta1 H1a/2002-N2
Hu-like H3/2002-N2
Low frequency but consistent detections of IV-A H3/2002-N2, IV-E H3/2002-N2, delta2 H1/1998-N2, IV-B H3/2002-N2, pdm H1/pdm N1, alpha H1/2002-N2, delta1b H1/2002-N2

Region 3 (Total HA/NA: 128)

Delta1a H1/2002-N2
Gamma H1/Classical N1
IV-A H3/2002-N2
Hu-like-H3/2002-N2
Low frequency detections of Beta H1/Classical N1, Pdm H1/Pdm N1, Delta1b H1/2002-N2, Delta1b H1/pdm N1, Hu-like-H3/Classical N1
1 Hu-like16 H3/Hu-like N2

Region 4 (Total HA/NA: 129)

Delta1b H1/2002-N2
IV-A H3/2002-N2
Delta1a H1/N2-2002
Gamma H1/Classical N1
Pdm H1/Pdm N1
Low frequency but consistent detections of beta H1/2002-N2, Alpha H1/2002-N2, Hu-like H3/2002-N2

Region 5 (Total HA/NA: 6)

Two Delta 1b H1/2002-N2
Two Alpha H1/2002-N2
One Hu-like H3/2002-N2
One IV-A H3/2002-N2

Figure 8 shows the distribution of rRT-PCR subtyped accessions among the five regions for Q3 FY 2015 through Q3 FY 2017. Regions 1, 2, 3, 4, and 5 demonstrate H1N2 as the predominant subtype. For regions recorded as "unknown," H1N1 was the predominant subtype.

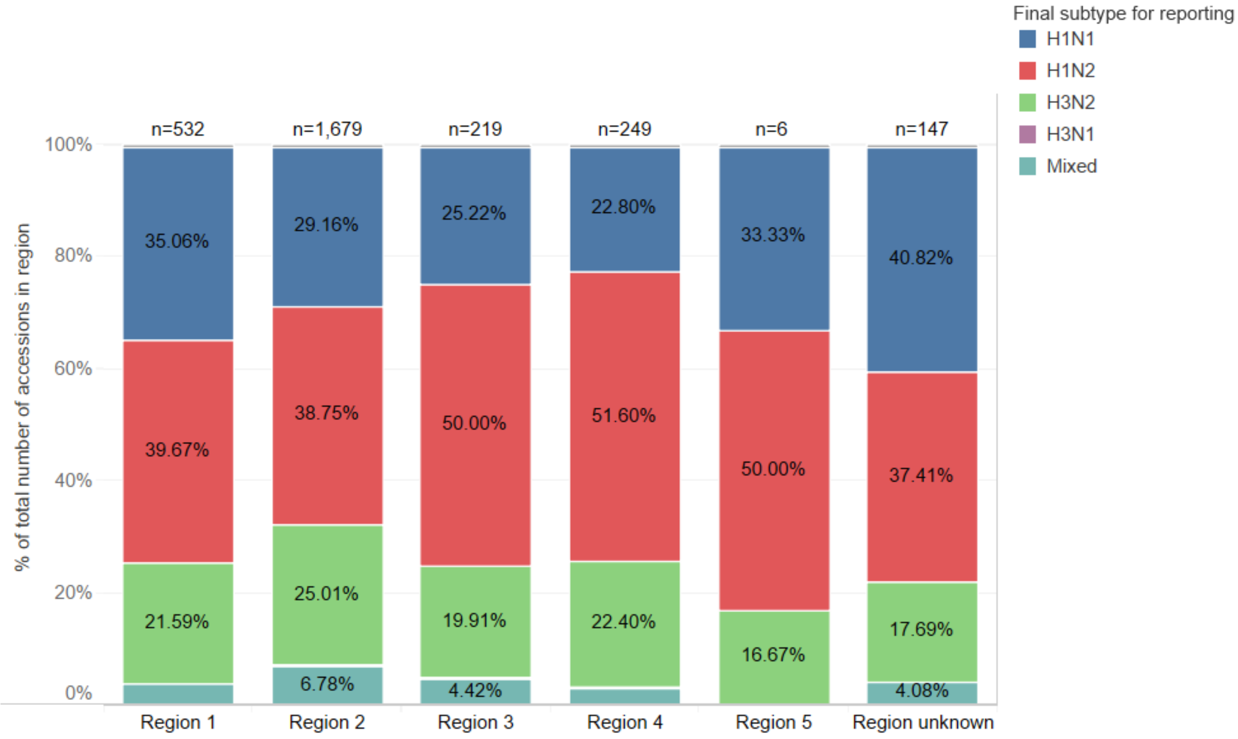


Figure 8. Percentage of subtyped accessions by region for FY 2015 Q3 through FY2017 Q3

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[2] of 202 isolates with published sequences in GenBank was characterized by phylogenetic analysis in Q3 FY 2017. 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.

---

[2] 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.

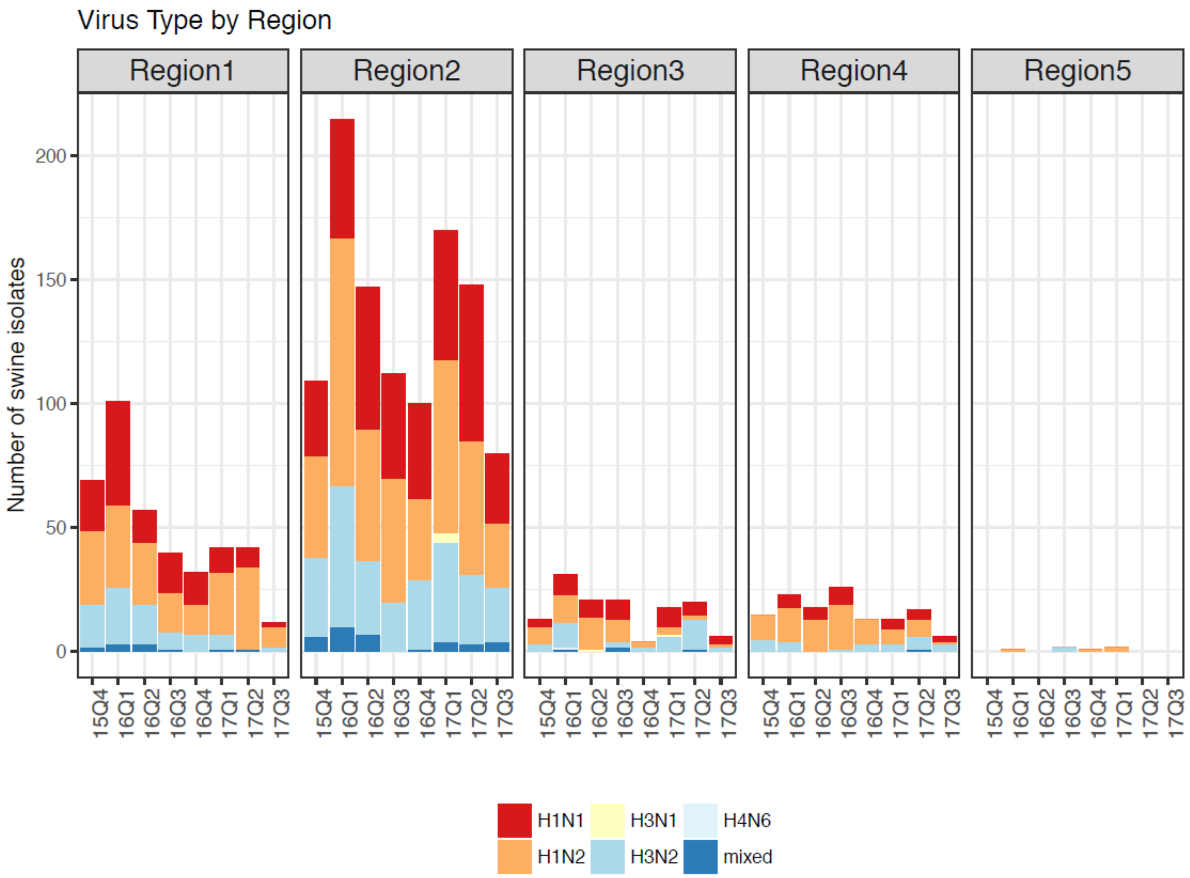


Figure 9. Virus type by region 2-year summary Q4 FY 2015 to Q3 FY 2017

Figure 9 demonstrates the four subtypes H1N1, H1N2, H3N1, H3N2, and mixed subtypes across the five regions. There was also a H4N6 reported in FY 2016 Q1. 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 10), there continued to be detections of alpha with two aa deletions (n=11). Delta 1a, delta 2, and gamma viruses are the predominant H1.

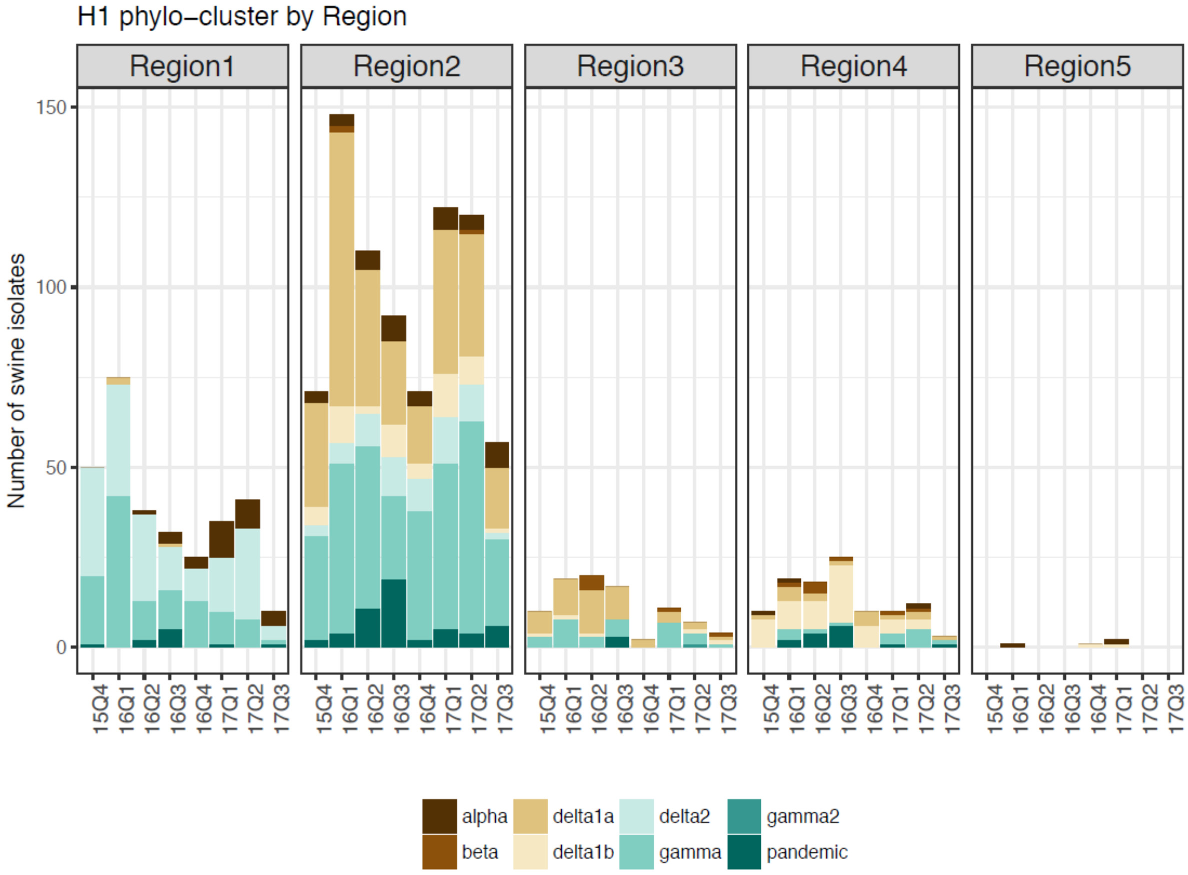


Figure 10. H1 phylo-cluster by region – 2-year summary Q4 FY 2015 to Q3 FY 2017

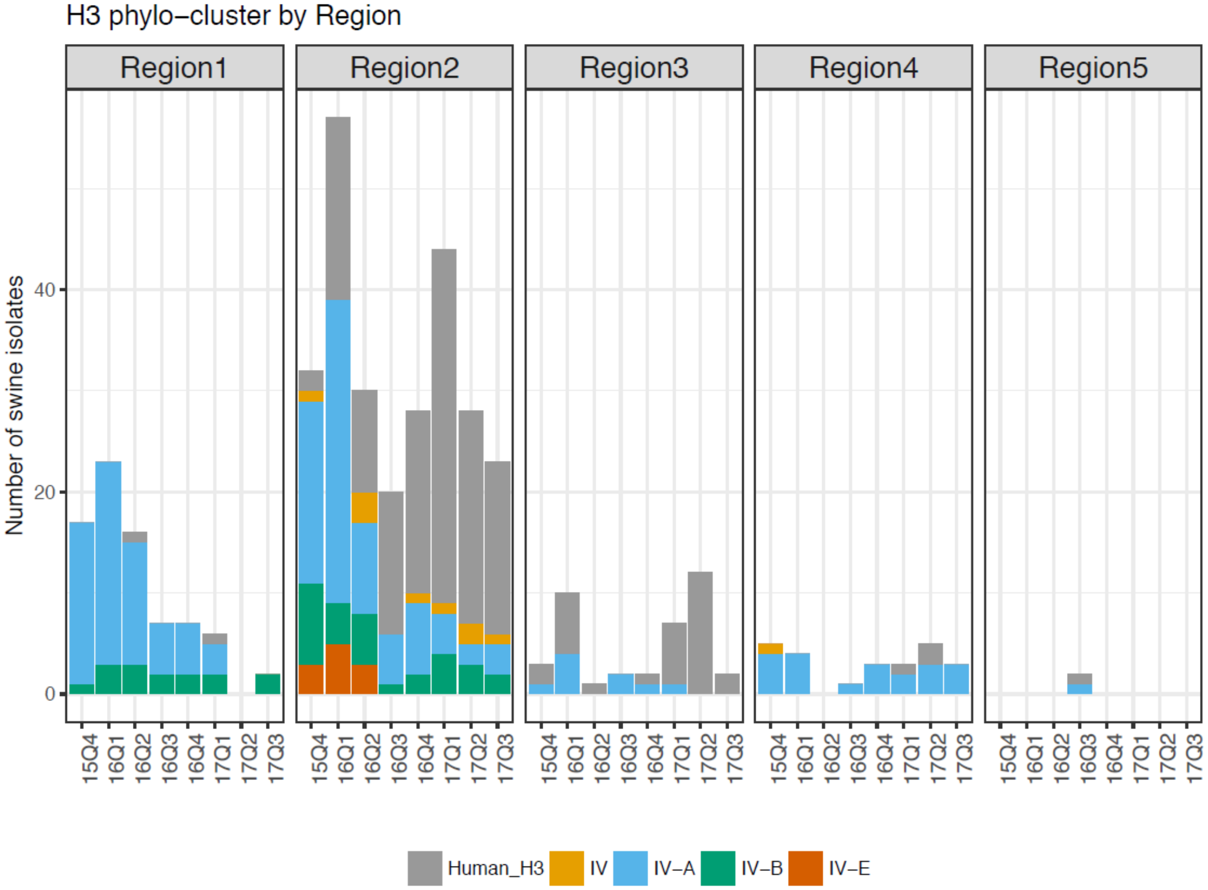


Figure 11. H3 phylo-cluster by region, 2-year summary Q4 FY 2015 to Q3 FY 2017

In Q3 FY 2017, there were detections of human-like H3 in Iowa, Illinois, Minnesota, Missouri, and Oklahoma. Out of 29 H3s in Q3 FY 2017, 18 are human-like H3s. Human-like H3 is the predominant H3.

National phylogenetic NA gene information

NA gene information remained the same in Q3 FY 2017. 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 89 percent of N2 collections.

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 through the surveillance system with public health, animal health, and academic researchers to facilitate genetic analysis and research on viruses of interest. The NVSL Diagnostic Virology Laboratory maintains a repository of the viruses submitted into the surveillance system and provides these viruses upon request.

In the third quarter of FY 2017, the NVSL Diagnostic Virology Laboratory provided 68 isolates to six institutions, one governmental, one academic, and three pharmaceutical, and one international (academic). NVSL received 232 isolates into the repository (Table 3). Table 4 reports the total number of isolates available in the repository by subtype for sharing.

Table 3. Virus isolates received in repository

Virus isolates in the repository	
2017 YTD	700
2016	1,046
2015	883
2014	765
2013	820
TOTAL TO DATE	3,982

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	1,575
H3N1	15
H1N1	2,044
H1N2	1,920
Mixed	313
TOTAL	5,867

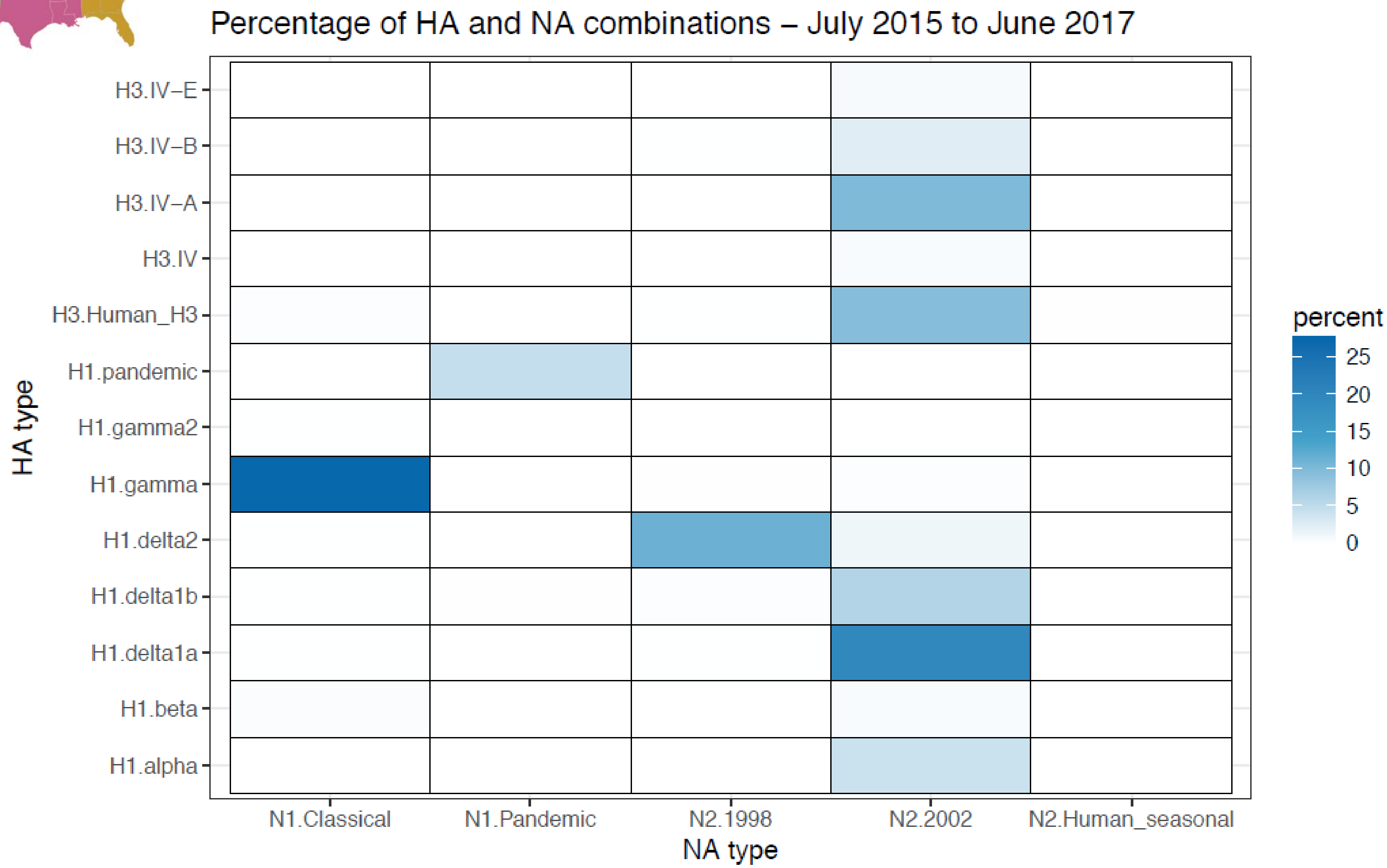
Conclusion

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.

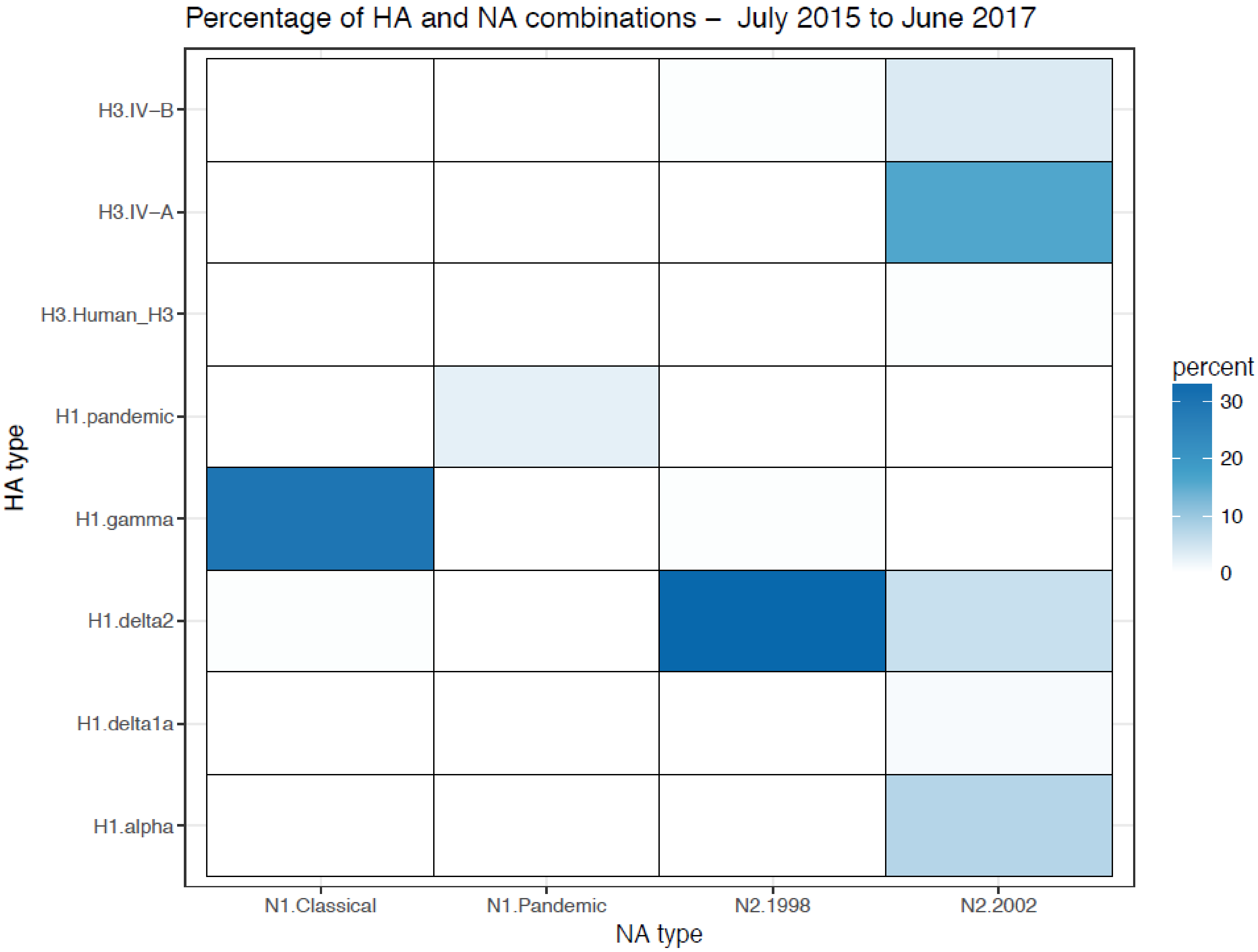
Appendix 1. Regional Charts of HA and NA Combinations by Percentage

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 2015 to June 2017. 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.

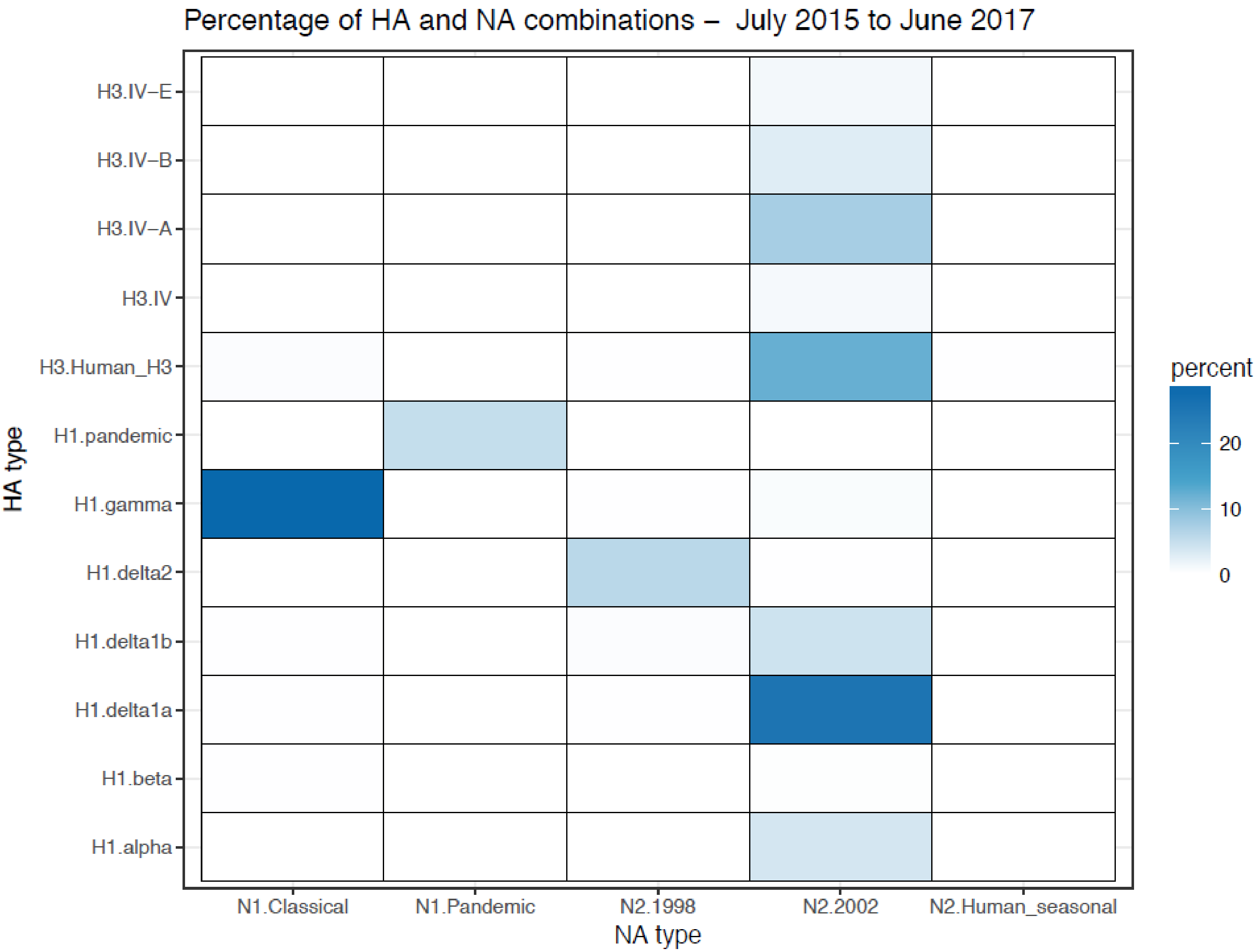
Total HA & NA combinations – 1,697



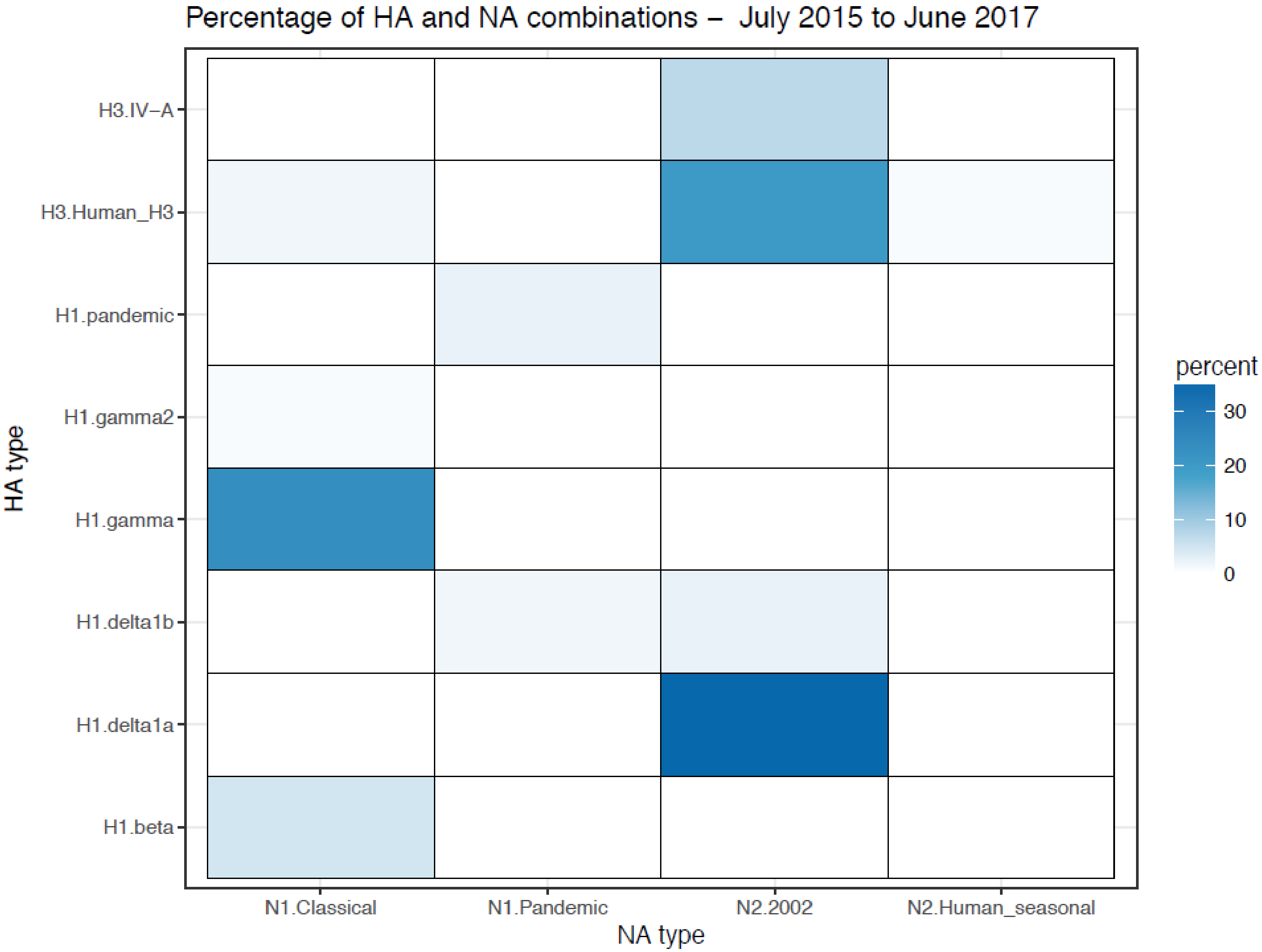
Region 1. Total HA & NA combinations – 382



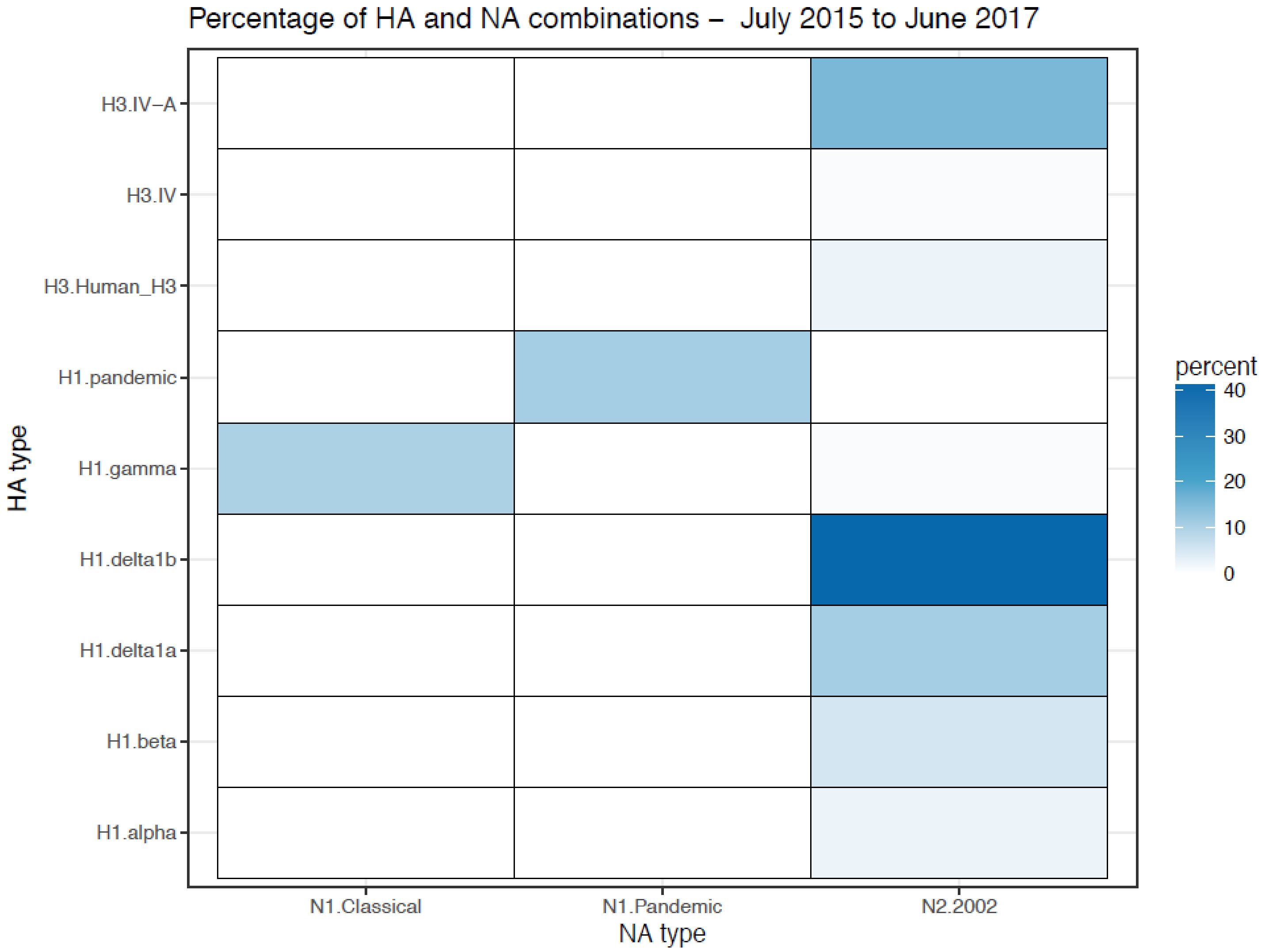
Region 2. Total HA & NA combinations – 1,050



Region 3. Total HA & NA combinations – 128



Region 4. Total HA & NA combinations – 129



Region 5. Total HA & NA combinations –6