USDA Surveillance for Influenza A Virus in Swine: Summary of Results

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Introduction

Classic H1N1 "swine origin" influenza virus was the sole endemic influenza A subtype found in the U.S. swine population for 70 years. However, human seasonal H3N2 influenza viruses emerged in U.S. swine in 1998 as a triple reassortment virus with genes of human, swine, and avian virus lineages. Since then, multiple introductions of human viruses have been documented with reassortment of gene segments subsequently occurring in swine each time, leading to increasing genetic diversity in influenza A virus populations in U.S. swine. This rising virus heterogeneity has in turn complicated the control of influenza infections in the U.S. swine population.

The public health community and swine industry have become increasingly aware of sporadic cases of human influenza A infections with viruses shown to be genetically similar to swine isolates. In 2008 the U.S. Centers for Disease Control and Prevention (CDC), the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS), and USDA-Agricultural Research Service (ARS) entered into an Interagency Agreement (IAA) for a pilot surveillance study of influenza A virus isolates in swine. The purpose of the pilot program was to gain an understanding of the diversity of influenza A isolates found in the U.S. swine population.

This effort had just begun when the influenza pandemic emerged in April of 2009. In response a more permanent USDA-led national influenza A virus surveillance plan for swine was implemented to monitor for evidence of swine infection by the Influenza A (H1N1) pdm09 virus. This virus subsequently became established in both human and swine populations, and influenza surveillance objectives in swine have now evolved toward documenting shifts and reassortments in the whole range of influenza A virus populations affecting swine.

From 2005 to 2011, the CDC reported 348 cases of variant influenza infection (all subtypes) in people caused by viruses thought to have originated from swine (http://www.cdc.gov/flu/swineflu/variant-cases-us.htm). In calendar year 2012, 313 cases of variant influenza infection (all subtypes) in people were reported in the United States, most with

extensive contact with swine at exhibitions. Most of these infections involved closely related (but not identical) influenza A H3N2 variant "A(H3N2)v" viruses containing matrix (M) genes similar to the M gene found in the 2009 human pandemic virus.

These recent events have served to further focus public and animal health interest on the potential for spillover of influenza A infections from swine to humans and humans to swine. Fortunately, the USDA Influenza A Virus in Swine Surveillance Program has provided investigators, diagnosticians, and researchers with a growing array of subtyping and sequence data from swine isolates to better inform investigations of these incidents.

From a swine health standpoint, the USDA Influenza A in Swine Surveillance Program has provided a much improved picture of the diversity of isolates found in the U.S. swine herd. This information can now be used to inform vaccine decisions, develop and/or improve diagnostic assays, and to aid research efforts to better understand and control influenza A infections in swine. At the practitioner and producer level, this program provides an economical means to obtain detailed phylogenetic information on at least 3 genes (H, N, and M) from herd isolates entered into the program. This information can be used for improved herd-level decisions related to epidemiological analysis, vaccine selection, and biosecurity protocols.

Objectives

The current objectives of the USDA Influenza A in Swine Surveillance Program are: [1] Monitor genetic evolution of endemic swine influenza viruses to better understand endemic and emerging influenza virus ecology; [2] Make virus isolates available for research and establish an objective database for 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.

This paper highlights results obtained from samples submitted for the surveillance program through April 2013.

Material and Methods

There are three components influenza surveillance in swine; [a] Surveillance of swine observed with influenza-like illness (ILI) on farms from which samples are submitted for laboratory testing, [b] Surveillance of swine epidemiologically linked to a human case of novel influenza A virus, and [c] Surveillance of swine observed with signs of ILI at points of concentration or comingling events, particularly where there is potential high exposure to humans. Approved samples include nasal swabs from live pigs and/or lung tissue from mortalities. In some cases oral fluid samples are accepted by laboratories granted a deviation from approved program protocols.

Up to 10 samples per submission are sent by attending herd veterinarians (or regulatory veterinarians in some cases) to participating National Animal Health Laboratory Network (NAHLN) laboratories for diagnostic workup. All samples are screened with a Matrix PCR

assay specific for all influenza A viruses. For laboratory accessions with matrix-positive results, up to 2 samples demonstrating the highest levels of nucleic acid are selected for simultaneous virus isolation and further diagnosis via a set of subtyping PCR assays to indicate the subtype (H1N1, H1N2, H3N2, and/or undetermined). Any viruses isolated are shipped to the National Veterinary Services Laboratories (NVSL) in Ames Iowa to be placed in the National Influenza Virus Repository. (Virus isolates can be obtained from the repository by contacting NVSL Diagnostic Virology Laboratory personnel at 515-337-7526.)

Successful virus isolates are further characterized via sequencing of the hemagglutinin (H), neuraminidase (N), and matrix (M) genes, either at NAHLN labs or at NVSL. Gene sequence data is then electronically submitted to GenBank, a publically available gene sequence database maintained by the National Institutes of Health (NIH), making the data available to researchers for further genetic characterization and research studies.

Epidemiological data collected at the NAHLN lab includes total animals and specimens tested by date, state of sample origin, reason for submission, tests conducted on the samples and their results, as well as sequence information if applicable. No producer or submitting veterinarian information is shared, unless written permission is granted. These data are transmitted weekly to the VS, NAHLN Program Office and tabulated monthly by the VS National Surveillance Unit (NSU) for analysis and reporting. Submitting laboratory information is stripped from sample data to assure anonymity of samples.

Results and Conclusions

Between October 1, 2010 and May 31, 2013, 28,398 samples from 7,670 laboratory accessions have been entered into the USDA Influenza A in Swine Surveillance Program. There were 2,797 accessions containing one or more Matrix-positive samples. Virus was isolated from one or more samples in 1,245 laboratory accessions, and subtyping results are recorded for 1,927 accessions. A total of 1,460 samples have been sequenced, with results entered into the GenBank influenza A dataset.

Figure 1 provides data from the surveillance program through April 30th, 2013. Figure 2 illustrates number of positive cases by month of collection for each subtype. Finally, figure 3 shows the number of GenBank accessions made via the USDA Surveillance Program by month of collection.

Discussion

The voluntary and anonymous USDA National Influenza A Virus in Swine Surveillance Program has demonstrated steadily increased participation and gained acceptance within the swine industry and public health community. Much valuable data has been generated which shows a high degree of diversity within the population of influenza A viruses isolated from U.S. swine. Detailed phylogenetic analyses have demonstrated extensive reassortments of virus segments within the population, with several likely introductions of human-origin influenza A viruses into the influenza virus pool in swine since 1998. This complex and dynamic population

illustrates why achieving stable and lasting control of Influenza A infections in swine has remained a challenge.

It is beyond the scope of this paper to discuss these issues in detail. However, these findings highlight the value of continuing this surveillance and characterization effort. It is only through collection of a wide variety of samples from diverse geographical areas over time that researchers can better understand changing patterns of influenza A virus (IAV) diversity and the epidemiological and genetic factors that may drive those changes.

Although the current system has tremendous value and is providing meaningful information on the current flu situation in swine, a major shortcoming of this surveillance program is an inability to derive true infection prevalence or more precise locational and associated epidemiological data from anonymous (state level) submissions. Influenza A is not a reportable disease in swine in the United States and some producers remain reluctant to share more detailed information with the USDA. It remains the role of university researchers, laboratory diagnosticians, and ultimately private practitioners to better understand within herd, within production system, and more localized geographical patterns of infection, spread, and virus evolution.

Despite these shortcomings, this program has played an invaluable role in providing information on influenza A virus infections in swine, contributing to a more holistic understanding of the shared nature of influenza A infections within and between affected species. A baseline has been established on which all future changes in viruses detected in swine can be compared. Knowledge gained will be utilized to devise efficacious solutions both within the swine population and also at the interface between swine and other potential hosts.

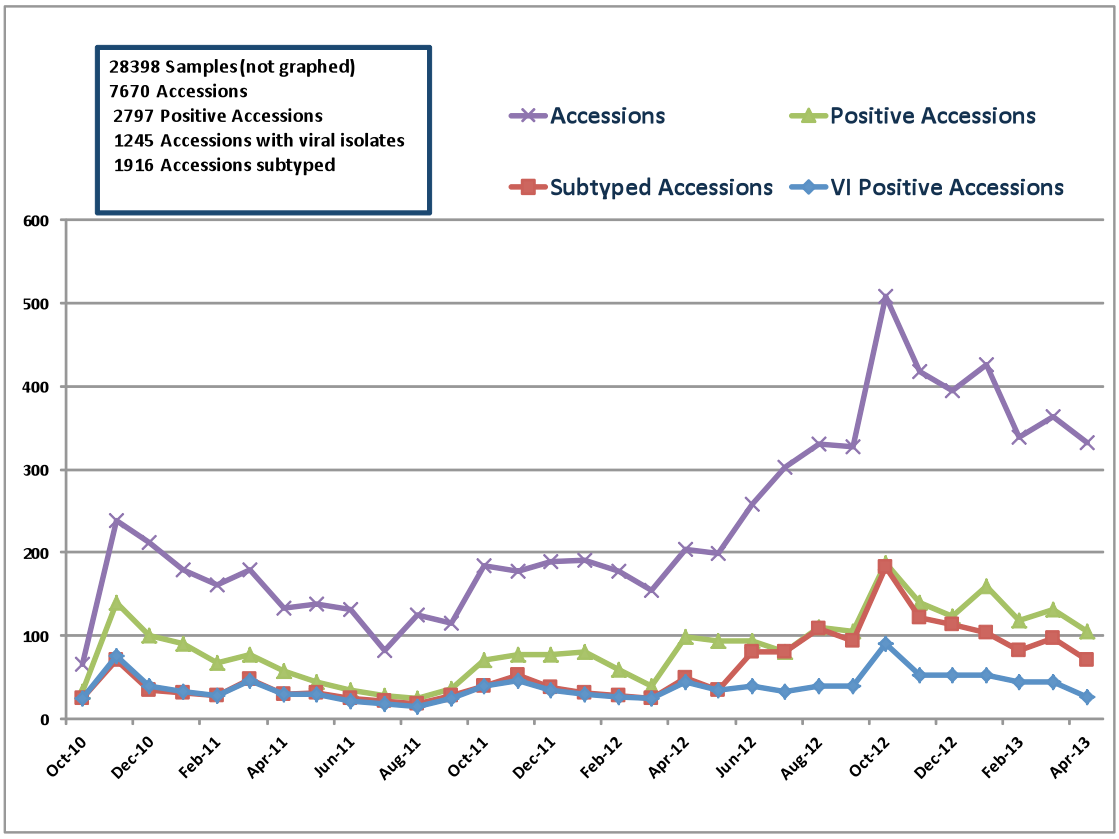

Figure 1. USDA USDA IAV Surveillance Program Isolation and Characterization Activities between October 1rst , 2009 and April 30th, 2013

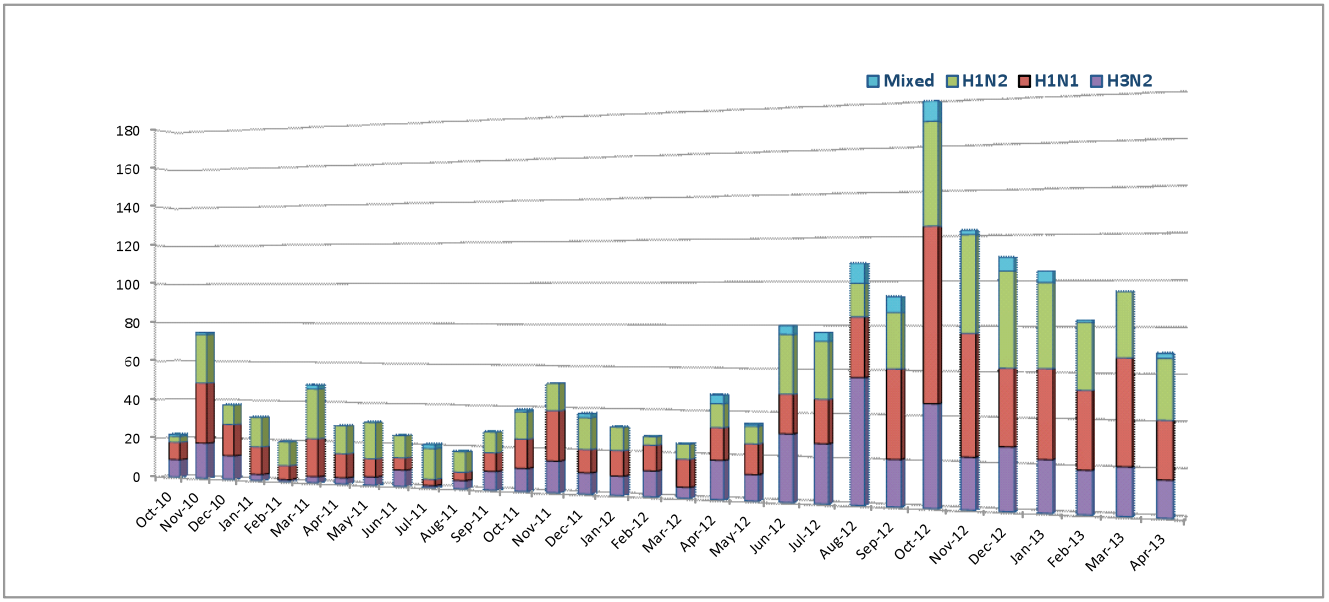

Figure 2. USDA IAV Surveillance Program: Subtypes Identified by month between October 1rst, 2009 and April 30th, 2013

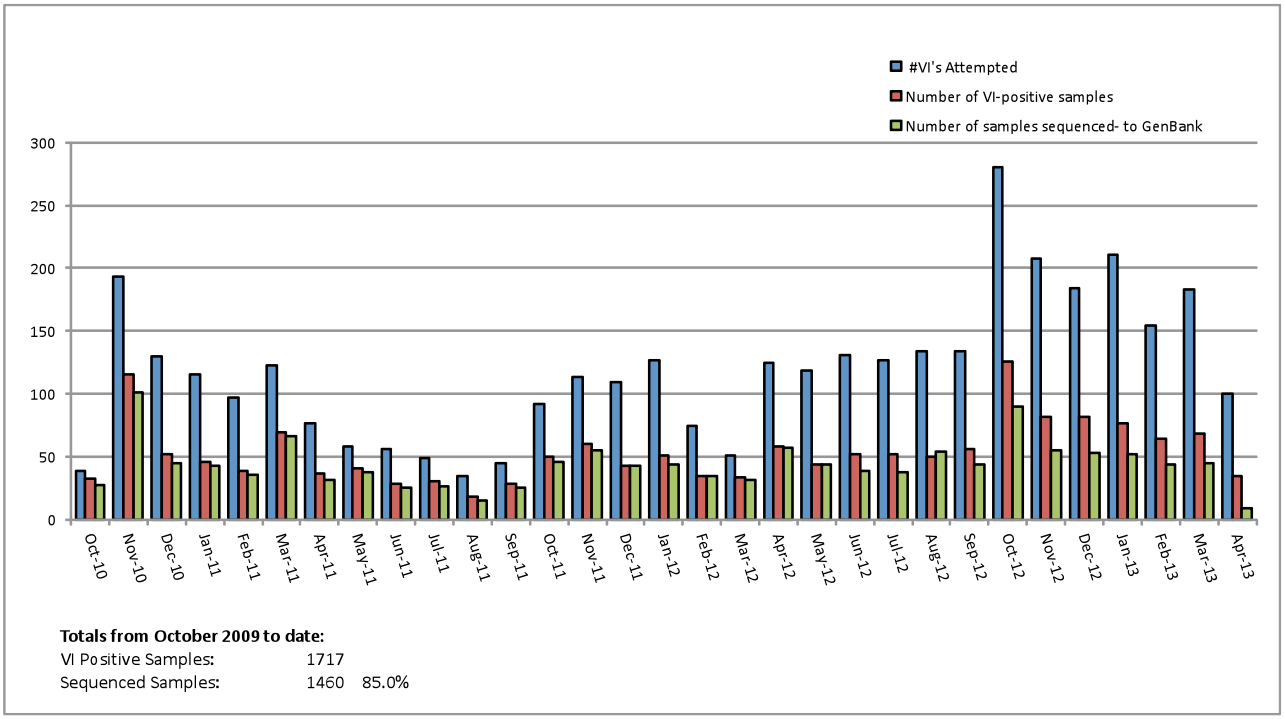

Figure 3. USDA IAV-Surveillance Program: GenBank Accessions submitted by month between October 1rst, 2009 and April 30th, 2013