

Appendix A: National Surveillance Plan

National Surveillance Plan for Swine Influenza Virus in Pigs

July 14, 2010
Version 3.2

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services



Table of Contents

EXECUTIVE SUMMARY	3
INTRODUCTORY INFORMATION	5
1.1 DISEASE DESCRIPTION	5
1.2 RATIONALE AND PURPOSE FOR SURVEILLANCE.....	6
<i>Rationale for surveillance.....</i>	6
<i>Purpose of surveillance.....</i>	6
1.3 SURVEILLANCE OBJECTIVES	6
1.4 EXPECTED OUTCOMES: PRODUCTS, DECISIONS, AND ACTIONS	7
1.5 STAKEHOLDERS AND RESPONSIBLE PARTIES	7
POPULATION DESCRIPTION AND SAMPLING METHODS.....	8
1.6 POPULATION DEFINITIONS	8
1.7 CASE DEFINITIONS.....	9
1.8 DATA SOURCES AND 1.9 SAMPLING METHODS FOR EACH SURVEILLANCE STREAM.....	10
A. <i>Case-compatible swine accessions submitted to veterinary diagnostic laboratories</i>	10
B. <i>Targeted surveillance of sick pigs at first points of concentration or comingling events.....</i>	11
C. <i>Surveillance of swine populations epidemiologically linked to a human case of SIV</i>	11
<i>Confidence in detection of novel SIV</i>	12
<i>Education and Outreach.....</i>	12
ANALYSIS, REPORTING AND PRESENTATION.....	12
1.10 DATA ANALYSIS, REPORTING AND INTERPRETATION	12
1.11 DATA PRESENTATION AND REPORTING	13
IMPLEMENTATION, BUDGET AND EVALUATION.....	14
1.12 SURVEILLANCE SYSTEM IMPLEMENTATION: PRIORITIES, TIMELINES, AND INTERNAL COMMUNICATIONS	14
<i>Priorities</i>	14
<i>Timelines.....</i>	14
<i>Internal Communications.....</i>	14
1.13 RESOURCES AND BUDGET.....	15
1.14 SURVEILLANCE PLAN PERFORMANCE METRICS	15
1.15 SURVEILLANCE SYSTEM EVALUATION	16
ATTACHMENT: BACKGROUND INFORMATION ON SWINE INFLUENZA VIRUSES AND PANDEMIC H1N1 OUTBREAK VIRUS.....	17
<i>Etiology of swine influenza</i>	17
<i>Background information regarding the Pandemic H1N1 2009 Virus outbreak</i>	17
<i>Clinical signs related to swine flu viruses.....</i>	18
<i>Epidemiology of swine flu viruses.....</i>	18
<i>Ecology of animal influenza viruses</i>	19
<i>Methods for control of endemic swine flu viruses.....</i>	21
<i>Diagnosis of swine influenza.....</i>	21
REFERENCES	25

Executive Summary

This revised national surveillance plan for influenza virus in swine (including the pandemic H1N1 2009 virus) will monitor genetic changes in endemic, emerging, and novel influenza virus isolates from pigs exhibiting influenza-like illness (ILI). Participation in this surveillance program is recommended by the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS) due to genetic diversity in influenza virus strains affecting swine and other species. This heterogeneity complicates individual herd swine influenza virus (SIV) control options. Influenza genomic changes must be monitored to increase knowledge and provide improved influenza diagnostic and control options in swine and may also be of benefit to other species.

The objectives of the surveillance program are to:

- Monitor genetic evolution of SIV to better understand endemic and emerging influenza virus ecology;
- Make available SIV isolates for research and to establish an objective database for genetic analysis of these isolates and related information; and
- Select proper isolates for the development of relevant diagnostic reagents, updating diagnostic assays, and vaccine seed stock products.

Current components of SIV surveillance include:

- Case-compatible swine accessions submitted to veterinary diagnostic laboratories. This surveillance will cover on-farm swine populations exhibiting ILI;
- Surveillance of groups of swine exhibiting ILI at first points of concentration or commingling events such as auctions, markets, fairs, or other swine exhibition events; and
- Surveillance of swine populations epidemiologically linked to a confirmed isolation of SIV in a human. This stream covers swine that are linked with a human SIV infection (including the pandemic H1N1 2009 virus).

Short-term expected outcomes include the establishment of State-level baseline data for SIV genomics in U.S. swine. This information will be used to:

- Make timely, informed, and scientific decisions about disease control and prevention measures, human health implications, and trade issues;
- Assist researchers and the animal health industry in developing relevant diagnostic reagents, targeted swine influenza diagnostic assays, and effective vaccines.

A response plan has been developed in cooperation with public health officials for suspected SIV zoonotic transmissions from swine, including fairs and exhibitions. Swine investigations will be coordinated through the VS and State cooperative field structure.

Long-term outcomes may include:

- Additional influenza A surveillance information in broader initiatives such as VS 2015 Surveillance for Action and One Health activities and the American Veterinary Medical Association's (AVMA) One Health Initiative;
- Further research and understanding regarding the ecology and epidemiology of swine influenza infection; and
- A better understanding of epidemiological factors and procedures that either limit or enhance the mutation and spread of SIV in the swine population.

Introduction

Endemic strains of swine influenza virus (particularly of the sub-type H1N1) have been circulating in U.S. swine populations for over 75 years. Various SIV surveillance efforts have been ongoing to better understand the virus and manage animal health due to the economic impact of uncontrolled endemic SIV on swine producers. University, State, and private diagnostic laboratories maintain and update extensive databases of swine influenza viruses, including genomic sequences. However, these efforts have provided only a limited national picture of SIV, and there are challenges associated with proprietary restrictions on isolate sharing.

Animal health and public health partners recognized the need for a more integrated and coordinated surveillance strategy resulting from common concerns related to mutation and reassortment of influenza A virus gene segments and documented sporadic zoonotic episodes of human SIV infection. VS, in collaboration with the Centers for Disease Control and Prevention (CDC) and other stakeholders, initiated the development of a pilot SIV surveillance program via an interagency agreement (IAA) established in July 2008. This collaborative SIV surveillance effort was in the early stages of implementation in 2009.

A novel influenza A virus now labeled the pandemic H1N1 2009 virus, was identified in human cases of influenza in North America in early 2009. Subsequently this virus spread globally in human populations and was declared a human pandemic by the World Health Organization (WHO) in June 2009. This event heightened public health interest in surveillance for influenza viruses in multiple species, including pigs. As a result the pilot SIV surveillance project was quickly modified to include surveillance for pH1N1 (2009).

1.1 Disease Description

SIV is a respiratory disease of swine caused by a type A influenza virus. SIV is commonly found in swine herds in North and South America, Asia, and Europe. Endemic SIV of the H1N1 subtype has been circulating in U.S. swine populations for over 75 years. SIV has evolved from a seasonal disease caused by a single, relatively stable genotype to an endemic year-round respiratory disease caused by multiple SIV genotypes (H1N1, H1N2, and H3N2 subtypes) of multiple species origins (pigs, humans, and avian).

Documented accounts of pandemic H1N1 2009 virus outbreaks and experimental infections of swine describe symptoms, morbidity, and mortality similar to the endemic strains of H1N1 influenza currently circulating in U.S. swine. Thus, this plan does not differentiate surveillance objectives or activities for endemic SIV from pH1N1 (2009).

See the attachment on page 17 of this document for detailed background information on swine influenza viruses in swine.

1.2 Rationale and Purpose for Surveillance

Rationale for surveillance

SIV in swine is not a reportable or regulated disease; however, the disease has had an increasing economic impact on the swine industry in recent years. New subtypes or strains of SIV that result from viral genetic shift or drift can result in an increased threat to animal health. Scattered reports of human SIV infection have also escalated concerns over its zoonotic potential.

Emerging SIV variants that may be a health threat can be identified by monitoring changes in circulating swine influenza virus strains. This knowledge allows animal and human health experts to update diagnostic tests and diagnostic reagents, anticipate vaccine component requirements, and develop response plans (if necessary).

Furthermore, under some circumstances, an influenza virus in swine may qualify as an emerging disease, as defined by the World Organization for Animal Health (OIE). An official OIE notification of infection in swine or other species is specified in the OIE Terrestrial Animal Health Code to meet world animal health reporting requirements.

Human infection with a novel influenza A virus is a human notifiable condition based on its pandemic potential (International Health Regulations 2005, Council of State and Territorial Epidemiologists List of National Notifiable Conditions). Novel influenza A viruses are defined as viruses isolated from humans that cannot be sub-typed by standard human diagnostic methods. Thus, by definition, novel human flu viruses likely originate from animals such as birds or pigs. Public health officials are very interested in investigating the epidemiological origin of human influenza infections.

Purpose of surveillance

Some information on SIV in swine is currently available, but this information is not centralized, contains significant gaps, and cannot be easily aggregated for analysis. This surveillance plan describes standardized national methods to collect, manage, and analyze epidemiological and genomic data to assess current swine influenza viruses at the State level and monitor the evolution of these viruses over time. Information collected will be used to assess the SIV status in U.S. swine and take appropriate precautions to protect livestock and potentially other species.

1.3 Surveillance Objectives

The objectives of this surveillance program are to:

1. Monitor genetic evolution of endemic SIV to better understand endemic and emerging influenza virus ecology;
2. Make available SIV isolates for research and to 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.

1.4 Expected Outcomes: Products, Decisions, and Actions

Short-term expected outcomes include the establishment of State-level baseline data for endemic and emerging SIV genomics in U.S. swine. This information will be used to:

- Make timely, informed, and scientific decisions about disease control and prevention measures, human health implications, and trade issues; and
- Assist researchers and the animal health industry in developing relevant diagnostic reagents, targeted swine influenza diagnostic assays, and effective vaccines.

Any swine investigations related to suspected SIV zoonotic transmissions will be coordinated through the VS and State cooperative field structure.

Long-term outcomes may include:

- Additional influenza A surveillance information in broader initiatives such as VS 2015 Surveillance for Action and One Health activities and the American Veterinary Medical Association’s (AVMA) One Health Initiative;
- Further research and understanding regarding the ecology and epidemiology of swine influenza infection; and
- A better understanding of epidemiological factors and procedures that either limit or enhance the mutation and spread of SIV in the swine population.

1.5 Stakeholders and Responsible Parties

Stakeholders in SIV surveillance include industry representatives and individuals responsible for designing, implementing, managing, and/or disseminating information. Specific parties that may have an interest in this surveillance plan include:

Stakeholders	Interest
National Pork Board (NPB)	Industry scientific issues
National Pork Producers Council (NPPC)	Industry policy issues
State pork associations	State-specific industry issues
Pork producers	Animal health and production issues
State Veterinarians	State regulatory control programs
American Association of Swine Veterinarians (AASV)	Private swine veterinarian/practitioner issues
Swine practitioners	Herd health
Commercial companies	Manufacture and sales of SIV vaccines, commercial reagents and assays in the U.S.
Health and Human Services (HHS)-CDC	Human health interface issues

State and local public health departments	Novel SIV infections in their jurisdictions
Public health groups	Role of SIV in zoonotic threats
Department of Homeland Security (DHS)	Pandemic preparedness
USDA-APHIS-VS	Protecting animal health and potential trade implications

Responsible Parties	Responsibility
<p>USDA-APHIS-VS</p> <ul style="list-style-type: none"> National Animal Health Policy and Programs <ul style="list-style-type: none"> • National Center for Animal Health Programs (NCAHP) • National Center for Import and Export (NCIE) Eastern and Western Regions: Directors, swine epidemiologists, and area veterinarians-in-charge (AVICs) and field staff Centers for Epidemiology and Animal Health (CEAH) <ul style="list-style-type: none"> • National Surveillance Unit (NSU) • Center for Animal Health Information and Analysis (CAHIA) Office of the Chief Information Officer (OCIO) National Veterinary Services Laboratories (NVSL) National Animal Health Laboratory Network (NAHLN) Center for Veterinary Biologics 	<ul style="list-style-type: none"> Cooperative data sharing <ul style="list-style-type: none"> • Policy, budget and implementation • Import, export and international health status management • Field implementation and reporting • Development, evaluation, and revision of the surveillance plan; data analysis • Risk-based analysis • Spatial analysis • IT systems for SIV surveillance information management • Diagnostic laboratory support; reference laboratory services and budgeting; sample testing and data reporting • Sample testing and data reporting • SIV vaccine and commercial reagent licensing and testing
National Animal Disease Center, Agriculture Research Service (NADC-ARS)	<ul style="list-style-type: none"> • Genome sequencing; studies in pigs; data reporting
Selected diagnostic labs	<ul style="list-style-type: none"> • Case identification and sample submission
State Veterinarians	<ul style="list-style-type: none"> • Jointly responsible with AVIC for field implementation of SIV Procedures Manual
CDC	<ul style="list-style-type: none"> • Human–swine interface case identification and funding • Cooperative data sharing

Population Description and Sampling Methods

1.6 Population Definitions

The surveillance program will target the following swine populations:

1. **Case-compatible swine accessions submitted to veterinary diagnostic laboratories.** This surveillance will cover on-farm swine populations exhibiting ILI. Producers, veterinarians, or other personnel who observe pigs exhibiting ILI on farms should collect and submit nasal swabs from live sick pigs, or lung tissues from pig mortalities for SIV testing.
2. **Swine exhibiting ILI at first points of concentration or commingling events such as auctions, markets, fairs, or other swine exhibition events.** This surveillance targets primarily small farm and backyard herds moving pigs to sites with an increased potential for disease spread and/or elevated human exposure. Animal health officials or licensed veterinarians that observe pigs with ILI at these events should be aware of influenza virus in pigs and its zoonotic potential. Groups of swine exhibiting ILI should be examined and appropriate samples should be submitted to a veterinary diagnostic lab participating in the SIV surveillance program.
3. **Swine populations epidemiologically linked to a confirmed isolation of SIV in a human.** Animal health officials, in cooperation with public health investigators, may collect samples from swine that are thought to be linked with a human influenza A infection (including the pandemic H1N1 2009 virus). The extent of swine sampling will be determined on a case-by-case basis and performed in cooperation with the licensed veterinarian having a valid client-patient relationship with the owner/operation (if applicable).

Sampling of feral swine may be performed over a defined time to assess the role of feral swine in the maintenance and emergence of influenza viruses in domestic swine populations.

1.7 Case Definitions

Test eligible case:

- A pig or swine herd exhibiting clinical signs consistent with influenza infection; *OR*
- A pig or swine herd epidemiologically linked to positive human cases (with or without ILI in swine); *OR*
- A pig or swine herd epidemiologically linked to confirmed influenza virus-infected herds of interest.

Presumptive positive case:

A pig or swine herd that meets test eligible criteria AND tests positive using the NVSL/NAHLN-approved matrix polymerase chain reaction (PCR).

Confirmed positive case:

- A presumptive positive case *that* has successfully undergone virus isolation with isolate confirmed as influenza A.

Additional specific criteria for a confirmed pandemic H1N1 2009 case:

- A presumptive positive case in which confirmatory testing occurs at NVSL with isolation of a virus and the isolate confirmed as pandemic H1N1 2009 virus based on genomic sequencing.

1.8 Data Sources and 1.9 Sampling Methods for each surveillance stream

Following are stream-specific data sources and sampling methods:

A. Case-compatible swine accessions submitted to veterinary diagnostic laboratories

Data Sources

Producers, veterinarians, or other personnel may submit samples from pigs exhibiting ILI for influenza diagnostic testing to participating veterinary diagnostic labs. This may be part of a diagnostic rule-out for respiratory disease.

Two data entry protocols exist for swine accessions tested for SIV. Submission information from qualifying swine accessions will be entered either as anonymous SIV surveillance or traceable SIV surveillance:

Anonymous data submission protocol: Data entered under the anonymous protocol only provide enough information to identify the State of origin. All swine submissions (containing appropriate nasal swabs or lung tissue sent to participating NAHLN diagnostic laboratories) will be entered under the *anonymous* surveillance protocol unless *the* laboratory has written owner consent that data will be entered in the traceable protocol.

Traceable data submission protocol: Data entered under the traceable protocol provides enough information to allowing premises and submitting veterinarian or producer identity. The swine herd owner *must designate* in writing that *samples are* to be submitted through the *traceable* option of this sampling stream.

Details of data element requirements for *anonymous* and *traceable* surveillance protocols are fully described in the SIV procedure manual.

Sampling Methods

Producers, veterinarians, or other personnel, including persons under the direct supervision of a licensed veterinarian, may collect and submit nasal swabs and/or lung tissue from swine meeting a test eligible case definition described under section 1.7 of this document. Animals to be sampled should be in the acute phase of the disease, febrile with serous nasal discharge and cough. Up to 10 case-compatible animals may be sampled per epidemiological unit. The samples should be submitted to a participating veterinary diagnostic laboratory according to protocols detailed in the SIV procedure manual.

B. Targeted surveillance of sick pigs at first points of concentration or comingling events

Data Sources

Veterinarians who observe resident pigs displaying ILI at swine events (e.g., fairs, expos, etc), markets, auctions or zoos should report the findings to the appropriate State animal health officials. The attending veterinarian, in cooperation with regulatory officials, should submit samples from these animals for SIV diagnostic testing to participating veterinary diagnostic labs for analysis per established protocol. A detailed description of epidemiological data to be provided by submitting veterinarians is found in the SIV procedure manual. Samples submitted under this stream will be entered under the anonymous protocol unless the owner of the swine designates traceable data entry.

Sampling Methods

Animal health officials may collect nasal swabs and/or lung from swine meeting test eligible case definition as described under section 1.7 of this document. Sampled animals should be in the acute phase of the disease, febrile with serous nasal discharge and cough. Up to 10 case-compatible animals may be sampled per epidemiological unit. The samples should be submitted to a participating veterinary diagnostic laboratory according to protocols detailed in the SIV procedure manual.

C. Surveillance of swine populations epidemiologically linked to a human case of SIV

These cases are investigated on the farm only with the consent of the pig owner/producer.

Data Sources

Animal health officials cooperating with public health investigators will determine whether swine may be epidemiologically linked to human infections on a case-by-case basis. Any necessary on-farm investigations will occur with herd owner consent in cooperation with an attending licensed veterinarian with a valid client-patient relationship with the swine operation (if applicable). A detailed description of epidemiological data to be provided by submitting veterinarians is

found in the SIV procedure manual. Data from these investigations will be entered under the traceable surveillance protocol.

Sampling Methods

With the consent of the owner, and under the direction of the State Animal Health Official (SAHO), personnel may collect nasal swabs and/or lung from swine meeting test eligible case definition as described under section 1.7 of this document. Sampled animals should be in the acute phase of the disease, febrile with serous nasal discharge and cough. Up to 10 case-compatible animals may be sampled per epidemiological unit. The samples should be submitted to a participating veterinary diagnostic laboratory according to protocols detailed in the SIV procedure manual.

Confidence in detection of novel SIV

Introduction of a new SIV strain into an immunologically naïve herd is expected to result in at least a twofold increase in background morbidity. Assuming 95 percent sensitivity and 100 percent specificity of the prescribed swine influenza testing regimen, testing of 10 sick animals (febrile with serious nasal discharge and cough) provides 95 percent confidence in detecting SIV in at least one of the targeted samples, if the virus is present in at least *30 percent of the sick animals* in that the epidemiological unit.

Education and Outreach

VS and industry will work together to provide appropriate education/communication materials to producers, swine veterinarians, selected industry representatives, diagnostic labs, and State and Federal veterinarians. Pertinent topics to be included in the education campaign are: the importance and purpose of SIV surveillance; case definitions; submission procedures for samples; and the requested epidemiological information for each of the streams.

Analysis, Reporting and Presentation

1.10 Data Analysis, Reporting and Interpretation

Data Management

All SIV surveillance data generated will be managed by a VS animal health surveillance data management system. These data will include observational data, laboratory test results data, and epidemiological elements as described in the procedure manual (e.g., anonymous and traceable surveillance protocols for case compatible diagnostic lab accessions).

Data for SIV testing are currently being collected on NAHLN lab-specific spreadsheet templates and forwarded to the NAHLN coordinator for collation before forwarding to

NSU for analysis. The methodology is viewed as a temporary data management solution until data can be collected via a permanent APHIS data management system that meets data and security requirements.

Virus sequence data for the H, M, and N-genes will be obtained and deposited into *GenBank*. NVSL and/or the NAHLN testing laboratory will coordinate GenBank data entry for sequences obtained as more fully described in the procedures manual.

Surveillance Data Analysis and Interpretation

CEAH-NSU will be responsible for SIV surveillance data analysis, working in collaboration with all stakeholders. Analysis will be provided to VS management and all pertinent VS units. Information and selected samples will also be shared with the CDC (per the existing IAA) and with industry and other stakeholders as appropriate. Data release will meet all Federal privacy law requirements and appropriate VS policy statements.

An accession of one to 10 individual samples from discrete production sites is considered the appropriate epidemiological analytical unit. NSU will complete the following analyses, based on the availability of submitted epidemiological data:

- Summary reports of number of samples and accessions collected and subtypes isolated in each data stream with accompanying descriptive statistics;
- Surveillance system sensitivity analysis;
- Performance and evaluation of each data stream;
- Economic benefit/cost analysis.

Due to the potentially sensitive nature of data, protocols for assuring data confidentiality and security will be established. Confirmed stakeholder and management-approved summary surveillance data may be posted on the National Animal Health Surveillance System (NAHSS) Web site or other USDA websites for sharing with the public. Reports containing national-level summary data should also be included in the annual U.S. Animal Health Report to demonstrate the surveillance efforts.

1.11 Data Presentation and Reporting

Monthly State and national level surveillance reports will be generated to keep stakeholders informed of the influenza status in swine, including virus subtype frequency changes that may be occurring. Reporting may later move to a quarterly basis if appropriate for the national SIV situation.

Annual summary reports will be generated during normal reporting cycles. Annual reports will minimally include (as data are available):

- Number of surveillance accessions and samples collected versus the expected numbers described in section 1.14 of the surveillance plan;
- Analysis of problems or issues within the sampling stream;

- A summary of individual State submission data;
- Review of compiled summary data reported; *and*
- Evaluation of sample stream efficacy and identification of needed changes.

Implementation, Budget and Evaluation

1.12 Surveillance System Implementation: Priorities, Timelines, and Internal Communications

Priorities

- To monitor genetic changes of SIV isolates from ILI in pigs;
- To isolate viruses suitable for vaccine and/or diagnostic assay updating and for assay reagent development;
- To share isolates with CDC per IAA;
- To collect, analyze, and disseminate accessible geographical and temporal epidemiological data related to SIV positive cases and the genomic sequences of interest to animal and public health officials; and
- To determine the distribution of the pandemic H1N1 2009 virus in U.S. swine (short term objective).

Timelines

- This program was implemented in May 2009, as requested by the USDA Secretary of Agriculture.
- Revisions to the procedures associated with case-compatible swine diagnostic laboratory accessions were made in the spring of 2010 with the goal of increasing sample numbers through anonymous sample submissions (e.g., samples identified only by the State of origin of the sample). A pilot study of influenza viruses in feral swine was initiated in 2010 to assess the role of feral swine in the maintenance of endemic influenza viruses and emergence of new influenza viruses in domestic swine populations.
- A formal evaluation of the procedural changes with the case-compatible swine diagnostic accession process will be performed 12 months after the implementation of the outreach effort. Further modifications may be considered at that time.

Communications

A SIV Pilot Working Group comprised of staff from NVSL, NCAHP, NAHLN, National Center for Animal Health Emergency Management, and NSU has been in existence since August 2008. This group will be expanded to include other VS staff as part of a swine health leadership group. The swine health leadership group will focus on the priorities identified above in final development of the surveillance plan, implementation activities, and addressing stakeholder concerns. Surveillance plan updates may arise from these discussions to address concerns. All proposed changes to the surveillance plan will be reviewed by the swine health leadership group.

The details of communication and reporting pathways of surveillance analysis results will be outlined in the implementation plan.

1.13 Resources and Budget

Detailed funding allocations were developed when funding was provided to APHIS through a one-time HHS funding allocation. Available funds from the CDC-funded pilot SIV surveillance project were utilized per the IAA prior to this allocation. *Importantly, APHIS has provided large amounts of in-kind funding (personnel, equipment, etc.) from multiple units* as this effort has unfolded. Blanket purchase or cooperative agreements with NAHLN labs and their associated diagnostic labs for submissions related to these criteria/case definitions are in place.

1.14 Surveillance Plan Performance Metrics

Laboratory-based surveillance relies on observation and reporting of clinical signs by an owner, producer, veterinarian, other animal health official (animal identification coordinator, wildlife biologist, game warden, etc.) or a participant in the animal industry (livestock hauler, market and slaughter plant personnel, etc). To be most effective, submission of sick pig samples should represent all swine production areas of the United States.

The USDA NASS 2007 Agricultural Census estimates 66,000 swine operations. A total sampling pool of 79,200 groups (i.e., epidemiological units) (120 percent of NASS estimate) is estimated, given that some farms involve multiple production sites for sampling. An estimated 50 percent incidence of respiratory illness (clinical signs) over a one year period is likely among these units. At 50 percent identification-participation, approximately 198,000 sample submissions (assuming 10 samples per group) are calculated for initial Matrix PCR screening from 19,800 sites.

Protocol is to further characterize two samples from influenza PCR matrix positive sets of 10. Assuming that 75 percent of the groups tested are matrix positive (1 or more of 10 samples positive), approximately 29,700 samples are expected for further testing to determine specific virus isolate identification.

[For budgeting purposes the above sampling volume analysis is assumed to include submissions from all sampling streams. Evaluation of actual sample submission and results of Matrix PCR screening will allow for greater accuracy in predicting future sample testing volumes.]

The targeted surveillance of sick pigs at livestock markets, swine exhibitions and fairs will be implemented in as many locales as possible with existing Federal and State personnel and livestock market and exhibition veterinarians.

The following metrics will be evaluated to assess the surveillance program and its ability to meet objectives stated in the plan. Initially these evaluations will be conducted

monthly; as the situation changes, reports will be provided on an appropriate timeline.

Metrics include:

- Evaluation of the numbers of samples and sample accessions submitted to NVSL through NAHLN labs;
- Evaluation of the reasons for sample submissions into the influenza surveillance program;
- Evaluation of isolates shared with CDC;
- Evaluation of isolate subtyping and sequencing results, including study in pigs by USDA's Agricultural Research Service-National Animal Disease Center, if applicable; and
- Evaluation of communications between NAHLN, NVSL, NADC, VS, and CDC and the swine industry.

1.15 Surveillance System Evaluation

In addition to the performance metrics identified above, surveillance will be evaluated for overall effectiveness in meeting the plan's outlined objectives and goals. CEAH-NSU personnel, in collaboration with the Swine Health Leadership Group, will assess implementation progress, actual obtained sample numbers, budgets, applicability of performance metrics, and attainment of stakeholder goals. Modifications to the plan will be made as necessary in consultations with stakeholders of the SIV Surveillance Database.

Attachment: Background Information on Swine Influenza Viruses and Pandemic H1N1 Outbreak Virus

SIV is the cause of an infectious respiratory disease of swine. Infection of pigs with SIV occurs throughout the world. It is commonly found in North and South America, Asia, and Europe, and has been reported in Africa. In the United States, SIV was first recognized in 1918 as a swine disease in Western Illinois; however, the virus was not isolated from swine until 1930. Since its identification in 1930, SIV has evolved from a seasonal disease caused by a single, relatively stable genotype to a year round, endemic respiratory disease caused by multiple SIV genotypes (Gramer, 2005).

Although SIV is not a notifiable disease to the OIE, it has a major economic impact on the swine industry in the United States as part of the swine respiratory disease complex. Furthermore, reassortant SIV is of increasing zoonotic concern due to its proclivity to exchange of genetic material with influenza viruses of other species, particularly human and avian hosts (genetic shift), and to rapidly mutate (genetic drift).

Etiology of swine influenza

Swine influenza viruses are classified as members of the family *Orthomyxoviridae*, genera *Influenzavirus A* (Type A). They are enveloped and have single stranded negative sense RNA with 8 genomic segments that allow for reassortment and production of novel virus mutants. The type designation (A, B, or C) is based upon the antigenic character of the virus envelope and the nucleoprotein within the virus particle. Type A influenza viruses are further divided into subtypes based on two surface glycoprotein antigens: hemagglutinin (HA) and neuraminidase (NA). Sixteen HA (H1-H16) and nine NA (N1-N9) antigens have been identified. Type A subtypes are designated according to their unique HA and NA surface antigen combinations. The most common subtypes found in North America are H1N1, H1N2 and H3N2 (Choi et al 2002; Webby et al 2004). Recently subtypes H3N1 and H2N3 have been identified (Lekcharoensuk et al, 2006; Ma, 2007).

Enveloped viruses such as SIV are susceptible to heating, drying, and chemical disinfectants that contain lipid solvents. Avian influenza A viruses have been shown to be stable for up to 4 weeks in water at 4 °C, and up to 5 days in water at 20 °C.

Background information regarding the pandemic H1N1 2009 virus outbreak

On April 17, 2009, cases of febrile respiratory illness in two children residing in adjacent counties in southern California were diagnosed with a novel influenza A (H1N1) virus infection by CDC. The viruses from the two cases were closely related genetically and thought to be of swine origin.

Neither of the initial cases had contact with pigs, and the infection source was unknown. Investigations were initiated in California to identify the source of infection and to determine whether additional people were ill from infection with similar influenza viruses. Investigations rapidly expanded to Texas and across the United States as additional human cases were found. Human infection with this novel H1N1 virus was eventually confirmed worldwide. The WHO has designated the virus as pandemic H1N1 2009 virus.

Clinical signs related to swine flu viruses

In swine, the disease course, nature, and severity of illness will vary with the strain and/or isolate of the virus, the age and immune status of the pig, the presence of concurrent viral infections, and whether the SIV infection is complicated by secondary infection. While often low or inapparent, morbidity can reach 100 percent, and mortality in confirmed cases can range from 1-3 percent in the absence of complications (AVMA 2007). Anecdotally, sporadic outbreaks with higher mortality have recently been reported.

Classical SIV clinical infection of swine presents as an acute upper respiratory disease. The incubation period is 1-3 days. Breeding animals that have been infected with influenza acquire active immunity to the virus and remain largely unaffected by subsequent re-infection by sufficiently homologous strains.

Respiratory signs include coughing (barking), nasal and/or ocular discharge, sneezing, and dyspnea. Hyperthermia in excess of 105 degrees F may be observed with associated anorexia, weight loss, lethargy, prostration, huddling and piling. Most pigs recover within 5-7 days in the absence of complications. Complicated infections can extend recovery times and dramatically increase the mortality rate. Boars and sows may experience impaired reproductive performance following infection. Reduced fertility in boars is a result of decreased semen quality and output. Sows may exhibit delayed return to estrus, abortion, or decreased litter size and viability of piglets depending on the stage of gestation when infected. Aborting sows are usually anorexic for 2 to 3 days and may have a fever up to 105 degrees F. Lactating sows may have reduced milk production resulting in adverse affects in nursing piglets.

Research studies at ARS and elsewhere and clinical reports indicate that swine infected with the pH1N1 (2009) virus are clinically indistinguishable from swine infected with endemic viruses (Vincent 2009). Clinical signs of SIV are not strain-specific, requiring advanced diagnostic techniques (PCR, gene sequencing) to differentiate infecting viruses.

Epidemiology of swine flu viruses

Endemic herds may be asymptomatic with sporadic outbreaks, usually during cooler months. Sows in herds with endemic SIV may have sporadic abortions and low conception rates. Epidemic SIV follows a seasonal rhythm, peaking during periods of the greatest environmental stress to the pigs. Disease onset is typically precipitated during periods of heat or cold stress. These phases can be related to the fall and winter months in the Midwest, and the late summer months in the South and Southeast. In the epidemic

form, infection is apparent in all age groups. Disease onset is acute and dramatic. Normal animals can become very sick within hours. The virus is primarily excreted through nasal secretions during the acute febrile stage of the disease. Pigs can begin shedding the virus within 24 hours of infection and may continue to shed for up to 10 days. In immunologically naïve herds, abortion rates can be widespread and reach as high as 10 percent very quickly. Abortion storms are characterized by high fevers, sows off feed, abortions, coughing, and death. The SIV-induced abortion storms pass in less than two weeks.

Management systems, husbandry procedures, and lax biosecurity practices can result in the introduction of SIV into a herd. Generally, introduction occurs when new stock or infected animals are moved or mixed into a herd. In production herds, the virus may persist through the infection of susceptible young pigs, no longer protected through maternal antibodies. Influenza viruses are spread easily by people and contaminated equipment moving between infected and non-infected herds.

Exposure to wildlife, shore birds and waterfowl, especially ducks, presents additional opportunities for the introduction of an influenza virus into a swine herd. Infected birds shed the virus through feces, sometimes for extended periods of time. Fecal contamination of drinking water sources, or lagoons used for wash-down procedures can inadvertently result in exposure. Since 1998, reassortants of SIV have included avian genes. Avian origin viruses (subtypes H4N6, H3N3, and H1N1) have also been found in swine populations (Widjaja 2004; Olsen 2003; Suarez 2002; Castrucci 1992; Webster 1992; Karasin 2000 and 2004).

There is anecdotal evidence for area spread of SIV infections; however, definitive epidemiological studies are lacking. Influenza viruses can be spread through both direct and indirect routes. Direct nose-to-nose contact can result in primary disease transmission within the herd, or from exposure to feral animals if the management system allows. Indirect transmission may occur through the inhalation of infected droplets that can be propelled short distances through coughing, the inhalation of aerosolized virus (dried droplet nuclei) over longer distances, shared feed and water, or through inanimate objects or fomites such as people, contaminated equipment, or vehicles.

Once an influenza virus is established in a herd, it is able to replicate and undergo genetic drift and reassortment regardless of its origin. If genetic changes result in the generation of an antigenically different subtype, then the herd may be susceptible to the new subtype despite vaccination or immune status.

Ecology of animal influenza viruses

Aquatic birds, primarily waterfowl, shore birds, and golden terns, are a natural reservoir for all influenza type A infections. However, these viruses can infect a variety of mammals and birds, with many species serving as amplification hosts, including swine and humans.

Type A viruses are constantly undergoing small antigenic modifications as the result of point mutations in their genetic makeup. The segmented genome gives the viruses the inherent ability to exchange genetic material with other viruses through reassortment. The resulting reassortant viruses can pose a threat to swine health if the acquired genetic modifications lead to an antigenic change in the virus such that the existing immunity of swine populations is ineffective (Gramer 2006). As a result, endemic SIV can continually infect susceptible pigs, resulting in acute respiratory disease, poor growth performance, and mortality, despite vaccination.

Prior to 1998, influenza in U.S. pigs was caused by one predominant circulating virus known as classical swine H1N1. In 1998, new reassortant H3N2 strains of SIV were identified in several swine populations across the United States. These new strains were either double reassortants with genetic material from swine and human influenza strains, or triple reassortants of avian, human, or swine influenza strains (Gramer 2005). Since 1998, novel viruses with varying origins and combinations of genetic components of H1N1 and H3N2, now endemic in the United States, have emerged.

Swine support replication of both avian and human influenza viruses. This unique characteristic has labeled swine as a re-assortment (“mixing”) vessel for avian, human, and swine influenza subtypes, and thus a potential origin of a novel reassortant virus that may trigger a pandemic human influenza outbreak. However, recent studies indicate that other species, including humans, have receptor cells for both avian and mammalian influenza viruses in their respiratory tracts (Sjouke 2010). So swine are not uniquely at risk for dual avian-mammalian influenza infections.

Although virus transmission across species is rare, avian to pig and pig to avian transmissions of type A viruses have been documented. Serologic studies have shown that turkeys can carry antibodies to classical swine H1N1 (i.e., the predominant swine flu virus prior to 1998). Swine H1N1, H1N2, and more recently, H3N2, have been isolated from turkeys (Suarez 2002; Choi et al 2004). Genetic analysis of H1N1 viruses in turkeys and pigs shows a high degree of genetic exchange between the two species.

Swine influenza viruses are potentially zoonotic. There is a considerable amount of evidence establishing the bidirectional exchange of viruses between pigs and humans. In 1976, the zoonotic nature of swine H1N1 influenza viruses was confirmed when influenza viruses isolated from humans were found to be antigenically and genetically identical to an H1N1 SIV isolated from a recent swine influenza outbreak. Since that time, there have been multiple reports from North America of SIV subtypes being isolated from humans with ILI.

Five human cases of SIV in 2007 were in contact with swine displaying clinical signs of upper respiratory disease in public event settings with human-swine interactions. Both the people and the swine subsequently displayed signs of influenza infection. Sustainable transmission among people did not occur in any of these cases. Surface antigen testing identified swine influenza H1N1 virus in all cases. However, genetic sequencing

revealed triple reassortant isolates with swine, human, and avian components, similar to the predominant SIV circulating in pigs in North America (CDC communication).

Swine have been identified as a major intermediate host where avian influenza strains have adapted for replication in humans (Webby 2006). Currently, human-swine reassortant H1N2 and H1N1 influenza viruses are circulating in pigs throughout the United States (Gramer 2008). Recent findings have shown that new or novel reassortant influenza viruses have been transmitted between humans and pigs (Webby 2007, CDC 2007).

Methods for control of endemic swine flu viruses

Control of SIV related disease is handled through management practices, biosecurity measures, and vaccination programs. There are no cost-effective therapies, although antibiotics may be used for secondary infections. In non-infected herds, prevention is focused on maintaining a closed herd and incorporating good biosecurity procedures. On infected premises, an all-in, all-out production system can be used to keep age or weight-matched groups together throughout the production process, with cleaning and disinfection of facilities between groups. This method minimizes the potential for direct SIV transmission between groups of animals, and indirect transmission from equipment and the environment.

Preventive vaccination programs for SIV are commonly incorporated into management practices to produce active immunity and either reduce the risk of SIV entering into the herd or protect against respiratory disease associated with endemic SIV. Vaccination does not provide complete protection; however, it reduces viral shedding and lessens the severity of the infection. Vaccinated sows will confer some passive immunity protection to piglets.

Commercially licensed and autogenous vaccines are available in the United States. USDA-licensed vaccines are available as killed monovalent or polyvalent products. They have been proven to be effective against both H1N1 and H3N2 SIV infection of pigs and sows (Erickson 2001).

For vaccination programs to be most effective, the vaccine must include the SIV subtypes likely to cause disease due to variable levels of cross protection between SIV subtypes. Vaccine manufacturers and producers are challenged by continued antigenic drift and shift in circulating SIV subtypes, rendering older influenza vaccine subtypes non-protective. The need for rapidly updated effective vaccines becomes more critical as new trivalent reassortants emerge and diverge. In response to this dilemma, USDA-APHIS' Centers for Veterinary Biologics (CVB) recently issued guidance to currently licensed SIV vaccine firms, allowing for expedited regulatory procedures for the update of SIV strains in current USDA licensed veterinary vaccines (USDA VS 2007).

Diagnosis of swine influenza

Diagnosis can be based on a combination of clinical signs, typical gross and histopathologic lesions, and diagnostic tests that include serology, virus isolation and

nucleic acid or antigen-based tests. A definitive diagnosis requires detection of virus, virus nucleic acid, or viral antigens in the tissues or secretions of a clinically infected animal.

Postmortem gross lesions in uncomplicated cases of SIV are those of viral pneumonia. Lesions are usually limited to the apical and cardiac lobes of the lungs. Altered lung tissue is consolidated and darkly colored. The airways are likely to be distended and filled with blood-tinged fibrinous exudates. Associated mediastinal and bronchial lymph nodes are usually enlarged. Severe cases may involve more than one half of the lung tissue.

Diagnostic tests can be run on antemortem nasal swabs or on postmortem lung tissue from acutely infected animals. Maternal antibodies in piglets can complicate the diagnosis of disease by either inhibiting active piglet antibody production, or by suppressing virus production and odds of isolation.

Virus identification is best accomplished by collection of samples within 24-48 hours after development of clinical signs. Acute animals will be febrile and exhibiting a cough with serous nasal discharge.

Diagnostic methods for viral detection include virus isolation, antigen detection by immunoassay and molecular based assays such as RT-PCR and partial or full genomic sequencing. Virus isolation with SIV is challenging and requires critical timing for sample collection since the virus is only shed for about 3-5 days following infection. RT-PCR assays show excellent specificity, good sensitivity and can differentiate subtypes. However, full genomic sequencing is necessary to detect more subtle changes in the SIV genome (Personal Communication Amy Vincent 2008).

Detection of serum antibodies is a common method used to diagnose infection. Currently hemagglutination inhibition (HI) testing is the most common method used to diagnose SIV. An advantage of the HI assay is that it can discriminate between different subtypes and antigenic variants within a subtype. However, it is restricted to known subtypes, so its diagnostic value diminishes with the emergence of novel reassortant subtypes (Long et al 2004; Wu 2006; Janke 2000). Two commercial ELISA tests have been licensed for both H1N1 and H3N2 subtypes, but their ability to differentiate antibodies against different isolates has not been well documented. Newer ELISA antibody tests have been developed that can differentiate between virus exposure and vaccination (Wu 2006) but are not commercially available. There can be some cross reactivity with H1N1 and H3N2 SIV in some tests, such as ELISA or indirect immunofluorescence assay (IFA), because Type A influenza viruses share a common matrix protein and nucleoprotein. Serum neutralization (SN) assays measure the level of antibodies that are capable of neutralizing the virus however this test is labor intensive, virus specific, and is not generally used for routine diagnostic screening.

Possible zoonotic relevance

The United States has encountered several recent SIV events of possible zoonotic relevance. These include:

- (1) The current events surrounding the pandemic H1N1 2009 virus causing human clinical illness globally (described above). This unique reassortant virus contains genes of swine, avian and human lineage, and components originate from multiple continents (North America and Euro-Asia);
- (2) Triple reassortant (containing genes of avian, porcine, and human flu virus origin) swine influenza A (H1N1) infections and disease in humans (2007-2009) in Iowa, Illinois, Ohio, Texas, and South Dakota. Concurrent swine and human infections with an identical triple reassortant SIV genome were reported in Ohio in 2007; and
- (3) The first isolation and identification of an avian and swine virus reassortant H2N3 influenza A virus causing infection and disease in swine in the U.S. in 2006. This isolate was a cause for public health concern because the swine H2N3 virus was infectious and highly transmissible in swine and ferrets under experimental conditions. This strain has not been subsequently identified in either swine or humans and likely died out.

It is important to note that the increased detection of SIV infections in humans may reflect a real increase in human infections, or it could be the result of enhanced diagnostic capabilities at State public health laboratories. In June 2007, the Council for State and Territorial Epidemiologists (CSTE) reporting requirements were amended to make human infection with novel influenza A viruses, including SIV, a nationally notifiable condition.

Dr. Amy Vincent states the following in a 2008 American Association of Swine Veterinarians (AASV) paper: “Reassortant viruses arising from the mixing of genes from swine, human, and avian influenza viruses in the swine host have epidemic potential in the pig population and may have pandemic potential in the human population. This underscores the importance of limiting the introduction of new influenza viruses to the swine population and *monitoring for newly emerging viruses* (emphasis added)” (Vincent, 2008).

Dr. Marie Gramer states in her 2008 AASV paper: “It is important to note that most influenza viruses isolated from US swine are triple reassortant viruses, containing genes of avian, human and swine influenza virus origin. These triple reassortant influenza viruses of swine are postulated to have an increased affinity for reassortment. Furthermore, the presence of multiple lineages of influenza viruses circulating concurrently sets the stage for virus variation and the appearance of new strains, *reinforcing the need for regular subtyping, serotyping and genetic sequencing* (Gramer 2008).

Currently, veterinarians send samples from swine with clinical signs that are consistent with SIV to USDA-ARS, CDC, universities (including National Institute of Health Centers of Excellence) and NVSL. Due to intellectual property issues and lack of coordination among these entities, there is inconsistent communication among these entities that results in:

- Limited sharing of knowledge of these SIV detection events in swine;
- Unknown incidence of SIV infections in swine in the United States;
- Unknown animal or public health relevance of isolates; and
- Little context for evaluating the significance of genomic changes in SIV.

The U.S. pork industry supports an estimated 550,200 domestic jobs, generates more than \$97.4 billion annually in total U.S. economic activity, and contributes \$34.5 billion to the U.S. gross national product. In 2006, the United States exported 1,262,499 metric tons of pork valued at \$2.864 billion. Pork exports depend directly on open and transparent disclosure of the commercial segment's disease status to all trading partners.

A mutating zoonotic reassortant swine influenza virus is a potential risk to this vital industry for several reasons:

- Potential for pandemic human illness;
- Infections in the pig population can cause severe illness and death loss;
- Farm workers and their families may be at risk for transmitting influenza infection to or from the pigs for which they care. Caretaker attendance and animal care compliance could fall precipitously in the wake of the threat of an influenza pandemic;
- Neighboring communities may be put at risk for pandemic influenza infection by proximity to sick pigs and / or infected swine workers;
- Demand for pork domestically may drop significantly in the wake of public loss of confidence in its safety in the wake of a zoonotic swine influenza outbreak; and
- With 15 percent to as much as 25 percent of the current pork supply merchandised in export markets, the U.S. swine industry risks huge financial losses should trading partners impose bans or additional restrictions.

These findings have increased interest by VS, ARS, and CDC in establishing a formal surveillance process for selected influenza events and shared virus analysis in a timely and structured manner. This process will monitor occurrences and encourage viral genetic analysis, assisting in understanding the swine (animal) and human health epidemiology and relevance of selected SIV events.

VS-NVSL has been collaborating with CDC on these recent swine and human SIV detection events, but a more coordinated awareness and information sharing process with veterinary diagnostic labs is needed to better detect and monitor changes in SIV in U.S. swine populations.

References

- American Veterinary Medical Association (2007). Swine influenza background. (Accessed May 2, 2008). http://www.avma.org/public_health/influenza/swine_bgnd.asp
- Brown, I. H. (2000). The epidemiology and evolution of influenza viruses in pigs. *Vet. Microbiol* 74:29–46.
- Castrucci, M. R., I. Donatelli, L. Sidoli, G. Barigazzi, Y. Kawaoka, and R. G. Webster. (1992). Genetic reassortment between avian and human influenza A viruses in Italian pigs. *Virology* 193:503–506.
- Center for Disease Control and Prevention (December 17, 2007). Update: Influenza Activity — United States, September 30–December 1, 2007. *MMWR* (56) 49: 7-11.
- Choi YK, Goyal SM, Joo HS. (2002) Prevalence of swine influenza virus subtypes on swine farms in the United States. *Arch Virol*;147:1209–20.
- Choi YK, Lee JH, Erickson G, et al. (2004) H3N2 Influenza virus transmission from swine to turkeys, United States. *Emerg Inf Dis*;10: 2156–2160.
- Council of State and Territorial Epidemiologists (CSTE), (2009) CSTE List of Nationally Notifiable Conditions
<http://www.cste.org/dnn/LinkClick.aspx?fileticket=7CCxM20JUGg%3d&tabid=36&mid=1496>
- Easterday, B.C. and K. Van Reeth, Swine influenza, in: B.E. Straw, S. D’Allaire, W.L. Mengeling, D.J. Taylor (Eds.), *Disease of Swine* (8th Edition), Iowa State University Press, Ames, (1999), pp. 277-290.
- Erickson, G. Oct 15, 2001 The Growing Role of Swine Influenza Virus. *National Hog Farmer* (accessed May 2, 2008, at http://nationalhogfarmer.com/mag/farming_growing_role_swine/)
- Gramer M. R. (2005). Defining swine influenza virus. *J Swine Health Prod* 13(3):157-158.
- Gramer, M.R. (2006) Reassortant human/swine H1N1 and H1N2 influenza virus infections in US swine. American Association of Swine Veterinarians Conference Proceedings pp. 463-464.
- Gramer, M.R. (2008) An update on swine influenza ecology and diagnostics. American Association of Swine Veterinarians Conference Proceedings pp. 543-547.
- Janke, B. Diagnosis of swine influenza. (2000). *Swine Health and Production* 8(2):79-84.

Karasin, A.I., I.H. Brown, S. Carman, and C.W. Olsen. (2000). Isolation and Characterization of H4N6 Avian Influenza Viruses from Pigs with Pneumonia in Canada. *J.of Virol* 74(19):9322–9327.

Karasin AI, K. West, S. Carman, and C.W. Olsen. (2004). Characterization of avian H3N3 and H1N1 influenza A viruses isolated from pigs in Canada. *J Clin Microbiol.* 42(9):4349-54.

Lekcharoensuk P., K. M. Lager, R. Vemulapalli, M. Woodruff, A. L.Vincent, and J. A. Richt . (2006). Novel swine influenza virus subtype H3N1, United States. *Emerging Infectious Diseases* 12(5) 787-794.

Long, B. C., T. L. Goldberg, S. L. Swenson, G. Erickson, and G. Scherba. (2004). Adaptation and limitations of established hemagglutination inhibition assays for the detection of porcine anti-swine influenza virus H1N2 antibodies. *J Vet Diagn Invest* 16:264–270.

Ma W., A.L. Vincent, M.R. Gramer, C.B. Brockwell, K.M. Lager, B.H. Janke P. C. Gauger, D.P. Patnayak, R.J. Webby and J.A. Richt. (2007). Identification of H2N3 influenza A viruses from swine in the United States. *Proc National Academies of Sc* 104(52):20949-20954.

Olsen CW, A. Karasin, and G. Erickson (2003) Characterization of a swine-like reassortant H1N2 influenza virus isolated from a wild duck in the United States. *Virus Res.*;93:115–21.

Schwartz, K. (Ed). (2004). *Influenza*. Pp 67-69) in *Swine Disease Manual*. American Association of Swine Veterinarians: Perry Iowa.

Sjouke G.M. Van Poucke, Nicholls J.M., Nauwynck H.J. **and** Van Reeth, K. (2010). Replication of avian, human and swine influenza viruses in porcine respiratory explants and association with sialic acid distribution. *Virology Journal* 7:38.

<http://www.virologyj.com/content/7/1/38>

Suarez DL, Woolcock PR, Bermudez AJ, Senne DA. (2002) Isolation from turkey breeder hens of a reassortant H1N2 influenza virus with swine, human and avian lineage genes. *Avian Dis.* 46:111–21.

Swenson, S.L. and G. A. Erickson. 1999. Swine Health Fact Sheet: Swine influenza virus. National Pork Producers Council and the American Association of Swine Practitioners. <http://www.pork.org/PorkScience/Documents/swineinfluenza.pdf>

USDA Veterinary Services Memorandum No. 800.111, September 15, 2007. *Viral Strain Changes in Equine Influenza and Swine Influenza Vaccines (Killed Virus)*

http://www.aphis.usda.gov/animal_health/vet_biologics/publications/memo_800_111.pdf

U.S. House of Representatives. *Statement of National Pork Producers Council*. March 20, 2007 [cited; Available from:

<http://waysandmeans.house.gov/hearings.asp?formmode=printfriendly&id=6076>

Vincent, A.L. (2008). Experimental evaluation of novel swine influenza viral subtypes. American Association of Swine Veterinarians Conference Proceedings pp. 549-551

Vincent, A.L., Swenson, S.L., Lager, K.M., Gauger, P.C., Loiacono, C., Zhang, Y., (2009), [Characterization of an influenza A virus isolated from pigs during an outbreak of respiratory disease in swine and people during a county fair in the United States](#). *Vet Microbiol* 137, 51-59.

Webby R.J. (2006). Swine influenza in the United States and its emergence as a zoonotic pathogen. American Association of Swine Veterinarians Conference Proceedings pp. 375-378.

Webby R.J., K. Rossow, G. Erickson, Y. Sims, and R. Webster. (2004) Multiple lineages of antigenically and genetically diverse influenza A virus co-circulate in the United States swine population. *Virus Res* 103(1-2):67–73.

Webby, R.J, S.L. Swenson, S.L. Krauss, P.J. Gerrish, S.M. Goyal, and R.G. Webster. (2000) Evolution of swine H3N2 influenza viruses in the United States. *Journal of Virology*. 74:8243–8251.

Webster R.G., W.J. Bean, O.T. Gorman, T.M. Chambers, Y. Kawaoka. 1992. Evolution and ecology of influenza A viruses. *Microbiol Rev* 56:152–179.

Widjaja, L, S.L. Krauss, R. J. Webby, T. Xie, and R.G. Webster (2004). Matrix Gene of Influenza A Viruses Isolated from Wild Aquatic Birds: Ecology and Emergence of Influenza A Viruses. *J. Virology* 78(16): 8771–8779.

World Health Organization, International Health Code Regulations (2005) second edition. http://whqlibdoc.who.int/publications/2008/9789241580410_eng.pdf

World Organization for Animal Health (OIE) Terrestrial Animal Health Code (2009), Chapter 1.1 Notification of diseases and epidemiological information, Article 1.1.3 http://www.oie.int/eng/normes/mcode/en_chapitre_1.1.1.htm

Wu, W-H., Kim W., Kim J-J., and Yoon, K-J. (2006). Improved serology testing for swine influenza virus (SIV). *Proc. Amer. Assoc. Swine Practitioners*. P.465-466.

Yoon, K-J. and B. H. Janke. Swine Influenza: Etiology, Epidemiology, and Diagnosis. In Morilla, A, K.Y. Woods, J.J. Zimmerman eds. *Trends in Emerging Viral Infections of Swine*. Iowa State University Press, 2002.