NVSL AVIAN INFLUENZA UPDATE

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ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
NATIONAL VETERINARY SERVICES LABORATORIES

NATIONAL POULTRY IMPROVEMENT PLAN GENERAL CONFERENCE COMMITTEE MEETING,
PORTLAND, MAINE MAY 18, 2017
AI Surveillance in the United States: National System

NPIP breeding flocks
- Egg and Meat-Type chickens
- Turkeys, Exhibition Poultry, Upland Game birds and Waterfowl

NPIP production flocks
- Meat-type chickens & turkeys
- Table Egg Layers
- Upland Game birds and Waterfowl

Live Bird Marketing System
- Producers, distributors, and retail markets
- Backyard flocks, auctions, swap meets, etc.

Other Diagnostics
- Passive surveillance, export testing, foreign animal disease investigations
- Wild bird surveillance

>2 million tests/year
AM H7N9 2017

• H7N9 HPAI and LPAI started in early March 2017, identified initially due to clinical signs
• The AM H7N9 is of North American wild bird lineage and not related to the Asian lineage H7N9
• HPAI and LPAI viruses are highly similar across the entire genome except for the insertion in the HA gene of the HPAI virus:
  – likely derived from chicken host 28S ribosomal RNA
• A closely related H7N9 LPAI virus was recovered from a Wildlife Services Wild Bird Surveillance sample collected from a blue-winged teal in Wyoming as part of a live bird banding effort during September 2016.
Timeline of detections

**Diagnostic challenges**

- Two important elements:
  - a) the HA-subtype, which is ‘presumptive’ based upon the NAHLN lab result and ‘confirmed’ based upon NVSL result, and
  - b) the virus pathotype, which is ‘presumptive’ based upon the clinical presentation of the flock compared to the USDA HPAI case definition, and ‘confirmed’ based upon the HA cleavage site sequence at NVSL.

<table>
<thead>
<tr>
<th>Test</th>
<th>IAV</th>
<th>HA subtype</th>
<th>NA subtype</th>
<th>pathotype</th>
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<tbody>
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<td>NPIP AI AGID/ELISA</td>
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<td>NVSL AI HI/NI</td>
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<td>NAHLN IAV-M PCR</td>
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<td>NAHLN H5/H7 PCR</td>
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<td>NVSL Sequencing</td>
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<td>NVSL chicken pathogenicity test</td>
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</table>

= test can determine

= test cannot determine
Overall findings: AM H7N9

• Molecular, epidemiologic, and serologic (antibody) data suggest the AM H7N9 virus circulated in the area undetected in poultry prior to the initial HPAI detection; and

• Molecular and epidemiologic support exists for
  – a) secondary spread from the first HPAI site to the second, and
  – b) more than one independent introduction of AM H7N9 LPAI, for example the available data show that LPAI/HPAI viruses from TN appear to have a common source and cluster separately from the AL viruses.
Recall H7N8 HPAI/LPAI
Indiana January 2016

- 10 Jan 16: meat turkeys w/↓ water consumption
- 14 Jan 16: preliminary +H7 rRT-PCR and depopulation initiated the NEXT DAY
- Wild bird virus identified with 5/8 genes in common – high similarity
- Molecular analysis supports epidemiologic data for likely location of LPAI introduction

Virology, Volume 507, July 2017, Pages 216-219
Dong-Hun Lee, Mia Kim Torchetti, Mary Lea Killian, David E. Swayne
North American H7 Lineage

The recent H7 viruses are North American lineage from Cluster III (H7N8 and H7N9)

Schematic phylogenetic tree of the HA1 nucleotide sequences of H7 AIVs (maximum-likelihood method) Boxes represent the three major genetic clusters; the Eurasian lineage (EU) is represented by the large black triangle. AM = North American lineage

*Courtesy of Xi-Feng Wan et al, Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University*

North American wild bird lineage viruses are not related to the Asian lineage H7N9
What can wild bird precursors tell us?

• Type and frequency of virus detection

• Distance to nearest wild bird relative helps with understanding timing of introduction

Fewer changes between a wild bird precursor and poultry virus suggests recent introduction with quick detection (e.g. AM H7N8)
H7N2 in Cats at NYC Shelter

Rare bird flu strain infects 45 cats in single Manhattan shelter and may have spread to recently adopted felines.
H5 events in US -2017

- March 3, 2017: **H5N2** LPAI of wild bird lineage positive result from a commercial turkey flock in Wisconsin
- April 10, 2017: **H5N2** LPAI virus in a primarily duck mixed species backyard flock in Idaho
- Both the HA and NA are distinct from the **EA/AM H5N2 clade 2.3.4.4** viruses from 2015
Rapid Response

• USDA APHIS Veterinary Services is committed to rapid depopulation where HPAI is suspected

• The National Animal Health Laboratory Network plays a crucial role; **depopulation can be initiated at the state level with the following:**
  – A non-negative H5 or H7 virus detection (and samples that have been forwarded to the NVSL for confirmation), and
  – A flock that meets the **case definition**, and
  – Agreement of State and Federal officials.

• **NOTE:** NVSL confirmation is required to determine subtype and pathotype for official disease declaration – forward non-negative samples ASAP

Refer to [HPAI Redbook](#) for further information
Fastest route for confirmation

Submit duplicate samples

• NVSL leverages the Ct from NAHLN Lab PCR to target samples for rapid subtype/pathotype by partial sequence where sufficient RNA is present
Special thanks to the Avian team and our partners!
**WI-AV-0020**

Avian Samples for PCR and virus isolation


**Chickens/turkeys**

Up to 5 OP swabs in 3mls VTM or 11 swabs in 5.5mls VTM*

**Domestic waterfowl**

Up to 5 CL swabs in 3mls VTM

If sampling free ranging waterfowl – 1 OP and 1 CL swab in SAME TUBE may be preferred (and is recommended for wild waterfowl)

Pool by:
- the same premises
- the same species
- the same sampling route
Alternate Sampling

• OP swabs recommended for routine detection
• For egg production drops (potential for swine lineage viruses) consider additional sampling routes
  – Cloacal swabs (CL)
  – Oviduct swabs
  – Semen

For egg production drops, collect up to 5 CL swabs in 3mls VTM

Up to 5 OP swabs in 3mls VTM or 11 swabs in 5.5mls VTM*
How many swabs / how much media?

<table>
<thead>
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<th>Purpose</th>
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<tbody>
<tr>
<td>Up to 5 swabs in 3 ml</td>
<td>FAD investigations where AI/ND are suspected and detection of bacterial diseases is needed (FADDs are supplied with this media)</td>
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Option to collect 5 swabs/3ml and pool samples at the lab for AI testing allowing ongoing use of such samples for detection of bacterial diseases *(refer to 3.1.2 and footnote #2 of the current version of NVSL WI-AV-0020)*
# How many swabs / how much media?

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<tbody>
<tr>
<td><strong>Up to 5 swabs in 3 ml</strong> <em>No abx</em></td>
<td>FAD investigations where AI/ND are suspected and detection of bacterial diseases is needed (FADDs are supplied with this media)</td>
</tr>
<tr>
<td><strong>Either 5 swabs in 3ml (any for domestic species) or up to 11 swabs in 5.5 ml with abx for TR/OP swabs from gallinaceous poultry</strong></td>
<td>10K surveillance around an infected premise</td>
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<td>Surveillance before a premise is positive</td>
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<td>Surveillance outside a 10K ring</td>
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<td></td>
<td>Surveillance after cleanup</td>
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</table>
Blood Collection for Antibody Detection

A minimum 0.5ml – 1.0ml needed for HI/NI (not to exceed 1% bw of bird – contact the NVSL for testing options for small birds)

• Serum/plasma/yolk
  – 0.2 ml each needed for HI and NI
• Vein (wing, jugular, leg): up to 10 ml depending on bird size; 22–27 ga, 0.5–1” needle
• Heart (anesthesia required): large volume; 18–20 ga, 1.5–2” needle

Important tips –

• To increase the volume of sera obtained immediately after collecting blood, lay the tube on its side to increase surface area exposure to air at room temperature
• To obtain better overall sample quality and reduce hemolysis, remove the sera from the clot prior to shipping

Blood collection video
(stop at 1:50sec)

Helpful links:
http://www.fao.org/docrep/005/ac802e/ac802e0a.htm