NVSL UPDATE

AVIAN INFLUENZA AND NEWCASTLE DISEASE

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VETERINARY SERVICES
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US Poultry Surveillance

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
Approved and Authorized Labs

For avian influenza:

~100 NPIP-authorized labs provide routine surveillance testing (serology, ACIA, and PCR where approved) as indicated by NPIP Program Standard Part A(8).

For avian influenza and Newcastle disease:

Over 50 National Animal Health Network (NAHLN)-approved laboratories (most of which are also NPIP-authorized) conduct PCR testing in accordance with NVSL SOP-AV-0068 in support of:

- Foreign animal disease investigations (FADI) per VS Guidance 12001, and
- Surveillance associated with NPIP per 9 CFR 145.14(d) and 9 CFR 146.13(b), LBM per Uniform Standards, Interagency wild bird surveillance, or other state program.
Avian Health Cooperative Agreements – **Commercial** | up to Q1 FY18: estimated # tests by sector and year – *excludes NVSL testing*

### AGID Tests
- 2015: 904,801
- 2016: 763,431
- 2017: 948,769
- 2018: 163,988

### ELISA Tests
- 2015: 885,923
- 2016: 807,218
- 2017: 914,455
- 2018: 239,190

### ACIA Tests
- 2015: 34,151
- 2016: 17,845
- 2017: 13,884
- 2018: 2,426

### PCR Tests
- 2015: 45,923
- 2016: 39,029
- 2017: 46,036
- 2018: 11,462
# Avian Health Cooperative Agreements – LBM/BYD up to Q1 FY18: estimated # tests by sector and year – excludes NVSL testing

<table>
<thead>
<tr>
<th></th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGID Tests</td>
<td>9,620</td>
<td>6,002</td>
<td>6,333</td>
<td>2,684</td>
</tr>
<tr>
<td>ELISA Tests</td>
<td>2,483</td>
<td>2,658</td>
<td>1,866</td>
<td>185</td>
</tr>
<tr>
<td>ACIA Tests</td>
<td>2,483</td>
<td>2,658</td>
<td>1,866</td>
<td>185</td>
</tr>
<tr>
<td>PCR Tests</td>
<td>33,367</td>
<td>28,868</td>
<td>44,244</td>
<td>8,218</td>
</tr>
<tr>
<td>VI Tests</td>
<td>3,102</td>
<td>2,985</td>
<td>2,998</td>
<td>543</td>
</tr>
</tbody>
</table>

Source flock monitoring per LBM guidelines.
Where do my samples go... AND WHAT DO YOU DO WITH THEM??
NPIP Serologic Workflow (AI)

1. NPIP-Authorized lab receives sera for testing

   - Influenza A ELISA / AGID
     - Neg
       - If commercial poultry collect swab samples
       - STOP
     - Any Antibody detection is forwarded to NVSL*

   - Received at NVSL for confirmation
     - Confirming subtype is PRIORITY HI (1-16) & NI (1-9)
       - Neg
         - STOP
       - HA/NA Subtype

2. PCR needed to confirm virus status of flock (refer to Molecular workflow)
Serologic Test Protocol (AI)

Establishes prior exposure
Test cannot determine pathotype
Test cannot determine virus status

NPIP AGID/ELISA
- Detect antibody to NP

NVSL HI
- H1-H16
- Confirm antibody to HA

NVSL NI
- N1-N9
- Confirm antibody to NA
Molecular Workflow

NAHLN lab receives swab or tissue for testing

Influenza A and/or APMV-1 rRT-PCR *

Neg

Any Ct>0 is tested by H5/H7 or F assays and forwarded to NVSL

Received at NVSL for confirmation

Molecular (PCR / Sanger direct from sample where possible)

Neg

HA/NA subtype/pathotype

Virus Isolation

Neg

Positive

Virus characterization

*Generic and subtyping assays may be run in parallel for FADl RNA detection by any assay is forwarded to NVSL
Molecular Test Protocol

Repeatable detection increases confidence
PCR results inform risk of viral shedding
Cleavage site sequence confirms the virus

NAHNL lab rRT-PCR
- Conserved target (e.g. matrix gene)
- Specific (H5/H7/ND)

NVSL repeats rRT-PCR
- Specific (H5/H7/ND)
- +/- conserved target

NVSL Sequence
- Partial (H&N for IAV, F for ND)
- Full genome

NVSL Virus Isolation
- In vivo test
- Virus characterization

Occurs in parallel
# AI/ND Testing and Forwarding

## PCR (NAHLN) and Serology (NPIP) Testing

| Initiate testing for AI/ND | • Within 48 hours of receipt (2 business days) for routine/healthy flock  
| | • Same day or as indicated by SAHOs for FADI |

| Forward non-negative samples to NVSL | • Within 24 hours of non-negative IAV or APMV-1 result (1 business day) for routine/healthy flock  
| | • **Same day for detections by H5/H7 or vNDV assay** |

## NVSL Testing

| Turn around time | • **Priority 1 FADI**: PCR within 4 hours of sample receipt; cleavage site attempt within 9 hours  
| | • Testing for lower priorities initiated within 24 hours, subject to testing burden  
| | • HI for antibody is possible same day; NI typically requires two days |

| Virus recovery and characterization | • Virus isolation 7-14 days (**full characterization may take longer**)  
| | • Contact DVL-AV for full genome sequencing and *in vivo* test completion |
# Test Result(s) Inform Status

<table>
<thead>
<tr>
<th>Lab/Test</th>
<th>Purpose</th>
<th>Interpretation for Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAHLN PCR</td>
<td>Detection of virus-specific RNA</td>
<td>Presumptive flock/premises • Refer to Response Option b</td>
</tr>
<tr>
<td>NVSL PCR</td>
<td>Monitors assays and demonstrates repeatability</td>
<td>• Establishes the presence of reportable disease (e.g. H5/H7/vND)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Resampling where the lowest Ct≥38 or not repeatable</td>
</tr>
<tr>
<td>Cleavage site sequence</td>
<td>Defines virulent viruses per the Select Agent Program – regardless of</td>
<td>• NVSL establishes the virus virulence(^c) per the USDA case definition, serving as</td>
</tr>
<tr>
<td>from sample and/or virus</td>
<td>the lab that generates it</td>
<td>✓ Confirmation of the flock/premises status, and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>✓ Official disease declaration for trade purposes</td>
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</table>

\(^a\) H5/H7 or vNDV PCR assay

\(^b\) Depopulation where state and federal agree

\(^c\) where no virus or sequence is obtained the virulence is determined by the clinical presentation and available test results
Determining Risk When Cts are High and/or not Repeatable

Collect additional samples to determine where virus is and how much is being shed

Conduct risk assessment to review factors such as performance records, production data, and status of virus shedding:

- Risk for spread is related to factors such as the amount of virus being shed and the duration of time that viable virus remains
- Virus detection: The greater the proportion of birds positive for virus (e.g. repeatable PCR Ct <38 and/or demonstration of viable virus recovery) the greater the risk
- Clinical presentation: Clinical presentation (including performance and production) can be used to determine when initial infection occurred

Environmental conditions: Weather, bedding type, ventilation, and management conditions can affect how long virus will survive in the environment
FY2018 Wild Bird Surveillance

>500 samples received

66 viruses recovered

H7 predominated in this period
No detections of HPAI since Dec 2016

<table>
<thead>
<tr>
<th>Date</th>
<th>State</th>
<th>Species</th>
<th>Surveillance type</th>
<th>Subtype</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar-18</td>
<td>MO, TX</td>
<td>commercial poultry</td>
<td>active</td>
<td>H7N1 LPAI</td>
<td>Quarantine, depopulation, C&amp;D, 10 km surveillance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1 TX, 1 MO)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sep-18</td>
<td>CA</td>
<td>commercial turkeys</td>
<td>passive</td>
<td>H7N3 LPAI</td>
<td>Quarantine, depopulation, C&amp;D, controlled market, 10 km surveillance</td>
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2018 March: AM H7N1 LPAI

26 Feb: Routine pre-slaughter commercial turkey flock in Jasper County MO

2 Mar: Broiler breeder flock in Texas (TX) confirmed by H7 and N1 antibody from sera

Viruses >99% similar across the entire genome

More than one independent introduction
Phylogeny of recent H7s from US poultry

- 2018 H7N1 LPAI MO, TX
- 2017 H7N9 LPAI TN, AL, KY, GA
- 2016 H7N8 LPAI IN
- 2018 H7N3 LPAI CA
- Mexico H7N3 HPAI
Other FY 2018 Events

Turkeys - wild bird lineage virus/exposure:
- MI H2N7 (Eurasian H2)
- PA & MD H2N3
- Swine lineage H1 (IA, MO), H3 (NC)

Other poultry
- Backyard exhibition birds: ducks WA, PA H5N2 LPAI; chicken CA vNDV
- AR H6N1 broiler breeder
- PA H1 broiler chicken (antibody only)
- FL H6N2 LBMS
- NJ, NY, PA H2N2 LBMS
LBM H2N2 HA Phylogeny

Older viruses to left, younger to the right
- Red = 2014
- Blue = 2015
- Green = 2016
- Pink = 2017-18

Not closely related to recent H2s from wild birds
2018 H2N3 PA, MD

Facilities within the same company with sites located on either side of state border

Birds moved to slaughter once negative for virus

purple = LBM/BYD
green = wild bird
red = poultry
APMV-1 in 2018

Phylogeny of representative viruses

- **2018 pigeon/dove**
  PPMV-1 from AZ, CA, ID, ME, MN, MT, NV, PA, TX, WA, and WI

- **2018 cormorant** from IL, MA, and MN – also reported in NY

- **2018 vNDV CA**
  backyard exhibition birds
Newcastle Disease Virus

Newcastle Disease virus (ND) is the cause of regular, frequent epizootics throughout Africa, Asia, Central America, and parts of South America and is considered a Foreign Animal Disease (FAD) in the United States.

- Why not Exotic ND (END)? Because it specifically referred to viscerotrophic velogenic Newcastle disease virus (vvNDV).
- Virulent ND or vND is intended to encompass all reportable virulent strains (e.g. viscerotrophic and neurotrophic).

Vaccination of birds against ND is common in the Americas, including the United States; the classical vaccine strains, are distinguishable from other viruses by genome sequencing.
Species Susceptibility

**Chickens** are one of the most susceptible species to disease caused by vNDV; US commercial poultry are vaccinated.

**Turkeys** are typically more resistant than chickens.

**Psittacine** species: variable susceptibility, potential to chronically shed virus has been reported; data supporting virus maintenance in these species is lacking.

**Pigeons and doves** in the US maintain a unique substrain (PPMV-1); unvaccinated poultry may be infected.

**Double-crested cormorants** maintain a unique subgroup of vNDV in the US, which can infect and cause disease in poultry.
Case Definition and Flock Status

Suspect: Domesticated bird or flock having:
- Clinical signs compatible with vND, OR
- Detection of APMV-1 (e.g. matrix target) by rRT-PCR; OR
- Epidemiological information indicating exposure to vNDV

Presumptive: suspect + detection of vNDV by the fusion-target rRT-PCR test

NOTE – a negative virulent test in the face of clinical signs requires further virus characterization by sequence and/or in vivo testing.

Confirmed: Domesticated bird or flock from which vNDV has been identified at NVSL
- Presumptive + identification of multiple basic amino acids (either directly via protein or by deduction through sequencing) in the fusion gene; AND/OR
- ICPI in day-old chicks (Gallus gallus) of 0.7 or greater

By NAHLN or NVSL
The CA 2018 virus (genotype Vb) is related to older Mexican-lineage viruses from Central America village poultry (Belize 2008, Honduras 2007), and the U.S. (smuggled parrot 1996, backyard CA 2002).

- Preliminary analysis of CA2018 virus isolates supports a single introduction followed by secondary spread.
- Lack of epidemiologic and contemporary sequence data contribute to the uncertainty surrounding the origin of the outbreak.
- Evolutionary analysis of available sequences with the CA2018 and CA2002 viruses suggest ongoing circulation of the virus; however, where and in what population remains unclear.

The virus is not related to classic vaccine strains, available data from vaccinated poultry in Mexico (2000-2010), species-adapted viruses from columbids (pigeons, doves), nor closely related to those from double-crested cormorants.
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*Remember* to send duplicate samples to your NAHLN lab and to NVSL in parallel for the fastest confirmation!
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