NVSL AI/ND UPDATE

JUNE 2018

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Where do my samples go... AND WHAT DO YOU DO WITH THEM??
NPIP Serologic Workflow (AI)

NPIP-Authorized lab receives sera for testing

Influenza A ELISA / AGID

Neg

Any Antibody detection is forwarded to NVSL*

If commercial poultry collect swab samples

Received at NVSL for confirmation

Confirming subtype is PRIORITY
HI (1-16) & NI (1-9)

Neg

HA/NA Subtype

STOP

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

- Serology informs suspect flock status
- Collect swabs to determine virus status of flock
- H5/H7 exposure confirmed by NVSL HI testing
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
NAHLN lab receives swab or tissue for testing

Conserved target (AI/ND) rRT-PCR *

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

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

Received at NVSL for confirmation

Molecular (PCR / Sanger direct from sample where possible)

Virus Isolation

Neg

HA/NA subtype/pathotype

Positive

Virus characterization

Neg
Molecular Test Protocol

PCR informs presumptive flock status

Options for rapid response available

Flock status confirmed by NVSL per USDA case definition via cleavage site sequence
**Molecular Test Protocol**

Disease confirmed through repeatable detection
PCR results inform risk of viral shedding
Cleavage site sequence confirms the virus

- **NAHLN lab rRT-PCR**
  - Conserved (e.g. matrix)
  - Specific (H5/H7/ND)

- **NVSL repeats rRT-PCR**
  - Specific (H5/H7/ND)
  - +/- conserved (e.g. matrix)

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

- **NVSL Virus Isolation**
  - In vivo test
  - Gen/ant.char

Occurs in parallel
Monthly Summary Data from the National Wild Bird Avian Influenza Surveillance Program:

July 2008 to January 2018

Graph 1. Percent of wild duck samples positive for low pathogenic Type A influenza viruses as determined by RT-PCR.

peak H5s

peak H7s
### Samples received from Interagency wild bird surveillance 2016-2018

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Total wild birds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampled since Jul 2015</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atlantic</td>
<td>10,663</td>
<td>9,074</td>
<td>8,012</td>
<td>27,749</td>
</tr>
<tr>
<td>Mississippi</td>
<td>13,792</td>
<td>9,973</td>
<td>8,616</td>
<td>32,381</td>
</tr>
<tr>
<td>Central</td>
<td>9,202</td>
<td>7,516</td>
<td>7,175</td>
<td>23,893</td>
</tr>
<tr>
<td>Pacific</td>
<td>11,868</td>
<td>9,175</td>
<td>7,632</td>
<td>28,675</td>
</tr>
<tr>
<td>American Oceania</td>
<td>24</td>
<td>9</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td><strong>Total birds sampled</strong></td>
<td>45,549</td>
<td>35,747</td>
<td>31,438</td>
<td>112,734</td>
</tr>
</tbody>
</table>

~10-11% detected by fluA PCR

~1% detected by H5/H7 PCR go to NVSL – others to NWRC
NVSL virus recovery from Interagency Wild bird surveillance 2016-2018

Isolates are archived at NWRC
### 2017-18 H5/H7 events

<table>
<thead>
<tr>
<th>Date</th>
<th>State</th>
<th>Surv stream</th>
<th>initial sample</th>
<th>Subtype</th>
<th>Species</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar-17</td>
<td>WI</td>
<td>COMM</td>
<td>swab</td>
<td>H5N2 LPAI</td>
<td>turkey</td>
<td>Quarantine, depopulation, C&amp;D, 10 km surveillonce</td>
</tr>
<tr>
<td>Mar-17</td>
<td>TN, AL, KY, GA</td>
<td>COMM, BYD (AL)</td>
<td>swab</td>
<td>H7N9 HPAI/LPAI</td>
<td>broiler breeder (COMM), duck &amp; guinea (BYD)</td>
<td>Quarantine, depopulation, C&amp;D, 10 miles surveillonce</td>
</tr>
<tr>
<td>Mar-18</td>
<td>MO, TX</td>
<td>COMM, BYD (MO)</td>
<td>swab</td>
<td>H7N1 LPAI</td>
<td>turkey, broiler breeder, chicken</td>
<td>Quarantine, depopulation, C&amp;D, 10 miles surveillonce</td>
</tr>
<tr>
<td>Apr-17</td>
<td>ID</td>
<td>BYD</td>
<td>sera</td>
<td>H5N2 LPAI</td>
<td>duck</td>
<td></td>
</tr>
<tr>
<td>Sep-17</td>
<td>WA</td>
<td>BYD</td>
<td>swab</td>
<td>H5N2 LPAI</td>
<td>chicken, duck</td>
<td></td>
</tr>
<tr>
<td>Oct-17</td>
<td>PA</td>
<td>BYD</td>
<td>swab</td>
<td>H5N2 LPAI</td>
<td>duck</td>
<td></td>
</tr>
<tr>
<td>Dec-17</td>
<td>OH</td>
<td>Upland Game</td>
<td>swab</td>
<td>H5N2 LPAI</td>
<td>duck</td>
<td></td>
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<tr>
<td>Nov-17</td>
<td>FL</td>
<td>LBM</td>
<td>swab</td>
<td>H5 PCR only</td>
<td>environment</td>
<td>n/a</td>
</tr>
</tbody>
</table>
LBM H2N2
HA Phylogeny

Simple tree... in general:
- Horizontal distance more significant than vertical
- Older viruses to left, younger to the right
- Red = 2014
- Blue = 2015
- Green = 2016
- Pink = 2017

Have a few H2s from wild birds - HA not related

2015 wild bird H2s
ID, MN, & NC BYD
Wild type virus introduced to domestic waterfowl (Muscovy?) ducks prior to 2014 ...

Data for viruses since 2014 suggests:
- First introduction circulated from late 2014 until 2016
- Second introduction (same virus lineage) circulating from late 2016 to present day
- Opportunity to adapt to poultry remains
- Risk of reassortment upon co-circulation with other virus remains

Not currently a public health concern – not related to previous pandemic H2s, but data are shared with CDC for ongoing evaluation
Tests *inform* Status *leading to* Response

Per the USDA case definition for AI (or ND), a **suspect** status is based upon the clinical presentation compared to USDA case definition with or without test result(s).

The **status** progresses from **suspect** to **presumptive** based upon **NAHLN** PCR testing, and is **confirmed** based upon NVSL testing.

APHIS is committed to rapid depopulation where HPAI or vNDV is suspected. **Response Option:** depopulation can be initiated for a **presumptive** premises with the following:

- The flock meets the USDA **case definition**,  
- NAHLN H5/H7/F-assay detection (and samples that have been forwarded to NVSL for confirmation), *and*  
- Agreement of State and Federal officials.
Update on vNDV in California

On May 16, 2018, the California Department of Food and Agriculture (CDFA) detected virulent Newcastle disease in a small flock of backyard exhibition chickens.

USDA Confirms Additional Cases of Virulent Newcastle Disease in Backyard Birds in California

The United States Department of Agriculture’s (USDA) Animal and Plant Health Inspection Service confirmed two additional cases of virulent Newcastle disease in backyard birds in San Bernardino County, California.

A complete list of confirmed cases is available on our website at www.aphis.usda.gov/animalhealth/vnd.

Virulent Newcastle disease has not been found in commercial poultry in the United States since 2003.

No human cases of Newcastle disease have ever occurred from eating poultry products. Properly cooked poultry products are safe to eat. In very rare instances, people working directly with sick birds can become infected. Symptoms are usually very mild and limited to conjunctivitis. Infection is easily prevented by using standard personal protective equipment.
General points

Newcastle Disease (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 neurotropic)

The virus can infect many species of domestic and wild birds. Chickens are highly susceptible; other gallinaceous birds such as turkey, quail, and guinea are also susceptible

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
Global Newcastle disease distribution
January-July 2017 (OIE WAHIS)
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.
Newcastle disease case definition

**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
APMV-1 are: highly diverse, have a broad range of virulence, and can infect many avian species

**Current diagnostics** distinguish APMV-1 viruses by virulence per OIE — *Infection of poultry caused by a virus of APMV-1 that meets the following:*

- Intracerebral pathogenicity index (ICPI) of ≥ 0.7 in day-old chicks
- Presence of multiple basic amino acids at a key site in the F2 protein plus phenylalanine at residue 117 in the F1 protein
  - ‘Multiple basic amino acids’ = at least three arginine or lysine residues between residues 113 and 116
- Diagnostic use of PCR to aid in detection of virulent viruses and sequencing to confirm virus lineage
Class II, genotypes V, VI, VII, and VIII are the predominant worldwide and contain only virulent viruses

- **Class I & Class II, genotype I** viruses are predominantly of **low virulence** and include some used as live vaccines (QV4/66 and Ulster/67)
- **Class II, genotype II** includes viruses of low virulence and include vaccine viruses such as LaSota, B1 and VG/GA, and the neurotropic virulent chicken TX GB/1948 (challenge strain)
- Other genotypes represent historic viruses no longer seen
  - Class II, genotypes I, II, III, IV and IX represent viruses 1930–1960
Vaccination: protection from challenge

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**D.R. Kapczynski, D.J. King / Vaccine 23 (2005) 3424–3433**

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Fig. 2. Survival of commercial broiler-breeder chickens and broilers receiving field vaccination against NDV and challenged with CA02. Chickens were infected via eye drop/intranasal route with $10^{5.9}$ EID$_{50}$/bird CA02 and mortality observed over a 2-week period.
Vaccination: viral shed

*D.R. Kapczynski, D.J. King / Vaccine 23 (200)*

Fig. 3. Comparison of CA02 mean virus titers from oral and cloacal swabs following challenge with CA02 virus in experiment III. Commercial broiler-breeders and broilers received field NDV vaccination and were challenged with $10^{5.9} \text{EID}_{50}$/bird CA02. Oral (A) and cloacal (B) swabs were sampled on the days indicated. NT: not tested.
# 2017 APMV-1 at NVSL

<table>
<thead>
<tr>
<th>APMV-1 Diagnostic Test</th>
<th>Total tests 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemagglutination-inhibition (HI) antibody</td>
<td>2,038</td>
</tr>
<tr>
<td>Real-time RT-PCR (M, F target)</td>
<td>946</td>
</tr>
<tr>
<td>APMV-1 (positive/total samples)</td>
<td>210/3,511</td>
</tr>
<tr>
<td>Molecular pathotype (Sanger)</td>
<td>233</td>
</tr>
<tr>
<td>In vivo pathotype (ICPI)</td>
<td>49</td>
</tr>
<tr>
<td>Whole genome sequencing (count by isolate)</td>
<td>161</td>
</tr>
</tbody>
</table>
Field case definition developed for CA 2002

Defined as >2 of any 5 birds demonstrating ANY of:

- Hemorrhagic tracheitis
- Hemorrhage at junction of proventriculus
- Hemorrhage of cecal tonsils
- Diptheritic lesions
Phylogenetic analysis CA 2018

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

- NOTE: temporal and geographic gaps in available data, especially from these areas and affected species, increase the uncertainty of the virus origin

The virus is not related to classic vaccine strains, vaccinated poultry strains from Mexico available (2000-2010), species-adapted viruses from columbids (pigeons, doves), nor closely related to those from cormorants.

The estimated evolutionary rate of CA 2018 compared to CA 2002 is consistent with the expected rate, and corresponds to 15-20 years of viral evolution.
V.2.1/V_b/AY562987/gamefowl/USA/CA_211472/2002
V.2.1/V_b/JN872192/chicken/USA/California_211472_4_/2002
V.2.1/V_b/EF520718/gamefowl/USA_/CA_212676/2002
V.2.1/V_b/JN942045/turkey/Belize/4438_4_/2008
V.2.1/V_b/JN872194/chicken/Honduras/498109_15_/2007
bird/California/18_016505_001/2018

97.5-98% identical

SLIDE COURTESY K. DIMITROV, USDA ARS SEPRL
PATHOGENESIS AND TRANSMISSION EXPERIMENT WITH THE CALIFORNIA 2018, BELIZE 2008 AND CALIFORNIA 2002 NDV

Challenge viruses:
- chicken/USA/CA/212676/2002
- chicken/Belize/4224-3/2008
- chicken/USA/CA/016505-001/2018

Inoculation route – 0.1 mL in the choanal cleft and a touch of the gavage needle on the left eyelid

Challenge 5 birds with either $10^2$, $10^4$, or $10^6$ EID$_{50}$/bird of each respective virus
Challenge 9 birds with CA18 $10^6$ EID$_{50}$/bird

swab (OP and CL) collection from directly challenged birds daily 1 to 4 dpc, and at 7dpc
Euthanize 3 birds per day from group #10 and collect tissues – 1dpc, 2 dpc, 3 dpc

3 (2) SPF contact birds added per group at 2 dpc
all contact birds swabbed (OP and CL) at 2 and 5 days post contact

SLIDE COURTESY K. DIMITROV, USDA ARS SEPRL
**CLINICAL SIGNS CONTACT 3-WEEK-OLD SPF CHICKENS**

**FIRST CLINICAL SIGNS APPEARED AT 4 DAYS POST CONTACT**

<table>
<thead>
<tr>
<th></th>
<th>Eyelid edema</th>
<th>Conjunctivitis</th>
<th>Lethargy</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA/02 10^2</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>CA/02 10^4</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>CA/02 10^6</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Belize/08 10^2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Belize/08 10^4</td>
<td>Yes</td>
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<td>Yes</td>
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<tr>
<td>Belize/08 10^6</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>CA/18 10^2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CA/18 10^4</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>CA/18 10^6</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

-: not observed

**SLIDE COURTESY K. DIMITROV, USDA ARS SEPRL**
NDV survival in the environment

doi:10.1017/S0950268812002610
NDV survival in chicken carcasses

Chickens inoculated with virulent NDV (Pakistan/VIIIi) and euthanized when clinical signs were apparent (~3-4 days post challenge)

Carcasses were stored at ambient temperatures (~75°F) for 18 days

8 tissues and fluid draining from the carcasses were collected at the time of euthanasia and at 6, 12, and 18 days post euthanasia (DPE)

- Heart, spleen, feather pulp, breast muscle, thigh muscle, skin, small intestine, lung, fluid
- Each sample is processed for virus viability and titer
  - Only some tissues have been tested at 12 DPE
  - In all tested tissues viable virus has been detected

DATA COURTESY E. SPACKMAN, USDA ARS SEPRL
Preliminary data, sample processing is in progress

NDV survival in carcasses

DATA COURTESY E. SPACKMAN, USDA ARS SEPRL
Helpful References

Other helpful references

- Newcastle Disease Brief
  [English PDF]
- Newcastle Disease Fact Sheet
  [English PDF]
- Comparison Chart for HPAI and Newcastle Disease
  [English PDF]
- Biosecurity and Disinfection Disinfection 101
  [English PDF]
- Characteristics of Selected Disinfectants
  [English PDF]
Special Thanks to:

• CAHFS
• USDA ARS SEPRL
• SAHOs, NPIP and NAHLN Labs
• Team Avian, Diagnostic Virology Lab

*Remember* to send duplicate samples to your NAHLN lab and to NVSL in parallel for the fastest confirmation!


