Southeast Poultry Research Laboratory

• Emerging and Exotic Avian Viral Disease Research Unit
  – David Suarez, David Swayne, Erica Spackman, Mary Pantin-Jackwood, Darrell Kapczynski, Claudio Afonso

• Endemic Viral Disease Research Unit
  – Laszlo Zsak, Michael Day, Qingzhong Yu, and Stephen Spatz
Replacement/Modernization of the Biocontainment Laboratory and Consolidated Poultry Research Facility, Athens, Georgia

• Proposed FY14 President’s budget
  – Only building project requested by ARS
  – Not included in either the House or Senate appropriations bill

• Proposed FY15 President’s budget
  – Only building project requested by ARS
  – Neither House or Senate initial budget markups included money for new building
BUILDINGS AND FACILITIES

2014 appropriation ................................................................. – – –
2015 budget estimate ............................................................... $155,000,000
Provided in the bill: .............................................................. 155,000,000

Comparison:
2014 appropriation ................................................................. +155,000,000
2015 estimate ................................................................. – – –

COMMITTEE PROVISIONS
For ARS Buildings and Facilities, the Committee provides an appropriation of $155,000,000 for priorities identified in the USDA Agricultural Research Service Capital Investment Strategy, April 2012.
We Need Your Help
Improved NDV rRT-PCR diagnostics

David Suarez
Matrix PCR Test

• Matrix test was developed using available sequence in a conserved gene, but with bias towards Mexican lineage NDV viruses

• Newcastle disease viruses are found world-wide and displays considerable sequence divergence

• Published reports of decreased sensitivity or false negatives because of sequence divergence J Clin Microbiol. 2010 May;48(5):1892-4.

• Goal to develop additional NDV screening tests with high sensitivity and specificity
**Single Nucleotide Polymorphisms (SNP) Analysis**

<table>
<thead>
<tr>
<th>Position</th>
<th>Score</th>
<th>Cons</th>
<th>A</th>
<th>T</th>
<th>G</th>
<th>C</th>
<th>Dele # Seq</th>
<th>Boxcar</th>
</tr>
</thead>
<tbody>
<tr>
<td>4202</td>
<td>4</td>
<td>C</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>253</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4203</td>
<td>0</td>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>254</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4204</td>
<td>100</td>
<td>T</td>
<td>0</td>
<td>163</td>
<td>2</td>
<td>89</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4205</td>
<td>4</td>
<td>A</td>
<td>253</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4206</td>
<td>4</td>
<td>T</td>
<td>0</td>
<td>253</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4207</td>
<td>4</td>
<td>A</td>
<td>253</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4208</td>
<td>0</td>
<td>G</td>
<td>0</td>
<td>0</td>
<td>254</td>
<td>0</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4209</td>
<td>0</td>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>254</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4210</td>
<td>7</td>
<td>A</td>
<td>252</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4211</td>
<td>4</td>
<td>A</td>
<td>253</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4212</td>
<td>0</td>
<td>A</td>
<td>254</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4213</td>
<td>14</td>
<td>T</td>
<td>0</td>
<td>249</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4214</td>
<td>0</td>
<td>G</td>
<td>0</td>
<td>0</td>
<td>254</td>
<td>0</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4215</td>
<td>0</td>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>254</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4216</td>
<td>66</td>
<td>C</td>
<td>0</td>
<td>43</td>
<td>0</td>
<td>211</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4217</td>
<td>0</td>
<td>T</td>
<td>0</td>
<td>254</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4218</td>
<td>0</td>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>254</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4219</td>
<td>27</td>
<td>T</td>
<td>0</td>
<td>242</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4220</td>
<td>0</td>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>254</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4221</td>
<td>0</td>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>254</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4222</td>
<td>106</td>
<td>T</td>
<td>5</td>
<td>160</td>
<td>0</td>
<td>89</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4223</td>
<td>0</td>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>254</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4224</td>
<td>0</td>
<td>A</td>
<td>254</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4225</td>
<td>27</td>
<td>G</td>
<td>12</td>
<td>0</td>
<td>242</td>
<td>0</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4226</td>
<td>0</td>
<td>G</td>
<td>0</td>
<td>0</td>
<td>254</td>
<td>0</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4227</td>
<td>0</td>
<td>T</td>
<td>0</td>
<td>254</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>4228</td>
<td>135</td>
<td>T</td>
<td>33</td>
<td>148</td>
<td>73</td>
<td>0</td>
<td>0</td>
<td>254</td>
</tr>
</tbody>
</table>

- Variation scores were conducted on rolling average of 20 nucleotides (Boxcar averaging)
- Areas with an average SNP score of <13 were considered for RRT-PCR development
Selection Process

- 8 different clusters of conserved sequence were identified
- Primers and probes were tested with a small NDV panel looking at sensitivity and specificity

<table>
<thead>
<tr>
<th></th>
<th>2455 probe</th>
<th></th>
<th>2455+3 probe</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2432</td>
<td>2429</td>
<td>2432</td>
</tr>
<tr>
<td>NDV+2432</td>
<td>CAC AGC ATA TCA TGG ACA A</td>
<td>49.9</td>
<td>3.53</td>
<td></td>
</tr>
<tr>
<td>NDV+2429</td>
<td>GAA CAC AGC ATA TCA TGG AC</td>
<td>51.1</td>
<td>6.55</td>
<td></td>
</tr>
<tr>
<td>NDV+2455</td>
<td>AGG AGT CAC AAC TAT CAG CTG GTG</td>
<td>58.4</td>
<td>7.25</td>
<td></td>
</tr>
<tr>
<td>NDV+2455+3</td>
<td>AGG AGT CAC AAC TAT CAG CTG GTG CAA</td>
<td>61.6</td>
<td>7.33</td>
<td></td>
</tr>
<tr>
<td>NDV-2590</td>
<td>GCC TCC ATC ATA GAC ATC A</td>
<td>51.3</td>
<td>7.73</td>
<td>25.1/31.3/-</td>
</tr>
<tr>
<td>NDV-2587-1</td>
<td>TCC ATC ATA GAC ATC ATC GC</td>
<td>51.8</td>
<td>8.05</td>
<td>20.75/27.7/42.7</td>
</tr>
<tr>
<td>NDV-2587+2</td>
<td>CCT CCA TCA TAG ACA TCA TCG C</td>
<td>55.1</td>
<td>8.13</td>
<td>22.9/28.3/39.6</td>
</tr>
<tr>
<td>NDV-2587</td>
<td>CTC CAT CAT AGA CAT CAT CGC</td>
<td>52.8</td>
<td>8.52</td>
<td>28.8/29.86/-</td>
</tr>
</tbody>
</table>
## Combined Results

<table>
<thead>
<tr>
<th></th>
<th>+4100</th>
<th>+10496</th>
<th>+12170</th>
<th>+4100</th>
<th>+10496</th>
<th>+12170</th>
<th>+4100</th>
<th>+10496</th>
<th>+12170</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive/total</td>
<td>31/154</td>
<td>102/154</td>
<td>110/154</td>
<td>14/44</td>
<td>110/142</td>
<td>112/142</td>
<td>N/T</td>
<td>118/141</td>
<td>123/141</td>
</tr>
<tr>
<td>Ct Avg</td>
<td>31.63</td>
<td>32.79</td>
<td>32.25</td>
<td>34.37</td>
<td>34.97</td>
<td>34.37</td>
<td>N/T</td>
<td>33.01</td>
<td>33.38</td>
</tr>
</tbody>
</table>

Three new tests for Class II NDV were developed that have improved sensitivity and specificity compared to current NDV Matrix test.
Conclusions

• Several new rRT-PCR tests were developed using bioinformatics to identify conserved regions
• Empirical testing was used to find best performing tests
• Two different tests, both in polymerase gene, have better sensitivity and specificity than matrix test in experimental study
• Availability of alternative/adjunct tests are important for rapid diagnostics
• Banking of tests in Veterinary stockpile recommended
Novel recombinant bivalent vaccines protect chickens against infectious laryngotracheitis and Newcastle disease

Wei Zhao, Stephen Spatz, Zhenyu Zhang, Guoyuan Wen, Maricarmen Garcia, Laszlo Zsak and Qingzhong Yu
Controls of ND and ILT

- Newcastle disease (ND) and Infectious laryngotracheitis (ILT) are highly contagious respiratory diseases of chickens
- Vaccination combined with restrict biosecurity practice has been the recommended strategy for controlling ND and ILT
- ND vaccines: LaSota, B1, C2, VG/GV, ... Effective when used properly
- ILT vaccines:
  - Live-attenuated vaccines
    - Chicken embryo origin [CEO]
    - Tissue culture origin [TCO]
    Both are effective, but with concerns of safety, particularly CEO. Establish latency.
  - Vectored vaccines
    - FPV-LT: express ILTV gB and UL-34
    - HVT-LT: express ILTV gI and gD
    Safe, but less effective: partial protection, barely reduce challenge virus loads in the trachea.

Thus, there is a need to develop a safer and more efficacious ILT vaccine.
Approach and advantages

- Use Newcastle disease virus (NDV) LaSota vaccine-based recombinant virus as a vector
- Express ILTV antigenic proteins: gB and gD
- Advantages of LaSota vaccine as a vector:
  - Safe, stable and worldwide usage
  - Respiratory tissue tropic replication
  - Local and systemic immunoresponses
  - Mass-administration at low cost
Construction of NDV/ILTV cDNA clones

**PCR**

**In-fusion PCR cloning**

**pLS-GFP vector**

**pLS/ILTV-gB or gD**

**ITLV genomic DNA**

**gB or gD**

**T7**

**TCAAG**

**TTAGAAAAAAA**

**ACGGGTAGAA**

**GCCACC**

**ATG CA**

**TGA**

Gene end

Gene start

Kozak

ILTV gB or gD ORF
### Biological assessments of rLS/ILTV-gB, -gD

<table>
<thead>
<tr>
<th>Virus</th>
<th>MDT(^a)</th>
<th>ICPI(^b)</th>
<th>HA(^c)</th>
<th>EID(_{50})(^d)</th>
<th>TCID(_{50})(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rLaSota</td>
<td>110hs</td>
<td>0.15</td>
<td>1024</td>
<td>6.8×10(^8)</td>
<td>3.5×10(^7)</td>
</tr>
<tr>
<td>rLS-GFP</td>
<td>120hs</td>
<td>0.00</td>
<td>2048</td>
<td>3.1×10(^8)</td>
<td>9.8×10(^7)</td>
</tr>
<tr>
<td>rLS/ILTV-gB</td>
<td>120hs</td>
<td>0.00</td>
<td>2048</td>
<td>3.8×10(^8)</td>
<td>1.6×10(^7)</td>
</tr>
<tr>
<td>rLS/ILTV-gD</td>
<td>112hs</td>
<td>0.01</td>
<td>2048</td>
<td>6.8×10(^8)</td>
<td>3.8×10(^7)</td>
</tr>
</tbody>
</table>

\(^a\) MDT: Mean death time in embryonated eggs.
\(^b\) ICPI: Intracerebral pathogenicity index in day-old chickens.
\(^c\) HA: Hemagglutination titer.
\(^d\) EID\(_{50}\): The 50% egg infectious dose in embryonated eggs.
\(^e\) TCID\(_{50}\): The 50% tissue infectious dose on DF-1 cells.
Vaccination and challenge against ILTV in SPF chickens

15 one-day-old SPF chickens/treatment

IO/IN

Monitor vaccine side effects

Inoculated w/ 100 µl of:
Groups 1 and 2: PBS
Groups 3 and 4: rLS-GFP (10^6 ID_{50}/bird)
Groups 5 and 6: rLS/ILTV-gB (10^6 EID_{50}/bird)
Groups 7 and 8: rLS/ILTV-gD (10^6 EID_{50}/bird)

ED/IT

Observe clinical signs daily

Collect blood samples at 21 or 28 DPV

Intra-tracheal and ocular swabs

Challenged w/200 µl of pathogenic ILTV (63140):
Groups 1, 3, 5 and 7: at 21 DPV (10^4 TCID_{50}/bird)
Groups 4, 6 and 8: at 28 DPV (10^4 TCID_{50}/bird)

qPCR

At 4 DPC
### ILTV CHALLENGE SCORING SYSTEM

<table>
<thead>
<tr>
<th>CLINICAL SIGNS</th>
<th>GRADE</th>
<th>SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory (R)</td>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>3</td>
</tr>
<tr>
<td>Conjunctivitis (C)</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>3</td>
</tr>
<tr>
<td>Depression (D)</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>3</td>
</tr>
<tr>
<td>Mortality</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>9</td>
</tr>
</tbody>
</table>
Clinical sign scores

Total clinical sign scores after ILTV challenge at 21 DPV

Days post-challenge

Total clinical sign scores after challenge at 28 DPV

Days post-challenge
Vaccination/challenge against ILTV in commercial broilers

20 three-week-old commercial broilers/treatment

Group 1: PBS
Group 2: rLS-GFP (10^6 ID_{50}/bird)
Group 3: rLS/ILTV-gB (10^6 EID_{50}/bird)
Group 4: TCO (Intervet: LT-IVAX, 1 dose)
Group 5: CEO (Intervet: TrachivaxH, 1 dose)

Collect blood samples at 21 DPV
Observe clinical signs daily

Challenged with:
200 µl of pathogenic ILTV (63140) at 21 DPV (10^4 TCID_{50}/bird)

Intra-tracheal and ocular swabs

qPCR

HI

Monitor vaccine side effects

Collect blood samples at 21 DPV

Measure body-weight at 21 DPV (42 days of age)

Measure body-weight at 9 DPC (51 days of age)

At 4 DPC
Clinical sign scores

Total clinical sign scores after ILTV challenge

<table>
<thead>
<tr>
<th></th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
<th>D6</th>
<th>D7</th>
<th>D8</th>
<th>D9</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>0</td>
<td>16</td>
<td>69</td>
<td>123</td>
<td>90</td>
<td>59</td>
<td>33</td>
<td>15</td>
</tr>
<tr>
<td>rLS-GFP</td>
<td>0</td>
<td>11</td>
<td>50</td>
<td>101</td>
<td>60</td>
<td>22</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>rLS/ILTV-GB</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CEO</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>TCO</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Protection against NDV challenge

10 one-day-old SPF chickens/treatment

IO/IN

Inoculated with 100 µl of
- Group 1: PBS
- Group 2: rLaSota
- Group 3: rLS/ILTV-gB (10^6 TCID<sub>50</sub>/bird)
- Group 4: rLS/ILTV-gD (10^6 TCID<sub>50</sub>/bird)

Blood samples at 14 DPV

IO/IN

Challenged with 100 µl of velogenic NDV (CA02, 10^5 EID<sub>50</sub>/bird)

Monitor clinical signs/mortality daily

HI
Serum antibody responses of chickens following vaccination and survivors after NDV challenge

<table>
<thead>
<tr>
<th>Expt. 3</th>
<th>Antibody responses</th>
<th>Survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seropositive birds</td>
<td>NDV HI titer a</td>
</tr>
<tr>
<td>PBS</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>rLaSota</td>
<td>10/10</td>
<td>4.8 ± 1.4</td>
</tr>
<tr>
<td>rLS/ILTV-gB</td>
<td>10/10</td>
<td>3.4 ± 1.3</td>
</tr>
<tr>
<td>rLS/ILTV-gD</td>
<td>10/10</td>
<td>3.8 ± 1.2</td>
</tr>
</tbody>
</table>

aHemagglutination inhibition (HI) titer was expressed as log2 of the mean ± standard deviation from samples taken at 14 days post-vaccinatio.
Summary

• Generated NDV LaSota strain-based recombinant viruses containing the ILTV gB or gD gene
• These recombinant viruses slightly attenuated, but retained similar growth dynamics and stability as the parental virus
• Expression of the ILTV gB and gD proteins in infected cells was detected by IFA
• Vaccination of SPF chickens and commercial broilers with the recombinant vaccines conferred significant protection against virulent ILTV and NDV challenges.
• The results suggest that rLS/ITLTV-gB and rLS/ITLTV-gD are safe, stable and effective bivalent vaccines
Enteric Diseases

Michael Day
Turkey and chicken intestinal virome analysis
- **Comparative metagenomic analyses of SPF sentinels placed in the field**
- **Marked differences in intestinal microbiomes—notably enteric picornaviruses**
Characterization/propagation of novel turkey enteric picornaviruses

- initially described at SEPRL via metagenomic analysis
- comparative metagenomics suggests a role in enteric disease syndromes (PEC, PES)
- shedding/association with performance problems and weight loss in experimental birds

Significant weight depression in commercial poulets inoculated with picornavirus + homogenates
In collaboration with industry stakeholders, investigated emerging turkey enteric coronavirus
- Distinct geographical isolates in the Southeast with at least two pathotypes
- Not related to concomitant outbreak of infectious bronchitis in chickens

Characterization of the novel turkey enteric picobirnaviruses (PBVs)
- initially described at SEPRL via metagenomic analysis
- design and validation of an RT-PCR assay for this novel virus
- phylogenetic analysis suggests the poultry PBVs may be new species

Antigen Capture kits for AIV detection

Erica Spackman

• Data are not available on how the clinical condition corresponds to HPAIV detection by LFD.

➢ How does clinical condition relate to virus detection with LFD?
Inoculate by Intra-choanal route

Two replicates with different isolates:
A/chicken/Jalisco/12283/1012 H7N3 HPAIV
Short mean death time
A/chicken/PA/1370/1983 H5N2 HPAIV
Long mean death time

50% of dead bird not tested immediately, but held for 12Hr to determine whether there was any difference in detection if a carcass was not tested immediately after death

Used low dose (~$10^4$ EID$_{50}$/bird) so birds would not die immediately
50 chickens per isolate
Oral swabs collected every 12 hours and chickens scored for clinical condition: Healthy, Sick, Dead

Swabs tested immediately with LFD
All testing with VetScan, FluDetect not available
Detection titer based

CK/PA/83 H5N2

Titer EID50/ml

% pos

n=334
Healthy
Sick
Dead
Dead+12 hr

Titer EID50/ml

# of chickens

n=334

CK/PA/83 H5N2
Avian Influenza Virus Type A Antigen Test Kit

VetScan®
Avian Influenza Virus Rapid Test

(-) Control Test
(+ Control

10^{4.7} \text{ 2-fold difference } 10^{5.0}
The difference in detection between the isolates is related to titers of virus shed.

<table>
<thead>
<tr>
<th></th>
<th>CK/PA/1983 H5N2</th>
<th>n=334</th>
<th>CK/Jalisco/2012 H7N3</th>
<th>n=385</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>Sick</td>
<td>Dead</td>
<td>Dead +12hr</td>
</tr>
<tr>
<td>Total samples</td>
<td>174</td>
<td>118</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>% positive</td>
<td>28.7</td>
<td>50.8</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Take-home message

- Under different conditions birds will shed different titers of virus, however shed titers may not correlate with clinical presentation.
  - Healthy birds can shed sufficient titers to be detected by ACIA
  - Sick and dead birds may not shed sufficient titers
  - Carcasses which sat at ambient temperature an extra 12 hrs had the highest titers.

- Clinical condition is not a consistent indicator of amount of virus shed (implications for all detection tests)
Experimental infection of mallard ducks with different subtype H5 and H7 highly pathogenic avian influenza viruses

Mary Pantin-Jackwood, Mar Costa-Hurtado, Eric Shepherd, Diane Smith, Erica Spackman, Darrell Kapczynski, David Suarez and David Swayne
Questions

- Is the pathogenesis of H5N1 HPAIV in wild ducks unique to this lineage?
- Can other H5 or H7 HPAIV infect mallards and transmit to contacts?
- Can mallards be long-distance vectors for other HPAIV?
Pathogenicity and transmission of H5 and H7 HPAIV in mallards

**Virus inoculation**

- 10^6 EID_{50} intranasal

**Body weight and temperature and collect oropharyngeal and cloacal swabs for virus detection**

- Euthanize 2 birds and collect tissues for virus detection
- Add 3 contacts

- Bleed and euthanize remaining birds

**Serology**

- Quantitative real time RT-PCR (qRT-PCR) targeting influenza matrix gene

2 week-old Mallards (*Anas platyrhynchos*)

n=12

Southeast Poultry Research Laboratory

USDA
Pathogenicity and transmission of H5 and H7 HPAIV (non-H5N1) in mallards

<table>
<thead>
<tr>
<th>Type</th>
<th>Challenge virus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LPAIV</strong></td>
<td></td>
</tr>
<tr>
<td>H7N8</td>
<td>A/mallard/Ohio/421/1987</td>
</tr>
<tr>
<td>H7N7</td>
<td>A/mallard/Sweden/85/2002</td>
</tr>
<tr>
<td>H7N3</td>
<td>A/chicken/Chile/184240-1/2002</td>
</tr>
<tr>
<td>H7N3</td>
<td>A/chicken/Canada/314514-2/2005</td>
</tr>
<tr>
<td>H7N3</td>
<td>A/chicken/Jalisco/CPA1/2012</td>
</tr>
<tr>
<td><strong>H7 HPAIV</strong></td>
<td></td>
</tr>
<tr>
<td>H7N7</td>
<td>A/chicken/Victoria/1985</td>
</tr>
<tr>
<td>H7N7</td>
<td>A/chicken/North Korea/7916/2005</td>
</tr>
<tr>
<td>H7N7</td>
<td>A/chicken/Netherlands/1/2003</td>
</tr>
<tr>
<td>H7N1</td>
<td>A/turkey/Italy/4580/1999</td>
</tr>
<tr>
<td><strong>H5 HPAIV</strong></td>
<td></td>
</tr>
<tr>
<td>H5N2</td>
<td>A/chicken/Pennsylvania/1370/1983</td>
</tr>
<tr>
<td>H5N2</td>
<td>A/chicken/Queretaro/14588-19/1995</td>
</tr>
<tr>
<td>H5N8</td>
<td>A/turkey/Ireland/1378/1983</td>
</tr>
<tr>
<td>H5N3</td>
<td>A/tern/South Africa/1961</td>
</tr>
</tbody>
</table>
Results

- All virus-inoculated ducks became infected
- No clinical signs
- Some effect on body temperature and weight (not significant)
Virus shedding - LPAIV

A/mallard/Ohio/1987 H7N8 LPAIV

Serology: 8/8 positive. 4-6 log₂ HI titers
Transmitted to all contacts
Virus shedding - HPAIV

A/chicken/Chile/2002 H7N3 HPAIV

Serology 8/8 positive. 4-6 log₂ HI titers
Virus shedding - HPAIV

A/chicken/Jalisco/2012 H7N3 HPAIV

Serology: 8/8 positive. 4-7 log₂ HI titers

Characterization of the 2012 Highly Pathogenic Avian Influenza H7N3 Virus Isolated from Poultry in an Outbreak in Mexico: Pathobiology and Vaccine Protection

David F. Koppoczynski,† Mary Pantin-Jackwood,† Sofia G. Guzman,† Yadira Rioscorzo,† Erica Speckman,† Nancy Bertran,† David J. Stewart, David E. Swayne
Virus shedding - HPAIV

A/chicken/Queretaro/1995 H5N2 HPAIV

Serology 8/8. 3-8 log₂ HI titers
### Summary

<table>
<thead>
<tr>
<th>Type</th>
<th>Challenge virus</th>
<th>Duration of virus shedding</th>
<th>OP vs. CL</th>
<th>Transm. to contacts</th>
</tr>
</thead>
<tbody>
<tr>
<td>H7N8</td>
<td>A/mallard/Ohio/421/1987</td>
<td>14 d</td>
<td>CL</td>
<td>3/3</td>
</tr>
<tr>
<td>H7N7</td>
<td>A/mallard/Sweden/85/2002</td>
<td>14 d</td>
<td>CL</td>
<td>3/3</td>
</tr>
<tr>
<td>H7N3</td>
<td>A/chicken/Chile/184240-1/2002</td>
<td>14 d</td>
<td>CL</td>
<td>3/3</td>
</tr>
<tr>
<td>H7N3</td>
<td>A/chicken/Canada/314514-2/2005</td>
<td>14 d</td>
<td>CL</td>
<td>3/3</td>
</tr>
<tr>
<td>H7N3</td>
<td>A/chicken/Jalisco/CPA1/2012</td>
<td>14 d</td>
<td>CL</td>
<td>3/3</td>
</tr>
<tr>
<td>H7N7</td>
<td>A/chicken/Victoria/1985</td>
<td>11 d</td>
<td>CL</td>
<td>3/3</td>
</tr>
<tr>
<td>H7N7</td>
<td>A/chicken/North Korea/7916/2005</td>
<td>11 d</td>
<td>CL</td>
<td>3/3</td>
</tr>
<tr>
<td>H7N7</td>
<td>A/chicken/Netherlands/1/2003</td>
<td>11 d</td>
<td>=</td>
<td>3/3</td>
</tr>
<tr>
<td>H7N1</td>
<td>A/turkey/Italy/4580/1999</td>
<td>11 d</td>
<td>=</td>
<td>3/3</td>
</tr>
<tr>
<td>H5N2</td>
<td>A/chicken/Pennsylvania/1370/1983</td>
<td>14d</td>
<td>=</td>
<td>3/3</td>
</tr>
<tr>
<td>H5N2</td>
<td>A/chicken/Queretaro/14588/1995</td>
<td>4 d</td>
<td>OP</td>
<td>1/3</td>
</tr>
<tr>
<td>H5N8</td>
<td>A/turkey/Ireland/1378/1983</td>
<td>11d</td>
<td>OP</td>
<td>2/3</td>
</tr>
<tr>
<td>H5N3</td>
<td>A/tern/South Africa/1961</td>
<td>14 d</td>
<td>=</td>
<td>1/3</td>
</tr>
</tbody>
</table>
Could HPAIV-infected wild ducks transmit viruses to poultry?
Current Status of H7N3 HPAI in Mexico

Darrell R. Kapczynski

US DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Athens, Georgia
Historical perspective - 2012 HPAI Outbreak

- Jalisco region-layers mainly, responsible for appx 55% of all eggs produced in Mexico
- April 2012-High mortality in layer flocks. Initial fears of HP H5N2, so increased vax for H5. Continued mortality in flocks.
- June 13th, three outbreaks of H7N3 HPAI reported in Acatic and Tepatitlan.
- June 21st-1st official report to OIE of HP outbreak.
- Confirmed as H7N3.
- Stamping out policy/Vaccination for H7
- >22million birds died or depopulated.
- Declared controlled October 2012
- Est. cost $750 million 2012
- New outbreaks 2103
Characterization of HA from H7 AI isolates in America’s

HA1 HA2

Cleavage site

CPA CT/Mex/2817/06 PENPK
CPA Ck/Jalisco/CPA1/12 PENPKD/RKSRHRRTR/TRA

100% similar to chicken 28S gene

Likely a wild bird source for the LP to HP outbreak. The 2012 HP virus is a distant relative to the 2006 CT/Mex LP virus, but not likely a direct descendant.
Acute disease
Lesions in reproductive tract from laying hens affected by infection with virus H7N3

Dra. M. T. Casubon, Dr S Velazques 2013
Vaccine Control

- 2012-13 Government controlled vaccine production and distribution.
- 98% sequence similarity between vaccine and 2012 HPAI virus.
- Only three companies make vaccine based on CT/06 virus.
- In 2014 expanded to 6-10 vaccine companies.
- Growers submit swab samples from farm to receive vaccine.
- Growers now choose who to purchase H7N3 vaccine from.
- Recent isolates appear to drifting away from vaccine (<95% sequence similarity).
Antigenic cartography of H7 isolates
Egg production in layers following vaccination and challenge with H7N3 HPAI

![Graph showing egg production over time following vaccination and challenge.](image-url)
Conclusions

• This virus is still in Jalisco, and likely in other states. Thus still a direct concern to US poultry.

• The Mexican 2012 HPAI isolate was unique in that the basic amino acid sequences at HA cleavage site is derived from chicken RNA.

• Current Mexican H7 vaccine isolate fully protective against 2012 isolate but doubts in field about whether or not it is still efficacious. Reminiscent of H5N2 HPAI

• Vaccination protects from mortality but extends recovery of infectious virus. Role in transmission?

• Black market exists for 2nd and 3rd cycle layers.

• US eggs, egg products, and meat exports to Mexico have increased as a result of outbreaks of avian influenza.