HIGHLY PATHOGENIC H5 AVIAN INFLUENZA IN THE AMERICAS

SEPRL Avian Influenza Research Team
Southeast Poultry Research Laboratory
U.S. National Poultry Research Center, Athens, Georgia
• Since 1996 – H5N1 hemagglutinin changes – e.g. DRIFT (similar to human seasonal flu)
• Since 2008 – reassortment of NA genes (N5, N6, N8, N2, N3)
Origins H5N8 in Asia

- H5N8 Highly Pathogenic Avian Influenza was reported in South Korea in wild and domestic birds in early 2014 causing serious outbreaks in poultry
- Virus was reassortant with H5N1 goose/Guangdong lineage poultry virus and presumable a wild bird isolate
- HA cleavage site was typical of HPAI
- Isolates were classified as clade 2.3.4.4 viruses
- The H5N8 lineage of virus represents the third major spillover of HPAI from poultry to wild birds
H5N8 HPAIIV and derivatives: Eurasia

- South Korea
  - January 2014 reported in domestic birds
  - Numerous outbreaks through September 2014
- Japan
  - April 2014 H5N8
  - Outbreaks in chickens, swans, cranes and wild ducks
- China
  - October 2014 H5N8
  - Environmental sample and duck sample during routine surveillance
- Russia
  - December 2014
  - Wild duck isolate
- Europe
  - November 2014-Feb 2015 in Germany, Netherlands, Italy, UK, Hungary
- Taiwan
  - January 2015 H5N8, H5N2, and H5N23 in poultry
Movement of H5N8 into North America
New Eurasian H5 viruses in North America

The wholly Eurasian H5N8 virus has mixed with North American avian influenza viruses, creating new mixed-origin viruses: H5N2 and H5N1
HPAI Outbreaks by County

- H5N2 detected in Minnesota on March 4, 2015
- Outbreaks in turkeys and layer farms continued through June 2015
- Outbreaks in poultry contained in June with no new detections of virus reported
12/8/2015 to 6/12/2015 – H5 HPAIV in wild bird, backyard poultry and commercial poultry

- 309 detections (4 captive wild bird; 21 backyard; 209 commercial flocks, 75 wild birds)
  - 21 states affected (AR, CA, IA, ID, IN, KS, KY, MI, MN, MO, MT, NE, ND, NM, NV, OR, SD, UT, WA, WI, WY)
  - ~48.6 million commercial birds: Turkeys ~7.5 million, Chickens ~41.1 million
Why is this outbreak so much different than previous outbreaks

• Wild bird reservoir
  • First time in U.S. dealing with wild bird reservoir of HPAIV
  • The prevalence of infection in some wild duck species is higher than would normally be expected for a low pathogenic isolate
  • Allowed exposure of poultry to HPAIV in over 20 states

• Size of farms
  • Egg layer operations in midwest are typically a million+ bird operation and have lots of movement on and off the farms
  • Once virus introduced onto farm, the virus will adapt

• Logistical issues
  • Difficult to depopulate that large numbers of birds
  • Difficult to dispose of that large number of birds
  • Budget cuts at USDA reduced numbers of veterinarians
Public Health

- Currently no human cases of H5N8 or H5N2 viruses
- However, these viruses have genes from the Asian H5N1 lineage known to cause rare human infections, but with high case fatality rate
  - Human to human transmission extremely rare for H5N1 viruses
  - Unknown whether the genetic changes in these strains make them more or less of threat to human health
  - Initial studies in mammals show less virulence for H5N8 than H5N1 viruses
- Sensitive to Tamiflu and other antivirals based on *in vitro* testing
  - Treatment should be started as early as possible
- PPE needs to be used as if this were known to be human infectious
Pathogenesis and Transmission

Determine any differences in infectivity, transmissibility & pathogenesis of the intercontinental H5Nx clade 2.3.4.4 HPAIV for gallinaceous poultry and mallard ducks

Approach

- Used two earliest USA isolates:
  - A/Gyrfalcon/Washington/41088/2014 (H5N8)
  - A/Northern Pintail/Washington/40964/2014 (H5N2)
- Intranasal infectious dose and contact transmission
  - SPF White Leghorn chickens
  - Commercial broad-breasted white turkeys
  - Commercial Japanese quail, pheasant, Bob-white quail
  - Mallard ducks
- Pathogenesis studies: preclinical, clinical & dead birds for virus shedding, histopathology and IHC-virus distribution
Infectious dose and virus transmission in chickens, turkeys and quail

Challenge with $10^2 \times 10^4, 10^6$ EID$_{50}$ of H5N8 and H5N2

Add 3 contacts to each dose group

Oropharyngeal and cloacal swabs collected for virus detection

Exposure by simulated respiratory (natural) route of infection.

Daily: Record mortality, clinical signs, gross lesions

Virus detected by quantitative real-time RT-PCR assay and virus isolation

Serology to determine infection status
## Chickens: Infectious dose and transmission

<table>
<thead>
<tr>
<th></th>
<th>Log 10 Dose</th>
<th>Inoculated dead/total (MDT)</th>
<th>Contact dead/total (MDT)</th>
<th>CLD50 Log 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Gyrfalcon/Washington/4108 8/2014 H5N8 Dec 2014</td>
<td>2</td>
<td>0/5</td>
<td>0/3</td>
<td></td>
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<tr>
<td></td>
<td>4</td>
<td>2/5 (4d)</td>
<td>0/3</td>
<td>4.3</td>
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<tr>
<td></td>
<td>6</td>
<td>5/5 (4.1d)</td>
<td>0/3</td>
<td></td>
</tr>
<tr>
<td>A/Northern Pintail/Washington/40964/2014 H5N2 Dec 2014</td>
<td>2</td>
<td>0/5</td>
<td>0/3</td>
<td></td>
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<td>0/5</td>
<td>0/3</td>
<td>5.7</td>
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<tr>
<td></td>
<td>6</td>
<td>3/5 (3d)</td>
<td>0/3</td>
<td></td>
</tr>
<tr>
<td>A/Tk/AR/7791/2015 H5N2 March 2015</td>
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<td>0/3</td>
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<td>0/5</td>
<td>0/3</td>
<td>5.1</td>
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<tr>
<td></td>
<td>6</td>
<td>8/9 (2.3d)</td>
<td>0/2</td>
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<td>0/3</td>
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<td>3.5</td>
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<tr>
<td></td>
<td>6</td>
<td>8/8(2d)</td>
<td>2/2</td>
<td></td>
</tr>
</tbody>
</table>
Pathogenesis: Chickens

• **Clinical Signs**
  - Mild illness (~2-3d): ruffled feathers, listlessness, eyes partially closed
  - Severe illness including neurological signs by 4 DPI

• **Mortality**
  - 100% in birds that were infected
  - MDT=3-4 Days

• **Gross lesions**
  - Not observed in all chickens
  - Necrotic combs/wattles, hemorrhages on shanks, necrotic pancreas, splenomegaly with pale color, petechial hemorrhages on the myocardium and skeletal muscle, enlarged pale kidneys, periorbital edema
### Turkeys: Infectious dose and transmission

- **H5N8**: TLD$_{50}$ = 4-6 (~5) log$_{10}$, 100% transmission to contacts at 6 log$_{10}$ dose
- **H5N2**: TLD$_{50}$ = 4-6 (~5) log$_{10}$, 100% transmission to contacts at 6 log$_{10}$ dose

<table>
<thead>
<tr>
<th></th>
<th>Log 10 Dose</th>
<th>Inoculated dead/total (MDT)</th>
<th>Contact dead/total (MDT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Gyrfalcon/Washington/41088/2014 H5N8 Dec 2014</td>
<td>2</td>
<td>0/5</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0/5</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5/5 (9)</td>
<td>3/3 (12.5)</td>
</tr>
<tr>
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<td>0/5</td>
<td>0/3</td>
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<tr>
<td></td>
<td>4</td>
<td>0/5</td>
<td>0/3</td>
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<tr>
<td></td>
<td>6</td>
<td>5/5 (5.3)</td>
<td>3/3 (7.3)</td>
</tr>
</tbody>
</table>
Pathogenesis: Turkeys H5N2

• Susceptibility
  • Only those exposed to the highest dose were infected
  • 100% infection in contact transmission turkeys

• Mortality
  • 100%
  • MDT 5.3 days (long)

• Signs
  • Most had none until near death
    • Severe lethargy
    • Huddling, ruffled feathers
    • Neurological signs (torticollis)

<table>
<thead>
<tr>
<th>Dose (Log 10)</th>
<th>Inoculated dead/total (MDT)</th>
<th>Contact dead/total (MDT)</th>
</tr>
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<tbody>
<tr>
<td>H5N2</td>
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<tr>
<td>2</td>
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<td>0/3</td>
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<td>4</td>
<td>0/5</td>
<td>0/3</td>
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<tr>
<td>6</td>
<td>5/5 (5.3)</td>
<td>3/3 (7.3)</td>
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</tbody>
</table>
Pathogenesis: Turkeys H5N8

- Susceptibility
  - Only those exposed to the highest dose were infected
  - 100% infection in contact transmission turkeys

- Mortality
  - 100%
  - MDT 9 days (very long)

- Signs
  - Disease not apparent until they are close to death
  - Severe lethargy, huddling, ruffled feathers

- Gross lesions
  - No lesions typically associated with AIV

- Virus shedding: pending

<table>
<thead>
<tr>
<th>Log 10 Dose</th>
<th>Inoculated dead/total (MDT)</th>
<th>Contact dead/total (MDT)</th>
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<td></td>
<td>4</td>
<td>0/5</td>
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<tr>
<td></td>
<td>6</td>
<td>5/5 (9)</td>
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</tbody>
</table>
Pathogenicity and transmission of H5N8 and H5N2 HPAI viruses in Mallards

Objective: To examine the pathogenicity (infectious dose, clinical signs, lesions, duration of virus shedding) and transmission of the H5N8 and H5N2 HPAIV’s in mallards, comparing to an Asian lineage H5N1 HPAIV wild bird isolate and an American lineage LPAIV.

Study design:
• Two-week-old mallards (*Anas platyrhynchos*)
• Viruses:
  • A/GyrFalcon/Washington/41088/2014 (H5N8)
  • A/NPintail/Washington/40964/2014 (H5N2)
  • A/Whooper swan/Mongolia/244/2005 (H5N1)
<table>
<thead>
<tr>
<th>Challenge Dose</th>
<th>Log 10 Challenge Dose</th>
<th>Inoculated #pos/total</th>
<th>Mortality #pos/total</th>
<th>Contact #pos/total</th>
<th>DLD50 Log 10</th>
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<tr>
<td></td>
<td>4</td>
<td>5/5</td>
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<td>0/5</td>
<td>3/3</td>
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<tr>
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<td>7.5</td>
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<td>0/5</td>
<td>3/3</td>
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<td>3/3</td>
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<td>7.5</td>
<td>5/5</td>
<td>0/5</td>
<td>3/3</td>
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<td>4</td>
<td>5/5</td>
<td>0/5</td>
<td>3/3</td>
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<td>6</td>
<td>5/5</td>
<td>0/5</td>
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<td></td>
<td>7.5</td>
<td>5/5</td>
<td>0/5</td>
<td>3/3</td>
<td></td>
</tr>
<tr>
<td>A/Whooper swan/Mongolia/244/2005 H5N1</td>
<td>6</td>
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</tbody>
</table>

10/10 10/10
Japanese Quail: Infectious dose and transmission

- **H5N8**: $\text{QLD}_{50} = 2-4 \ (\sim 3) \ \log_{10}$, no transmission to contacts
- **H5N2**: $\text{QLD}_{50} = 3.6 \ \log_{10}$, 67% transmission to contacts at 6 $\log_{10}$ dose

<table>
<thead>
<tr>
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<th>Log 10 Dose</th>
<th>Inoculated dead/total (MDT)</th>
<th>Contact dead/total (MDT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H5N8</td>
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<td>0/3</td>
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<tr>
<td></td>
<td>4</td>
<td>5/5 (2.5)</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4/5 (2.5)</td>
<td>0/3</td>
</tr>
<tr>
<td>H5N2</td>
<td>2</td>
<td>1/5 (3)</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3/5 (3)</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4/5 (2.75)</td>
<td>2/3</td>
</tr>
</tbody>
</table>
Pathogenesis: Japanese quail

• Signs
  • Clinical signs – listlessness, ruffled feathers “sunk” eyes

• Mortality
  • 100% in birds that were infected
  • MDT=2.5-3 Days

• Gross lesions
  • Not observed in all quail
  • Mildly sick birds: necrotic pancreas, hemorrhagic duodenal loop, splenomegaly and mottled spleen; petechial hemorrhages on the myocardium; pulmonary hemorrhage
  • Found dead birds: similar lesions as above but more pronounced plus petechial hemorrhages on the proventricular (glandular stomach) mucosa
Conclusions

- The two early 2.3.4.4 HPAIV strains were not well adapted to gallinaceous poultry
  - $BID_{50}: 100-1,000$ times higher than previous H5N1 HPAIVs
  - Inefficient transmission to contacts except when placed with high challenge dose group
- Birds that became infected = died ($BID_{50} = BLD_{50}$)
- Longer time to death than historic H5N1 HPAIV
- More restricted virus replication and lesions
- Susceptibility (including mallard study)
  - Mallards > Japanese Quail > Turkeys > Chickens
- Later isolates were more adapted to chickens with lower $BID_{50}$
Conclusions

• The H5Nx viruses were able to experimentally infect and transmit easily in mallard ducks with no clinical disease
• Supports the role of wild birds in moving the virus to North America
• Initial outbreaks in poultry in U.S. appear to be point source introductions with little farm to farm spread of virus
• Later outbreaks have evidence of farm to farm spread as major contributor to spread
• Control efforts in U.S. remain as early detection and stamping out of infected flocks
• Virus is evolving, likely in part to the high density of birds on the farms it has affected
## Infectious dose and transmission - Original H5N2 and H5N8 viruses

<table>
<thead>
<tr>
<th>Species</th>
<th>% Mortality</th>
<th>MDT (days)</th>
<th>BLD$<em>{50}$ (log$</em>{10}$)</th>
<th>Transmission to contacts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chickens</strong> <em>(Gallus Gallus)</em></td>
<td>60-100</td>
<td>3 - 4</td>
<td>4.3-5.7</td>
<td>No or only in $10^6$ groups</td>
</tr>
<tr>
<td><strong>Turkeys</strong> <em>(Meleagris gallopavo)</em></td>
<td>100</td>
<td>5.3 - 9</td>
<td>5</td>
<td>Only in $10^6$ groups</td>
</tr>
<tr>
<td><strong>Japanese Quail</strong> <em>(Coturnix japonica)</em></td>
<td>80</td>
<td>2.5 - 3</td>
<td>3.0 - 3.6</td>
<td>Only H5N2 in $10^6$ group</td>
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<tr>
<td><strong>Pheasants</strong> <em>(Phasianus colchicus)</em></td>
<td>100</td>
<td>4.7 - 4.8</td>
<td>3.0 - 3.4</td>
<td>Yes, in $10^4$ and $10^6$ groups</td>
</tr>
<tr>
<td><strong>Partridge</strong> <em>(Alectoris chukar)</em></td>
<td>100</td>
<td>4.1 - 5.2</td>
<td>3.6</td>
<td>Yes, in $10^4$ and $10^6$ groups</td>
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<tr>
<td><strong>Pekin ducks</strong> <em>(Anas platyrhynchos var. dom.)</em></td>
<td>0</td>
<td>-</td>
<td>BID$<em>{50}$ = 3 log$</em>{10}$</td>
<td>Yes, in $10^4$ and $10^6$ groups</td>
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<tr>
<td><strong>White Chinese Geese</strong> <em>(Anser cygnoides)</em></td>
<td>25</td>
<td>7-7.5</td>
<td>&gt; 6</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Mallards</strong> <em>(Anas platyrhynchos)</em></td>
<td>0</td>
<td>-</td>
<td>BID$<em>{50}$ ≤ 2 log$</em>{10}$</td>
<td>Yes, in all groups</td>
</tr>
</tbody>
</table>

MDT=mean death time; BLD$_{50}$=Bird lethal dose$_{50}$
Other Research Activities

- Vaccines-covered by David Swayne
- Diagnostics
  - Real-time RT-PCR test specific for 2.3.4.4 lineage to provide rapid confirmation of HPAI infection
  - N2 antibody ELISA test to support DIVA vaccinations
- Wildbird surveillance
  - Sample testing from California and Alaska
- Network analysis of sequence information
Modifying C&D: on-farm inactivation of HPAIV

- Can we reduce time and cost of C&D by “cooking” the virus in the poultry house?
  - Similar to what we do for LT
  - Will test a range of temps and litter conditions
    - High temperatures not possible in colder regions
    - Does litter moisture affect time needed for inactivation
  - Develop inactivation curves
  - Aim to look at NDV as well
- Funded by USPEA and conducted by Spackman lab
Future

- Anticipate persistence of H5Nx HPAI in wild birds for at least 3-5 years
- Potential for introduction anywhere in the U.S.
- Increase in biosecurity and surveillance in poultry
- Increase surveillance in wild birds
- Increased resources to USDA to more quickly react to multiple outbreaks if they occur
- Research to better understand the virus and provide improved control tools
Contributors

• **SEPRL Avian Influenza Research Team:** David Suarez, Mary Pantin-Jackwood, Erica Spackman, Darrell Kapczynski, David Swayne, Kateri Bertran, Mar Costa-Hurtado, Donghun Lee, Marisela Rodriguez, Yue Wang, Eric DeJesus, Charles Balzli, Kira Moresco, Diane Smith, Aniko Zsak, Scott Lee, Suzanne Deblois, Cam Greene, James Doster, Megan Christian, Nicolai Lee, Rebekah Lee, Samantha Pallas, Melissa Scott, Bill Gagnon, Roger Brock, Ronald Graham, Gerald Damron, Keith Crawford

• **Industry Veterinarians:** David Rives, Eric Gonder, Raul Otalora

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