Vaccine and Disease Research Responses to the 2015-2016 HPAIV Outbreaks

SEPRL Avian Influenza Research Team

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Southeast Poultry Research Laboratory

- Agricultural Research Service (ARS) is the intramural research arm of the United States Department of Agriculture
- SEPRL is the ARS laboratory located in Athens, GA that studies Avian influenza and Newcastle disease virus, both Select Agents requiring BSL-3 facilities
- Reference Center on avian influenza and Newcastle disease virus for the World Organization for Animal Health (OIE) and the Food and Agriculture Organization (FAO)
SEPRL Influenza Outbreak Response

- Avian influenza is highly variable in the disease and mortality that it can cause depending on host, age, physiologic status, and environmental factors
- Controlled animal studies are needed to determine the species most susceptible to infection, typical disease pattern, and likelihood for transmission
- Diagnostic tests need to be evaluated for sensitivity and specificity and new tests may need to be developed
- Vaccines available in the near term need to be evaluated for possible use during an outbreak
Influenza A Virus

Segmented RNA virus
10 to 12 influenza proteins

9 proteins packaged in virion
HA, NA, M2-surface proteins
NP, PA, PB1, PB2, M1 and NS2 internal proteins

17 HA subtypes
10 NA subtypes

NS1 not packaged in virion
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 for initial studies:
  • 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** $10^2$, $10^4$, $10^6$ EID$_{50}$ of H5N8 and H5N2

**Add 3 contacts to each dose group**

**Daily: Record mortality, clinical signs, gross lesions**

**0 1 2 3 4 5 6 7 8 9 10 11-14 days**

**Oropharyngeal and cloacal swabs collected for virus detection**

**Exposure by simulated respiratory (natural) route of infection.**

**Serology to determine infection status**

Virus detected by quantitative real-time RT-PCR assay and virus isolation
## Chickens: Infectious dose and transmission

<table>
<thead>
<tr>
<th>Strain</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</td>
<td>2</td>
<td>0/5</td>
<td>0/3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2/5 (4d)</td>
<td>0/3</td>
<td>4.3</td>
</tr>
<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</td>
<td>2</td>
<td>0/5</td>
<td>0/3</td>
<td></td>
</tr>
<tr>
<td>A/Northern Pintail/Washington/40964/2014</td>
<td>4</td>
<td>0/5</td>
<td>0/3</td>
<td>5.7</td>
</tr>
<tr>
<td>A/Northern Pintail/Washington/40964/2014</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>
<td>2</td>
<td>0/5</td>
<td>0/3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0/5</td>
<td>0/3</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>8/9 (2.3d)</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td>A/Tk/MN/12582/2015 H5N2 April 2015</td>
<td>2</td>
<td>0/5</td>
<td>0/3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3/5 (2d)</td>
<td>0/3</td>
<td>3.5</td>
</tr>
<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:** $TLD_{50} = 4-6 \, (\sim 5) \, \log_{10}$, 100% transmission to contacts at 6 $\log_{10}$ dose
- **H5N2:** $TLD_{50} = 4-6 \, (\sim 5) \, \log_{10}$, 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/41 088/2014 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>
<td>A/Northern Pintail/Washington/40964/2 014 H5N2 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 (5.3)</td>
<td>3/3 (7.3)</td>
</tr>
<tr>
<td></td>
<td>Log 10 Challenge Dose</td>
<td>Inoculated #pos/total</td>
<td>Mortality #pos/total</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------------------</td>
<td>-----------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td><strong>A/Gyrfalcon/Washington/41 088/2014 H5N8 H5N8 Dec 2014</strong></td>
<td>2</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td><strong>A/Northern Pintail/Washington/40964/2014 H5N2 Dec 2014</strong></td>
<td>2</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td><strong>A/Whooper swan/Mongolia/244/2005 H5N1</strong></td>
<td>6</td>
<td>10/10</td>
<td>10/10</td>
</tr>
</tbody>
</table>
## Infectious dose and transmission - Original H5N2 and H5N8 viruses

<table>
<thead>
<tr>
<th>Species</th>
<th>% Mortality</th>
<th>MDT (days)</th>
<th>BLD&lt;sub&gt;50&lt;/sub&gt; (log&lt;sub&gt;10&lt;/sub&gt;)</th>
<th>Transmission to contacts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickens <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>Turkeys <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>Japanese Quail <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>
</tr>
<tr>
<td>Pheasants <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>Partridge <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>
</tr>
<tr>
<td>Pekin ducks <em>(Anas platyrhnchos var. dom.)</em></td>
<td>0</td>
<td>-</td>
<td>BID&lt;sub&gt;50&lt;/sub&gt; = 3 log&lt;sub&gt;10&lt;/sub&gt;</td>
<td>Yes, in $10^4$ and $10^6$ groups</td>
</tr>
<tr>
<td>White Chinese Geese <em>(Anser cygnoides)</em></td>
<td>25</td>
<td>7-7.5</td>
<td>&gt; 6</td>
<td>Yes</td>
</tr>
<tr>
<td>Mallards <em>(Anas platyrhnchos)</em></td>
<td>0</td>
<td>-</td>
<td>BID&lt;sub&gt;50&lt;/sub&gt; ≤ 2 log&lt;sub&gt;10&lt;/sub&gt;</td>
<td>Yes, in all groups</td>
</tr>
</tbody>
</table>

MDT=mean death time; BLD<sub>50</sub>=Bird lethal dose<sub>50</sub>
Conclusions

• The two early 2.3.4.4 HPAIV strains were not well adapted to gallinaceous poultry
  • BID\textsubscript{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\textsubscript{50} = BLD\textsubscript{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\textsubscript{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
- Virus is evolving, likely in part to the high density of birds on the farms it has affected
- Concern for persistence in wild bird population
Diagnostic tests

- Developed a real-time RT-PCR test to rapidly identify the clade 2.3.4.4 H5 gene as a rapid screening tool
- Developed an N2 antibody ELISA test
  - Baculovirus expressed protein for efficient and clean protein expression
  - ELISA accurately detects infected birds
  - Some cross reactivity with other influenza subtypes, but not N1 subtype
  - A N8 test is also in development
  - A competitive ELISA test is being developed that should make the test more specific and allow the test to be a multi-species test
Purpose of Vaccination

• Control of clinical disease (Vaccines don’t prevent infection)
  • Many countries use vaccination as a way to live with disease (China, Egypt, Indonesia, Mexico)

• Eradicate pathogen by breaking the transmission chain
  • Only select pathogens are suitable for eradication
  • Avian influenza outbreak lineages in poultry are eradicable
  • Vaccination can contribute to eradication
Value of Vaccination

• Vaccines can help eradicate an outbreak if properly implemented

• **Significant reduction of virus shedding is a key for control**
  • Decreases opportunity for transmission
  • Reduces potential of zoonotic infection

• Increased biosecurity, surveillance, quarantines, animal movement controls and education are still required if eradication is the goal

• Several countries that have become endemic for HPAI H5N1 have used vaccination as primary control tool
Negatives to vaccination

• Trade exports are negatively effected by vaccination!
  • Many countries have either total ban or partial ban on poultry exports because of HPAI
  • Most countries will further restrict imports of U.S. poultry if we vaccinate for at least temporary period
• Countries will use HPAI as a non-tariff trade barrier whether justified or not
• Vaccines have a cost
  • Production
  • Administration—often more than cost of vaccine
• Vaccination programs need to fit the industry requirements
Are Available Vaccines Adequate as Part of Eradication Program?

- Initial vaccine trials performed starting in January 2015 using representative H5N8 and H5N2 viruses
- Tested selected licensed vaccines or seed strains
  - Fowlpox vector with Turkey/Ireland/1983 insert
  - Herpesvirus of turkeys with Swan/Hungary/2006 insert
  - Licensed seed strains including Turkey/WI/1968 and TK/CA/2002 and several foreign RG vaccines
  - RNA particle vaccine with GyrFalcon/WA/1014 insert
  - Reverse genetics vaccine GyrFalcon/WA/1014 insert
- Key determinants to measure success of vaccine
  - Protection from clinical disease
  - Reduction in viral shedding in vaccinated birds compared to controls
US HPAI VACCINE STUDIES

Notice: Vaccines studies were funded by the USDA, and USDA derives no economic benefit from the use of any of the vaccines described, and does not endorse any specific vaccine

SEPRL & NVSL/NADC have completed over 25 H5 Vaccine Studies in support of outbreak
HA nucleotide sequence and phylogenetic analysis of vaccine isolates

- A-gyrfalcon-Washington-41088-6-2014
- A-chicken-Oregon-41613-2-2014
- A-baikal teal-Korea-Donglim3-2014
- A-turkey-Germany-R2474-L00899-2014
- A-chicken-Netherlands-14015526-2014
- A-duck-GD-S1322-2010 H5N1
- A-duck-Anhui-1-2006 H5N1
- A-swan-Hungary-4999-2006 H5N1
- A-turkey-Ireland-1983 H5N8
- A-turkey-Wisc-1968 H5N9
- A-turkey-CA-2002 H5N2

- Green = RE vaccines
- Blue = Commercial recombinant
- Red = USDA LPAI H5 seed isolates
Mortality:

Inactivated H5N8/H5N2 HPAI Vaccine

Homologous

- iH5N8xH5N8
- iH5N2xH5N8
- iH5N8xH5N2
- iH5N2xH5N2

Inactivated USDA H5 Master Seed

- Tk/Wisc xH5N8
- Tk/CA xH5N8
- Tk/CA xH5N2
- Tk/Wisc xH5N2

Inactivated Re5/Re6

Chinese

- Re5xH5N8
- Re6xH5N8
- Re5xH5N2
- Re6xH5N2
Interim Conclusions After Initial Studies

- Trials were not necessarily designed for field application
- Homologous vaccination worked the best, but you can’t use HPAI as vaccine seed in U.S.!
- North American H5 seed strains provide only partial protection and not recommended alone
- Licensed viral vectors provided only partial protection when administered as single dose
- Chinese reverse genetics viruses are closer genetically to U.S. isolates and provided better protection
- Matching the vaccine to field virus is always recommended!
- Consider alternative vaccines
2nd Generation Vaccine Trials

• Consideration of vaccines likely to be licensed and available quickly
• Requires commitment of manufacturer to license in the absence of defined vaccine market in the U.S.
• SEPRL, with financial and technical support from APHIS, committed to evaluating the most promising vaccines because of the ongoing outbreak
• Consideration for experimental design that could be practical for field use in layer chicken, broiler chicken, or meat turkey industries
• Changed challenge strain to TK/MN/15 which was more virulent and infectious (more stringent challenge)
Highly Pathogenic Avian Influenza Virus

Extract RNA

RT-PCR and cloning

Modify to Low pathogenic cleavage site

Inactivate virus and prepare vaccine

Grow vaccine in eggs

Rescue in cell culture

Vaccinate Chick!

PR8 backbone

Combine +

Vaccinate Chick!
# Age of Biotechnology for Avian Influenza Vaccines

<table>
<thead>
<tr>
<th>Recombinant Vectored-Live</th>
<th>Reverse Engineered</th>
<th>RNA Particle</th>
<th>Plasmid DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEVA, Merial</td>
<td>Zoetis</td>
<td>Harris Vaccine</td>
<td>Benchmark Biolabs</td>
</tr>
<tr>
<td>HVT</td>
<td>Reverse genetics H5</td>
<td>Alphavirus RPH5</td>
<td>AIV H5 Mod DNA</td>
</tr>
<tr>
<td>FPV</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

- **H5-hemagglutinin genetically & antigenically match the outbreak virus**
- **Can rapidly change hemagglutinin gene**
- **Rapidly obtain conditional license for non-replicating vaccines, unclear of speed for licensing of recombinant vaccines**
Results

• Homologous and the RG-Gyr Falcon killed adjuvanted vaccines provide excellent results with no clinical disease and large reduction in viral shedding after single vaccination in SPF chickens and commercial turkeys
• HVT-AI and Fowlpox vectored vaccines with partially matched hemagglutinin gene inserts had only partial clinical protection and high virus shedding
• RP studies provided good short term protection with single vaccination and strong protection with prime/boost approach
• Expectation that multiple vaccines needed in layer chickens and turkeys
Vaccine-Conclusions

• Vaccine response is related both to clinical protection and viral shedding if vaccinated birds are infected
• Homologous killed vaccines provided best protection
• Other killed vaccines had good clinical protection but concerns about levels of virus shedding
• Vectored vaccines with partially matched hemagglutinin had marginal protection on their own
• Interest in being able to Differentiate vaccinated from vaccinated and then infected animals (DIVA) vaccines
Practical Vaccination by Sector

- Turkeys, layers, broilers, and others
  - Broilers - Single vaccination in ovo or at day of age
  - Layers - Expectation of vaccination at day of age and some kind of booster at 3 or 4 weeks of age. Likely an additional booster will be needed
  - Turkeys - Expectation of vaccination at day of age in hatchery and some kind of booster at 3 or 4 weeks of age.
  - Gamebirds, waterfowl, zoo birds, falconry
DIVA

- **Differentiate** Infected from Vaccinated Animals
- **DIVA** principle primary application is to assure trading partners that livestock have not been exposed to infectious virus i.e. **differentiate vaccinated only and vaccinated and then infected poultry**
- Can also be used as surveillance tool for low virulence AIV to determine incidence of infection when vaccination is used
- Inexpensive, reliable, and high throughput differential serologic test needed to make DIVA surveillance testing viable
- For countries that do not export poultry, DIVA vaccination probably not a major priority
Future

- Other vaccine technologies may be on the market soon including baculovirus expressed proteins
- HVT-AI and fowlpox vaccines may be updated with H5Nx H5 gene and improved results are expected
- If vaccines are used they must greatly reduce virus shedding!
- Serologic DIVA surveillance should be possible that may help regain export markets if vaccination is used
- Must generate data on DIVA surveillance to get internationally recognized
Contributors


- **NVSL:** Mia Torchetti

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

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