6th Annual Biopreparedness Symposia Series
Protecting Our Heartland and Its Resources

Sidney - April 15 - 17, 2008
Kearney - May 6 - 8, 2008
Norfolk - June 3 - 5, 2008
Omaha - July 8 -10, 2008
Avian Influenza (AI)

- Review of Avian Influenza (AI)
- Nebraska’s Poultry Industry
- Surveillance
- Response
- Asian Strain H5N1 vs North American Strain
Avian Influenza (AI)

- Review of Avian Influenza (AI)
- Nebraska’s Poultry Industry
- Surveillance
- Response
- Asian Strain H5N1 vs North American Strain
Review of Avian Influenza

- Primarily a disease of birds
- Reservoir is in wild waterfowl, shore birds, and gulls
- Can infect many different species
- Ability to change slowly or rapidly
- Disease ranges from no visible signs (low path) to 100% mortality (high path)
Influenza Etiology

- Family: Orthomyxoviridae
- Genus
  - Influenza Type A* – humans, equine, swine, and avian
  - Influenza Type B – humans only
  - Influenza Type C – humans only
- *Influenza A the only type of significance in animals

Source: http://virology-online.com/presentations/Respiratory.ppt#283,13,Parainfluenza Virus
Two Surface Antigens

H - Hemagglutinin (1 -16)
N - Neuraminidase (1- 9)
Type A Influenza

- Orthomyxovirus
- RNA
- Single stranded
- Segmented
- Enveloped
### Type A Influenza Surface Antigens

**Hemagglutinin (H) Surface Antigens**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equine</td>
<td>x</td>
<td></td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swine</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avian</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>
### Type A Influenza Surface Antigens (continued)

#### Neuraminidase (N) Surface Antigens

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equine</td>
<td></td>
<td></td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swine</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avian</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>
Type A Influenza
Surface Antigens

16H X 9N = 144 combinations!

Only H5 or H7 have the potential to mutate to Highly Pathogenic Avian Influenza (HPAI)
Avian Influenza Distribution

All AI Subtypes

H5/H7 Subtypes

Highly Pathogenic H5/H7 Subtypes
Avian Influenza Viruses Have the Ability to Change

- **Antigenic Drift**: Changes in H or N - accumulated point mutations, nucleotide substitutions, insertions, and deletions
  
  *(Slow)*

- **Antigenic Shift**: Replacement of H or N - gene reassortment.
  
  *(Fast)*
Reassortment
(antigenic shift; how pandemic viruses are born)

RNA Segments + Major Antigen → Human adapted strain + H5N1 in poultry = Transmits among humans, Ag type from birds
Cross Species Spread of Flu

Swine not necessary (?)
Mechanism of pathogenicity in poultry

- Proteolytic enzymes (proteases) produced by host cells cleave the HA sequence to allow virus replication

- LPAI - proteases present in the respiratory or intestinal tracts, the infection remains localized

- HPAI - proteases present in many types of tissue, infection spreads systemically
Avian Influenza
Determining Pathogenicity

- Requires a live virus (isolate)
- Virus subtype determined by HI & NI tests
- If H5 or H7 – characterization is done by
  - chicken pathogenicity test, and
  - amino acid (genetic) sequence at the cleavage site.
Determining Pathogenicity continued

1. Chicken Pathogenicity Test
   - inoculate eight chickens (4 to 6 weeks of age) intravenously and observe for 8 days.
   - if 6, 7, or 8 chickens die, classified as “highly pathogenic avian influenza” (HPAI)
   and/or

2. Sequencing at cleavage sites
AIV Transmission

- Direct or indirect contact with these excretions or secretion
- No evidence of true vertical transmission
- Airborne transmission >100 yards seems unlikely
- Temperature and moisture levels have a marked effect on survivability of virus outside the host
- Infected birds shed virus in feces and respiratory secretions, primarily the first 7-10 days
AIV Transmission in Poultry

- Spread is largely by people who have direct contact with birds and their feces
- Farm-to-farm spread by movement of infected poultry or virus-contaminated equipment, materials, etc.
- Examples of high risk activities:
  - Live haul (birds & equipment)
  - Movement & spreading of litter
  - Dead bird disposal (rendering services)
  - Insemination & vaccination crews
Spectrum of Avian Influenza

Low pathogenic
- None to mild disease
- No clinical signs

Highly pathogenic
- Acute, systemic disease
- 100% Mortality
2007 Cases of H5 or H7 LPAI in the United States

- West Virginia – March – H5N2 - Turkeys
- Minnesota – April – H7N9 - Turkeys
- South Dakota – June – H5N2 - Geese
- Nebraska – June – H7N9 - Turkeys
- Virginia – July - H5N1 – Turkeys
- South Dakota – November H5N2 - Turkeys
Instances in which LPAI Viruses of Subtype H5 and H7 Mutated into HPAI viruses

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mexico</td>
<td>1994-1995</td>
</tr>
<tr>
<td>Italy</td>
<td>1999</td>
</tr>
<tr>
<td>Chile</td>
<td>2002</td>
</tr>
<tr>
<td>British Columbia, Canada</td>
<td>2004</td>
</tr>
</tbody>
</table>
Three HPAI Findings in U.S.

- 1924 - “Fowl Plague” affected live bird markets in the Northeastern U.S. (H7N?)
- 1983 - destruction of 17 million birds in Pennsylvania (H5N2)
- 2004 - quickly contained and eradicated in Texas (H5N2)
Disease in Poultry

- Strain dependent
- Runs the extremes
  - **Low Pathogenic**
    - Sub-clinical infection
    - Primarily respiratory
    - Decreased egg production
  - **Highly Pathogenic**
    - Apocalyptic: 100% mortality within 24 hours of exposure (no time for clinical signs to develop)
Avian Influenza (AI)

- Review of Avian Influenza (AI)
- Nebraska’s Poultry Industry
- Surveillance
- Response
- Asian Strain H5N1 vs North American Strain
Nebraska’s Poultry Industry
Nebraska’s Poultry Industry

1. Layer industry >12 million laying (7th) hens producing >3 billion eggs/year
2. Broiler industry produces > 16 million birds/year
3. Turkey industry produces >3.5 million birds/year
4. Farm flocks/Backyard Flocks/Hobby Flocks - unknown numbers -
5. Gamebirds 120 Controlled Shooting Area
Nebraska’s Layer Industry

- 12 million hens (7th in US)
- 3 billion eggs/year
  All eggs go for further processing
- Michael Foods, Wakefield
- Henningsen Foods, Omaha
- 22 contract growers
- 1 independent egg producer
Nebraska’s Turkey Industry

Nebraska Turkey Grower Cooperative
- 13 independent producers
- 3.5 million turkeys
- 53 million lbs. annually
- processing plant in Gibbon
Nebraska’s Broiler Industry

- MBA Poultry
  - 14 million Veg-Fed broilers - live weight 81 million lbs
  - 2 million Organic Broilers - live weight 11 million lbs
Back Yard/Hobby

2 Chicken hatcheries
Unknown number of:
- Farm flocks
- Hobby flocks
- Exhibition birds
- Game fowl
- Other???
Gamebird Industry
120 Controlled Shooting Areas
KFC witness

protection program
Avian Influenza (AI)

- Review of Avian Influenza (AI)
- Nebraska’s Poultry Industry
- Surveillance
- Response
- Asian Strain H5N1 vs North American Strain
Surveillance for Avian Influenza
Notifiable Avian Influenza (NAI)

“Avian influenza in its notifiable form (NAI) is defined as an infection of poultry *caused by any influenza A virus of the H5 or H7 subtypes or by any AI virus with an intravenous pathogenicity index (IVPI) greater than 1.2 (or as an alternative at least 75% mortality) as described...* NAI viruses can be divided into highly pathogenic notifiable avian influenza (HPNAI) and low pathogenicity notifiable avian influenza (LPNAI)”
Federal Agencies

- US Department of Health and Human Services (DHHS)
- US Department of Agriculture (USDA)
- US Department of the Interior (DOI)

State Agencies

- Nebraska Game & Parks Commission
- Nebraska Department of Agriculture
Surveillance

- AI is a reportable disease - 950 accredited veterinarians statewide
- LEDRS Veterinarians - 195 emergency responders
- 12 Foreign Animal Disease Diagnosticians (FADD)
- UNL-Veterinary Diagnostic Center (UNL-VDC) approved for AI testing
- National Veterinary Services Laboratory (NVSL) in Ames, IA is the National Reference Laboratory
FREE TESTING FOR AVIAN INFLUENZA IN NEBRASKA

Prevent the spread of Avian Influenza.
Keep the poultry industry in Nebraska strong and healthy.
Contact the Nebraska Department of Agriculture at 1-800-572-2437.
Surveillance

USDA provides funds for surveillance testing in:

- Commercial Poultry
- Non-commercial Poultry
  Birds for Exhibition
  Exotic Sales
  Backyard Flocks
- Gamebirds
Antibody Detection

- **Enzyme-linked immunosorbent assay (ELISA)** – screening test to detect antibodies.
- **Agar gel immunodiffusion (AGID) test** – screening test used to detect antibodies.
- **Hemagglutinin-inhibition (HI) test** – gold standard test specific antibodies for the 15 known H subtypes of influenza A virus.
- **Neuraminidase-inhibition (NI) test** – gold standard test for specific antibodies for the 9 known N subtypes of influenza A virus.
Antigen Detection

- **Matrix RRT-PCR** – screening test to detect most strains of avian influenza (all subtypes)
- **H5 specific RRT-PCR** – subtype-specific test used to confirm H5 avian influenza virus
- **H7 specific RRT-PCR** – subtype-specific test used to confirm H7 avian influenza virus
- **Antigen capture immunoassays** (Directigen, VMRD Flu Detect) – a quick test for on farm or in a laboratory to detect live or inactivated virus in clinical samples. A positive test only indicates an infection with influenza A virus (cannot identify a subtype).
Virus Isolation

- Virus isolation is the **gold standard** test to diagnose infections with AIV.
- subtyped using HI or NI test.
- Isolates identified as H5 and H7 subtypes are further characterized to determine if the isolate is HPAI or LPAI
  - the chicken pathogenicity test
  - determining the amino acid sequence at the cleavage site of the H protein
LPAI Surveillance – National Animal Health Laboratory Network (NAHLN)

More than 45 laboratories participate in the network. NAHLN labs:

- Provide laboratory services nationwide
- Provide laboratory data for reporting
- Respond to foreign animal disease outbreaks
- Focus on animal diseases
## Nebraska AI Surveillance Testing
### June 1, 2006 – May 31, 2007

<table>
<thead>
<tr>
<th></th>
<th>Number of Birds Tested</th>
<th>Number of Positive Tests</th>
<th>Number of Premises</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial</td>
<td>6,179</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Non-Commercial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>County Fairs</td>
<td>3,851</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td>Backyard Flocks</td>
<td>3,865</td>
<td>1*</td>
<td>212</td>
</tr>
<tr>
<td>Exhibitions</td>
<td>2,035</td>
<td>2**</td>
<td>45</td>
</tr>
<tr>
<td>Game Birds</td>
<td>1,070</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>17,000</td>
<td>3</td>
<td>376</td>
</tr>
</tbody>
</table>

*1 duck serologically positive, H3N2, farm flock

** 2 pheasants at an exotic sale, PCR positive but not H5 or H7
Nebraska AI Surveillance Testing

<table>
<thead>
<tr>
<th></th>
<th>Number of Birds Tested</th>
<th>Number of Positive Tests</th>
<th>Number of Premises</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial</td>
<td>8,341</td>
<td>1*</td>
<td>4</td>
</tr>
<tr>
<td>Non-Commercial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>County Fairs</td>
<td>3,497</td>
<td>0</td>
<td>84</td>
</tr>
<tr>
<td>Backyard Flocks</td>
<td>4,219</td>
<td>0</td>
<td>199</td>
</tr>
<tr>
<td>Exhibitions**</td>
<td>1,320</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Game Birds</td>
<td>330</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>17,707</td>
<td>1</td>
<td>320</td>
</tr>
</tbody>
</table>

* Positive Turkey Flock (H7N9) – one premises
** Includes exotic sales, swap meets, etc.
Avian Influenza (AI)

- Review of Avian Influenza (AI)
- Nebraska’s Poultry Industry
- Surveillance
- Response
- Asian Strain H5N1 vs North American Strain
Response Depends Upon Strain of AI

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broken leg</td>
<td>Shoot</td>
</tr>
<tr>
<td>Infected eye</td>
<td>Shoot</td>
</tr>
<tr>
<td>Splayed hoof</td>
<td>Shoot</td>
</tr>
<tr>
<td>Runny nose</td>
<td>Shoot</td>
</tr>
<tr>
<td>Fever</td>
<td>Shoot</td>
</tr>
<tr>
<td>Open sores</td>
<td>Shoot</td>
</tr>
<tr>
<td>Closed sores</td>
<td>Shoot</td>
</tr>
<tr>
<td>Swollen belly</td>
<td>Shoot</td>
</tr>
<tr>
<td>Ornery</td>
<td>Shoot</td>
</tr>
<tr>
<td>Swayback</td>
<td>Shoot</td>
</tr>
<tr>
<td>Erratic heart</td>
<td>Shoot</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>Shoot</td>
</tr>
<tr>
<td>Bad breath</td>
<td>Shoot</td>
</tr>
<tr>
<td>Mange</td>
<td>Shoot</td>
</tr>
</tbody>
</table>

Like most veterinary students, Doreen breezes through Chapter 9.
Response

Low Pathogenicity Avian Influenza (LPAI)

- Elevated biosecurity plan
- Flock plan
- Monitoring and Surveillance testing
- Control of movement of birds
- Cleanup plan
Response

Low Pathogenicity Avian Influenza (LPAI H5/H7)

- Notifiable Low-pathogenic AI (NLPAI)
- Elevated biosecurity plan
- Flock Plan
- Quarantine zone with testing
- Surveillance zone with testing
- Controlled Marketing or Depopulation
Infected & Surveillance Zones

Infected Premises

Buffer Zone

Infected Zone (2 mi.)

Surveillance Zone (5 mi.)
Response

Highly Pathogenic Avian Influenza (HPAI)

- Handled like a Foreign Animal Disease (FAD), USDA will be involved
- Quarantine flock
- Quarantine & Surveillance zone testing
- Depopulation
- Repopulation with surveillance
Avian Influenza (AI)

- Review of Avian Influenza (AI)
- Nebraska’s Poultry Industry
- Surveillance
- Response
- H5N1 Asian Strain vs North American Strain
Asian Strain of H5N1
a.k.a. The Bird Flu

“Let it be known that all H5N1 Avian Influenza Viruses are NOT created equal!”
Is the current situation with the Asian H5N1 a Pandemic?

- Current Asian H5N1 is a bird disease!
- No sustained person to person spread
Areas reporting confirmed occurrence of H5N1 avian influenza in poultry and wild birds since 2003

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Organisation for Animal Health (OIE) and national governments
Map Production: Public Health Mapping and GIS, Communicable Diseases (CDS) World Health Organization

© WHO 2007. All rights reserved
Risk Factors for Asian Strain H5N1

H5N1 outbreaks in 2005 and major flyways of migratory birds
Situation on 30 August 2005

Risk Factors for Asian Strain H5N1

Contact with Poultry
Biosecurity for Poultry Flocks

- Biosecurity - practices designed to prevent the spread of disease onto and off of your farm.
- Maintaining the facility to minimize traffic of biological organisms (viruses, bacteria, rodents, etc.) across its borders.
- Biosecurity - cheapest, most effective means of disease control available.
- No disease prevention program will work without it.
Biosecurity

How Poultry Disease Spreads

Farm 1

Vehicles
- Fuel
- Feed

Farm 2

Bird-to-bird
- Carcasses
- Manure
- Litter/debris
- Feathers

Poultry equipment
- Filler flats
- Hauling crates/coops
- Feeder, waterers

Farm 3

Farm 4

People
- Boots
- Clothing
- Handling

Vehicle equipment
- Manure spreader
- Tractor
- Truck
- Front-end loader
Risk Factors for Asian Strain H5N1

Reporting
Disease Control
Disposal
Vaccination for Avian Influenza

- Not routinely used in the poultry industry
- Too many strains to make it practical
- Vaccine is available and its use in outbreaks is a topic of a lot of discussion
- Use of vaccine with H5 or H7 must get USDA approval
Risk Factors for Asian Strain H5N1

Proper Handling of Poultry Products
Risk Factors for Asian Strain H5N1

Properly cooking poultry products kills all avian influenza viruses!

"Darren Smith and I'll be cooking chicken nuggets with Mars bars in a Coca Cola sauce"
The End