Avian Influenza and Newcastle Disease: Latest Cases, Epidemiology and Control

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Poultry Diseases

• Respiratory disease or decreased egg production
  – Acute to subacute viral causes
    • Infectious Bronchitis Virus
    • Infectious Laryngotracheitis Virus
    • Low Pathogenicity Avian Influenza (e.g. H9N2, H7N9, etc.)
    • Low virulent “Newcastle disease” (lentogenic APMV-1)
  – Bacterial causes
    • *Mycoplasma* spp.
    • Infectious Coryza
    • *Ornithobacterium* sp.
    • *Bordetella avium*
    • Fowl Cholera, others
  – Fungal – *Aspergillus* sp.
Poultry Diseases

• High mortality, systemic disease
  – Peracute viral diseases –
    • High Pathogenicity Avian Influenza Virus
    • Virulent Newcastle Disease Virus
  – Peracute septicemia – Fowl Cholera
  – Heat exhaustion
  – Water deprivation
  – Toxins (feed or waterborne); e.g. older sulfa drugs, sodium monofluoroacetate, nicotine
Issues/sequence:

1. Must identify the problem – e.g. diagnostic systems to identify the cause of clinical poultry health problem
   - Pathogen detection (rRT-PCR/virus isolation)
   - Antibodies to pathogen (HI or other tests)
2. Must know geographically and compartmentally where the problem is located – e.g. surveillance for the pathogen
3. Develop a solution – e.g. prevent, manage or eradicate the disease and infection
Critical Components:

1. Partnership – government (federal, state & local), industry and academia
2. Transparency – no hidden information
3. Trust among all partners
4. Technical competency & adequate lab services
5. Science should lead the process
6. Solution should be available to all producers/companies
7. Diagnostics must be decentralized with adequate capacity for the task and accreditation of labs
8. Diagnostics functions must be separated from research
9. Recognize solutions differ for each pathogen
Diagnosis

• Virus detection
  – rRT-PCR: matrix (screening) and H5/H7/H9/νNDV fusion specific
  – Virus isolation - will not be replaced: isolates needed for confirmation and characterization (sequencing)
• Antibody tests
  – ELISA/AGID – screening for type A influenza
  – Hemagglutination inhibition (HI) – subtype specific (H9, H5, NDV, etc.)
• Tests for other pathogens (lab based as clinical signs are not definitive)
Differentiation of NDV from AIV

- Both have hemagglutinating activity
- Replicate in embryonating chicken eggs
- Gross lesions similar
- Wide virulence range – low to high virulence
- NDV cross protective, AIV none
- NDV predictable, AIV not
- Vaccinate: NDV = widely, AIV = only in 6 countries for HPAI
- Eradication is goal for HPAI and vND
What Poultry Diseases are Reportable?

World Organization for Animal Health
(Office International des Epizooties, OIE)

• 178 Member countries: e.g. Turkey, EU, USA, etc.

• Three internationally controlled poultry diseases:
  – HPAI
  – H5/H7 LPAI
  – Virulent Newcastle disease

• H1-4, H6, & H8-16 LPAI are not notifiable, nor low virulent APMV-1: (ex. H9N2, lentogenic NDV)
Newcastle Disease

- **Avian paramyxovirus Type 1**: easily killed virus with heat and chemicals
- **One serotype (HI)**
- **Protein projections on the surface:**
  - Fusion glycoprotein
  - Hemagglutinin-neuraminidase (HN) glycoprotein
- **Vary in disease production (chickens):**
  - **Low virulent**: local - mild respiratory disease & egg drop – virus in respiratory & digestive organs/tissues;
    - Lentogenic
    - Asymptomatic (enteric)
  - **Virulent**: systemic - deadly disease
    - Velogenic: viscerotrophic and neurotropic
    - Mesogenic
80 countries: NDV; active, suspect or unresolved

(75 poultry or wild birds & poultry, 5 wild birds only)

July-Dec 2013

Jan-June 2014
NDV Outbreaks: Reported to OIE

• Many developing countries are endemic
• Few actual outbreaks reported except in NDV free countries
• Most countries use 6 month reports and delayed in filing with OIE
Why NDV is a problem worldwide?

- Virulent viruses have been worldwide since 1930s, causing severe disease and economic losses
- Virulent viruses continue to evolve with appearance of virulent variants & host range expansion (e.g. domestic geese)
- Global mobility: recent viruses from China, South America and South Africa are nearly identical
- Many reports of vaccine failure worldwide
- Vaccines are used as a management strategy against low virulent & virulent viruses
- But only one effective strategy to eliminate virulent NDV: Eradication through stamping-out
Results of over 50 years of vaccination: Eighteen Genotypes of Virulent Viruses Circulate Worldwide
Is Current NDV vaccination failing?

- Efforts to prevent Newcastle Disease (more than 50 years of vaccination) have not effectively eradicated NDV in countries where the virus is endemic.
- Effective control requires periodic vaccination every 3-4 weeks.
- Vaccine failures are still reported in 2-4 week old chickens and in highly vaccinated layers, even in countries with good veterinary structure.
- Virulent NDV can still replicate in the mucosal tissue of healthy vaccinated animals and escape into the environment.
Utilizing Phylogenetic Relationships Between Vaccines and Circulating Viruses:

All NDV belong to a single serotype but genetically diverse

- **B1 & LaSota** live and inactivated vaccines (Class II, genotype II) are widely used in the poultry industry. Isolated in the late 1940’s.

- Some other vaccine strains such as Ulster and V4 are Genotype I.

- But currently, viruses from genotypes V, VI, VII and XIII are causing outbreaks around the world and are genetically different from the vaccine strains.
## Amino acid differences fusion protein

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## Amino acid differences Hemagglutinin-Neuraminidase

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Cross protection with inactivated vaccines
Survival: Cross Protection of inactivated vaccines using current circulating viruses of Genotypes V, VII and XII
Cross Protection: Viral Shedding

Day 4 Oral

Titer (Log$_{10}$ EID$_{50}$/0.1 ml)

Sham- Malaysia
LaSota- Malaysia
Mexico - Malaysia
Malaysia- Malaysia
Peru - Malaysia
Sham- Mexico
LaSota - Mexico
Mexico - Mexico
Malaysia- Mexico
Peru - Mexico
Sham - Peru
LaSota - Peru
Mexico - Peru
Malaysia - Peru
Peru - Peru

0
1
2
3
4
5
6
7

a
b
b
b
b
b
b
b
b
Final Constructs

rLS-SA

rLS-PK
Results...

Survival after challenge

- BHI vs. PK8
- BHI vs. SA60
- rLS-PK vs. PK8
- rLS-SA vs. SA60
- LaSota vs. PK8
- LaSota vs. SA60

Survival (%)

Days after challenge
## Birds shedding vaccine and challenge virus

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Excreción viral (Pre- y post-desafío)

Shedding Results...
# Relationship between death time and specific antibody titers

## Challenged 14 dpv

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Avian Influenza

- **Orthomyxovirus**: easily killed virus with heat and chemicals
- **Protein projections on the surface**:
  - 16 hemagglutinin subtypes (i.e. H1-H16)
  - 9 neuraminidase subtypes (i.e. N1, N2, N3….N9)
- **Vary in disease production (chickens)**:
  - Low pathogenicity (LP): local - mild respiratory disease and egg drop – virus in respiratory & digestive organs/tissues; e.g. H9N2 LPAIV
  - High pathogenicity (HP): systemic - deadly disease (some H5 & H7) – virus in all organs/tissues; e.g. H5N1 HPAIV
## 35 HPAI Disease Events

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<td>H7N3</td>
</tr>
<tr>
<td>2004</td>
<td>S. Africa</td>
<td>H5N2</td>
</tr>
<tr>
<td>2005</td>
<td>N. Korea</td>
<td>H7N7</td>
</tr>
<tr>
<td>2007</td>
<td>Canada</td>
<td>H7N3</td>
</tr>
<tr>
<td>2008</td>
<td>England</td>
<td>H7N7</td>
</tr>
<tr>
<td>2009</td>
<td>Spain</td>
<td>H7N7</td>
</tr>
<tr>
<td>2011-3</td>
<td>S. Africa</td>
<td>H5N2</td>
</tr>
<tr>
<td>2012</td>
<td>Chinese Taipei</td>
<td>H5N2</td>
</tr>
<tr>
<td>2012-5</td>
<td>Mexico</td>
<td>H7N3</td>
</tr>
<tr>
<td>2012</td>
<td>Australia</td>
<td>H7N7</td>
</tr>
<tr>
<td>2013</td>
<td>Italy</td>
<td>H7N7</td>
</tr>
<tr>
<td>2013</td>
<td>Australia</td>
<td>H7N2</td>
</tr>
</tbody>
</table>

* § Largest epizootic in 50 yrs

* § Vaccine used in the control strategy

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35 HPAI Disease Events
HPAI (1/1/2013-12/30/2014): 24 countries

- **H5N1 HPAI (N2/N6/N8)**
  - 22 countries – poultry, wild birds, humans

- **H5N2 HPAI**
  - S. Africa – ostriches
  - Chinese Taipei – native chicken

- **H7N2 HPAI**
  - Australia - layers

- **H7N7 HPAI**
  - Italy – poultry

- **H7N3 HPAI**
  - Mexico - layers
Evolution of the Asian H5 Hemagglutinin

When discrete monophyletic groups begin to appear within a specific clade and those groups meet the nucleotide divergence criteria (as well as having bootstrap values >60), they are split into second order clades (but still considered part of the original first order clade). As a second order clade continues to evolve it may reach a similar level of genetic diversity at which point it may be split into third order clades and so on. The same clade designation criteria apply to first, second, and any higher order clade designations.
Distribution of H5N1 Subclades

H5N1 HPAI (22)
Bangladesh Japan
Bhutan N. & S. Korea
Cambodia Laos
Canada Libya
China Nepal
Egypt Netherlands
Germany Russia
Hong Kong United Kingdom
India USA
Indonesia Vietnam
Italy

6 genetic clades
1.1.2, 2.1.3.2, 2.2.1, 2.3.2.1, 2.3.4.4, (H5N8 H5N5, H5N6, H5N3, H5N2, H5N1), 7.2

Epicenter – S. Central & SE Asia, & NE Africa
One Predictable Issue About Avian Influenza Viruses – They Change

Drift ➔ H5N1 HPAI hemagglutinin clades
1.1.2 2.1.3.2 2.2.1 2.3.2.1 2.3.4.4 7.2

<table>
<thead>
<tr>
<th>Subclade</th>
<th>Poultry/Wild Birds Infections</th>
<th>Human Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1.2</td>
<td>Cambodia, Viet Nam</td>
<td>Cambodia (7)</td>
</tr>
<tr>
<td>2.1.3.2a</td>
<td>Indonesia</td>
<td></td>
</tr>
<tr>
<td>2.2.1</td>
<td>Egypt, Libya</td>
<td>Egypt (4)</td>
</tr>
<tr>
<td>2.3.2.1a</td>
<td>Bangladesh, India</td>
<td></td>
</tr>
<tr>
<td>2.3.2.1c</td>
<td>China, Indonesia, Lao, Viet Nam</td>
<td></td>
</tr>
<tr>
<td>2.3.4.4</td>
<td>China (H5N1/N6/N8), Japan &amp; Korea (Rep.) (H5N8); Lao (H5N6), Viet Nam (H5N6/N1), Canada Chinese Taipei, USA</td>
<td>China (H5N6) (1)</td>
</tr>
<tr>
<td>Unknown</td>
<td>Korea (Dem. Peoples Republic)</td>
<td>Indonesia (1)</td>
</tr>
</tbody>
</table>

Sources:
- Wild aquatic birds - 1° introduction
- Live poultry marketing system - 1° introduction and 2° spread
- Domestic ducks – maintenance host & source LPMS

Shift ➔ Reassortment of Genes

Other Avian Influenza Viruses from Wild Birds and Live Poultry Markets

- H5N1 (2.3.4.4)
- H5N2 (2.3.4.4)
- H5N3 (2.3.4.4)
- H5N5 (2.3.4.4)
- H5N6 (2.3.4.4)
- H5N8 (2.3.4.4)
Recent:

- H5N8 HPAI outbreaks in poultry and wild birds – S. Korea & Japan, winter 2014
- Spring 2014 virus moved to Siberia and west Alaska
- Fall 2014: H5N8 appeared Europe, North America
- Fall 2014: Reassortant H5N2 and H5N1 in North America
Fall 2014 viruses – intercontinental group:
• icA1 – western Russia, Europe, Japan
• icA2 – North America, Japan
• icA3 – Japan & Korea
Control: HPAI

Eradication is the only strategy for HPAI

Historical “Stamping-out” Program:

• Enhanced biosecurity → prevent HPAI introduction onto naïve farms or from leaving affected farms

• Diagnostics and surveillance → quickly find HPAI

• Elimination of infected poultry (culling) → stamp-out HPAI action plan

• Education → your individual responsibility

• Decreasing host susceptibility (vaccines/vaccination) → temporary solution (5 of 35 outbreaks)
• 30 epizootics used stamping-out alone, but 5 epizootics added vaccination as a additional control component
• Vaccination - immediate positive impact on HPAI prevention & management (disease & mortality)
• But stamping-out alone was associated with shorter eradication times than stamping-out + vaccination programs (Pavade et al. 2011)
• HPAI vaccination can be associated with complacency
14 countries vaccinated poultry against HPAI (2002-2010)

- **Preventive** (<0.2%): Mongolia, Kazakhstan, France, The Netherlands
- **Emergency** (<0.8%): Cote d’Ivoire, Sudan, PDR Korea, Israel, Russia, Pakistan
- **National/routine** (>99%): China (including HK), Egypt, Indonesia and Vietnam, plus added Bangladesh

---

**Doses of Vaccine (millions): 2002-2010 (Total >113b)**

OIE Performance of Veterinary Services (PVS) tool: Higher critical competencies associated with better HPAI control:

- Staffing of veterinarians and paraveterinarians
- Professional competencies & continuing education of vets
- Emergency funding
- Veterinary laboratory diagnosis
- Epidemiological surveillance
- Availability of veterinary medicines and biologicals
- Transparency
- Disease prevention, control and eradication measures

Outcome:

- Higher PVS scores were associated with shorter time to eradication, fewer outbreaks, lower mortality rate, and higher culling rate
Low Pathogenicity Avian Influenza

- Subtype: H1-16
- Global distribution in wild aquatic birds

H5/H7 LPAI in poultry is notifiable to OIE
- Some H5/H7 LPAI → HPAI
- Strategy: earlier detection and stamping-out (eradiation)
- Surveillance & reporting critical

H9N2 is NOT notifiable to OIE; control decided by country
• LPAI poorly documented but costly: 1) H9N2 LPAI Asia & Middle East, 2) H5N2 LPAI Mexico, Central America, and the Caribbean, & 3) sporadic w/other LPAI subtypes
• LPAI augment severity of other diseases such as mycoplasma, Newcastle disease, E. coli, salmonella, etc. → increased losses from mortality/morbidity, respiratory disease and loss of egg production
LPAI Control

• Control programs are economically based and similar to control of other respiratory diseases
  • Exposure avoidance (biosecurity)
  • Surveillance for detection
  • Controlling environmental factors (bacteria and other viruses, temperature/humidity, etc.)
  • ± vaccination

• Vaccines for control - field use:
  • H9N2 was the most common (10 countries) - China, Pakistan, Middle East and Egypt (billions of doses)

Swayne et al., OIE Scientific and Technical Review 30(3):839-870, 2011
## Comparison

<table>
<thead>
<tr>
<th>H9N2 LPAI</th>
<th>H5N1 HPAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Localized infection only: respiratory and egg drops &amp; egg quality changes</td>
<td>• Systemic infection: all organs including meat and eggs</td>
</tr>
<tr>
<td>• Low to high mortality – latter needs 2° pathogen</td>
<td>• Very high mortality in non-vaccinates (virus alone)</td>
</tr>
<tr>
<td>• Not reportable to OIE</td>
<td>• Reportable to OIE</td>
</tr>
<tr>
<td>• Goal – MANAGE since economic disease/decision</td>
<td>• Goal – ERADICATE because is devastating animal health dis. &amp; public health impact</td>
</tr>
<tr>
<td>• Vaccination commonly used</td>
<td>• Vaccination uncommon among most countries</td>
</tr>
</tbody>
</table>
What Can Vaccines Do?

Increase resistance to AIV infection
Reduce replication of AIV in respiratory & GI tract
Prevent illness and death in poultry

↓

Reduced environmental contamination
Reduced transmission to birds
Maintained livelihood and food security of rural poor

**Result:** Vaccines manage disease

**Negative:** Makes diagnosis and surveillance difficult
What is needed to have effective LPAI or HPAI vaccination program?

1) High potency vaccine
2) Antigenically relevant vaccine seed strains
3) Proper vaccination program
4) Adequate number of vaccinations
5) Monitor vaccinated populations for protective titers
6) Survey vaccinated populations to find vaccine resistant AIV (‘DIVA’)
1. High Potency Vaccines

- **Indirect Potency** – HI serology in SPF chickens
  - HI test using vaccine strain as antigen
  - Standards:
    - $\geq 1:32$ – prevent mortality
    - $\geq 1:128$ – prevent oropharyngeal shedding
- **Direct assay**: measure the amount of hemagglutinin protein in each dose of vaccine
  - 1-5 μg hemagglutinin/dose
  - 512-1024 HA units/dose
  - Use NDV vaccine standard = 50 PD$_{50}$

**Solution**: Government license vaccines and set minimum potency standard
2. Antigenically Relevant Vaccine Seed Strains

Antigenic Drift (Changing Virus):

Vaccine Seed Strains - Indonesia

- Historical H5 Vaccines – Similar antigenicity
- Drifting of HA away from root
  - Good protection: Ck/HK/220/97, Ck/Legok/03, VN/1203/04, Ck/WJ/HAMD/06
  - Intermediate protection: Ck/Papua/06
  - Poor protection: PWT/06

Swayne et al. J. Virol 2015
2. Antigenically Relevant Seed Strains

- **Egypt (2006-)**: Some comm. farms, HPAIV resistance to immunity from Mex/94 and Re-1 vax (Rauw et al. 2011)
- **China (2004-)**: (Chen 2009)
  - Re-1 (rg A/gs/Guangdong/1/1996 [H5N1] (0): 2004-8
  - Re-4 (rgA/ck/Shanxi/2006 [H5N1](7): 2006-7
  - Re-5 (rgA/dk/Anhui/1/2006 [H5N1](2.3.4): 2008-12
  - Re-6 (rgA/dk/Guangdong/S1322/2010 [H5N1] (2.3.2): 2012-
  - Re-7 (new 7.2 clade): 2014-
- **Vietnam**: 2011 2.3.2.1B resistant to immunity from Re-1 & Re-5 (now use Re-6)
- **Hong Kong (2008)**: clade 2.3.4 (Leung et al., 2013)
- **Mexico (LPAI)**: North American H5N2 (Lee et al, 2004; Eggert et al., 2010)

**Solution**: Change in vaccine seed strain with switch to custom reverse genetic strain to match field virus when resistance occurs
3. Proper Type of Vaccination Program

- Vaccination used in different ways:
  - *Zoo birds and captive held non-poultry* (i.e. 14 EU and 2 other countries H5 and H7)
  - *Single poultry farm* (ex. H5N1 Israeli ostriches)
  - *Ring vaccination zone* after outbreak (Pakistan, Mexico)
  - *Targeted for high risk poultry* – ex. outdoor ducks (France), free-range layers (the Netherlands)
  - *Focused sector-specific vaccination* – (ex. Italy in turkeys & capons 2003-2005 N. Italy H5/H7 LPAI)
  - *Routine/National vaccination* of poultry: China (including HK), Egypt, Vietnam and Indonesia for H5N1, 10 countries for H9N2

Swayne et al., OIE Scientific and Technical Review 30(3):839-870, 2011
3. Proper Type of Vaccination Program

Average National Coverage Rate (%) for All Years of AI Vaccine Usage

National vaccination difficult to achieve population immunity

Solution: Targeted vaccination (risk based & resource smart), using adequate field surveillance to locate areas of infection

4. Adequate Number of Vaccinations

1. Specific prime-boost two dose application protocols as minimum for meat birds if they have maternal antibody to influenza virus and/or the vector
2. Meat ducks, meat turkeys, chicken layers and breeders may need 3-4 vaccinations to get protective immunity
3. Licensing of recombinant vaccines for priming chicks in the hatchery (e.g. rHVT-H5 or rDVE-H5)
5. Monitor Vaccinated Populations for Protective Titers

• Hemagglutination inhibition assay (HI) – e.g. H5 and H7
  – Titers of ≥1:32, protect from death
  – Titers of ≥1:128, best protection from virus replication and shedding
  – Need ≥ 80% with protective antibody titers

• Vaccinated flocks should be boosted if < 80% have titers ≥ minimum established titer by control authority
6. Survey Vaccinated Poultry to Find HPAIV

**Virological Surveillance (‘Biosensor’)**

- **Identifiable, susceptible population**
  - Non-vaccinated sentinel birds that die
  - Daily mortality or sick vaccinated birds

- **Rapid sensitive detection methods for virus**
  - RRT-PCR: 3 hour laboratory test
  - Virus isolation on all RRT-PCR positive – HPAI virus growth in 48 hours & genetic/antigenic analysis

- **Serological surveillance: supplements virus surveillance**
  - Need special preparation: vaccine/field virus different N

**Solution:** Monitoring all vaccinated flocks for HPAIV in daily mortality for chickens – sample every 30 days
Conclusions

• Must identify the cause of the problem – e.g. accurate & rapid diagnostic systems to identify the cause of clinical poultry health problem
• Must know geographically and compartmentally where the problem is located – e.g. surveillance for the pathogen such as H9N2 LPAI or H5N1 HPAI or vNDV
• Develop a solution – e.g. prevent, manage or eradicate the disease and virus
• Solutions and goals will differ depending on LPAI vs HPAI or low vs high virulent ND
Conclusions

• Current NDV vaccines are not optimized for NDV management in endemic countries.

• Reduction of replication and transmission of challenge virus can be achieved by vaccines or vaccination strategies that produce higher levels of antibodies homologous to the challenge virus.

• Live recombinant vaccines expressing F and HN homologous to challenge viruses should provide better protection against survival than the conventional LaSota vaccine.

• If dosing and timing are suboptimal the current gold standard (Lasota vaccine) may not prevent disease.
Conclusions

- H9N2 LPAI will continue as a severe economic disease of poultry in Asia with vaccination as the primary control tool, but also must identify and control secondary diseases
- Stamping-out (without vaccination) is preferred method for HPAI control & is associated with shorter eradication times
- Vaccination will continue as a HPAI management tool for some countries to limit disease & infection, but will continue to delay eradication efforts, until changes in poultry production occur that favor stamping-out programs
- Vaccination will prevent disease and reduce infection rates, but will not eradicate the HPAI virus; i.e. the stamping-out part of control program (biosecurity, culling, education, and surveillance and diagnosis) leads to eradication
Conclusions

• Proper vaccines/vaccination characteristics:
  • Only high potency vaccines are licensed and used
  • Vaccines contain only antigenically relevant seed strains
  • Vaccines are applied in a targeted vaccination program, based on risk
  • Adequate number of vaccinations must be used based on age and species of poultry
  • Vaccinated populations must be monitored for protective titers and boosted if not protected
  • Vaccinated populations must have adequate surveillance to find vaccine resistant AIV, and such viruses should be analyzed for genetic and antigenic changes that impact vaccine seed selection and update when resistance occurs
Merci Beaucoup!