Studies on H7N9 virus infectivity and transmission in poultry and field assessment of epidemiology and control

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H7N9 LPAI: Spring 2013

- Human infections with hospitalization and deaths
- 77% human cases contact w/poultry
- No reports of deaths or isolations in poultry on farms (i.e. inadequacy of Syndromic Surveillance)
- 2 waves - loosely associated with Lunar New Year

Discussion areas in agricultural systems:
- Field Epidemiology
- Pathogenesis studies
Background: Poultry Production in China

- Poultry production changed in last 15-20 yrs.
- Two main poultry production systems:
  - Industrial poultry, primarily “white” chicken for meat (Cornish x White Rock) or eggs (Leghorns)
  - “Yellow” meat chickens for Live Poultry Markets (LPM)
- Other poultry in LPM
  - Domestic ducks and geese (outdoor reared)
  - Japanese quail
  - Miscellaneous gallinaceous poultry
  - Pigeons
  - Captive-reared “wild” waterfowl
Background: Poultry Production in China

• Industrial chicken meat production
  – Integrated production system: chicks, feed, veterinary care, processing
  – Contract growers must meet company biosecurity standards
  – High regulatory and consumer oversight
  – Short production cycle: 35-49 days
  – 20,000 – 200,000 chickens/farm
  – Outlets: supermarkets and restaurant chains
Background: Poultry Production in China

- “Yellow” chicken production
  - Semi-integrated production system: chicks from broker, feed and medicines
  - Contract growers or independent growers, indoor rearing
  - Low regulatory and consumer oversight
  - Long production cycle: 120d (70d, 90-100d & 120d)
  - High usage of vaccines and antibiotics
  - 1000-5000 chickens/farm
  - Low biosecurity and poor house construction
  - Outlets: Live Poultry Markets [LPM] (wholesale) >> house restaurants and retail markets
Live Poultry Market System

Farms – Outside District
• Wholesale Markets w/booths
  • 24hr turnover
  • Booths specialize by bird type or geographic area

Farms – Inside District

Retail Markets (1-5 stalls)

House Restaurants: receive live birds to hold
2013 H7N9 LPAI: Actions

• Over 1 million samples with 83 virus positive or H7 antibody positive
• 561,442 birds culled with compensation to owner
• Close Live Poultry Market once H7N9 was diagnosed
• Movement restrictions
• Cleaning and disinfection
• LPM reopened with new sanitary requirements
2013 H7N9 LPAI: Conclusions

- LPM systems are contamination sites and amplifiers of the virus, esp. wholesale markets in large cities
- Poultry farm source is unknown but was associated with “yellow” chicken production
- No new human infections in large urban areas where LPM system closed (i.e. eliminated exposure)
- The inability to find “infected” farms by “trace back” system is similar to New York City LPM system for H7N2 LPAIV
Changes between 1\textsuperscript{st} and 2\textsuperscript{nd} Wave:

- Cases in poultry workers and farmers
- Geographic spread south, west and north of human cases
Changes between 1\textsuperscript{st} and 2\textsuperscript{nd} Wave:

- Shift to environmental testing in LPM
- Geographic spread south, west and north for bird/environmental samples
- Positive chickens imported into Hong Kong and Macau – specialized farms

Sample origin could not be determined for 66 additional samples (35 in wave 1, 31 in wave 2). 73 additional positive virological samples (9 chickens, 64 environmental) were reported in peer-reviewed articles.
Poultry pathogenesis questions

• What species are susceptible to infection?
• Is there any clinical disease associated with infection?
• Do infected poultry shed large amounts of virus that make them likely vectors for human infections?
• What is the pattern of virus shedding?
• Does the virus become systemic and create a threat for food-borne (meat-eggs) transmission to humans?
Virulence

- IV inoculation of 10 chickens (IVPI) (A/Anhui/1/2013 [H7N9])
  - IVPI = 0.00, or low pathogenicity (n=3)
- No mortality in poultry at $\leq 10^6$ EID$_{50}$, a few chicken deaths at $10^8$ EID$_{50}$ dose
  - Lethality associated with kidney tropism (tubule necrosis with associated inflammation)
Pathogenesis studies (SPF or SAN birds)

Chickens (*Gallus g. domesticus*) 59-w/o
Japanese quail (*Coturnix japonica*), 4-w/o
Racing pigeons (*Columbia l. domestica*), 6-12 m/o

Pekin ducks (*Anas platyrhynchos*), 2-w/o
Mallard ducks (*Anas platyrhynchos*), 2-w/o
Muscovy ducks (*Cairina moschata*), 2-w/o
Embden geese (*Anser a. domesticus*) 2-w/o

7-11 birds

Virus was detected by quantitative real-time RT-PCR assay specific for the H7 gene

Pathogenicity of H7N9 influenza virus in chickens, quail and pigeons

No clinical signs

<table>
<thead>
<tr>
<th>Days Post-Inoculation</th>
<th>Chicken - OP</th>
<th>Chicken - Cloacal</th>
<th>Quail - OP</th>
<th>Quail - Cloacal</th>
<th>Pigeon - OP</th>
<th>Pigeon - Cloacal</th>
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Serology (11 DPI)

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<th>Birds</th>
<th>Results</th>
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<td>Chickens</td>
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<tr>
<td>Quail</td>
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<td>Pigeons</td>
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</table>

Pathogenicity of H7N9 influenza virus in chickens, quail and pigeons

Virus Titer (Log10 EID50) vs. Days Post-Inoculation

- Chicken - OP
- Chicken - Cloacal
- Quail - OP
- Quail - Cloacal
- Pigeon - OP
- Pigeon - Cloacal

Pathogenicity of H7N9 influenza virus in ducks and geese

- No clinical signs

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Serology (11 DPI)

- Pekin ducks: 6/8
- Mallard ducks: 5/8
- Muscovy ducks: 5/5
- Emden geese: 0/6

Pathogenicity of H7N9 influenza virus in ducks and geese

Virus Titer (log10 EID50) vs. Days Post-Inoculation

- Pekin - OP
- Pekin - Cloacal
- Mallard - OP
- Mallard - Cloacal
- Muscovy - Oral
- Muscovy - Cloacal
- Geese - Oral
- Geese - Cloacal
Histopathology and viral antigen staining in tissues

- Replication mostly limited to upper respiratory tract
- No evidence of systemic infection. Lungs, muscle, and egg contents negative by VI and RT-PCR

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Chickens</th>
<th>Quail</th>
<th>Pigeons</th>
<th>Pekin ducks</th>
<th>Mallard ducks</th>
<th>Muscovy ducks</th>
<th>Embden geese</th>
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Three birds examined at 3 DPI. Lesions/viral antigen staining

Histopathology and viral antigen staining in tissues

Quail-Nasal epithelium

Chicken-Nasal epithelium

Histopathology and viral antigen staining in tissues

Quail-trachea

Quail-nasal glands

Muscovy ducks - intestinal epithelium

Average weight gain in sham versus H7N9 vaccinated groups
Transmission of H7N9 influenza virus in quail, pigeons, and Pekin ducks

$10^2, 10^4, \text{and } 10^6$ EID$_{50}$ dose of Anhui/1/2013

Japanese quail

Pigeons

Pekin ducks

5 birds

Oropharyngeal and cloacal swabs collected for virus detection

3 birds (contacts)

$0 \quad 1 \quad 2 \quad 3 \quad 4 \quad 5 \quad 6 \quad 7 \quad 8 \quad 9 \quad 10 \quad 11$ days

Serology

Transmission of H7N9 influenza in quail, pigeons and Pekin ducks

Infectious dose and transmission in chickens to characterize adaptation to chickens

Challenge with $10^1$ through $10^8$ EID50 of Anhui/1/2013

Add 3 contacts to each dose group

Oropharyngeal and cloacal swabs collected for virus detection

Euthanize 3 birds in $10^6$ group and collect tissues for histopathology, IHC and virus detection

Serology

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

Erica Spackman, et al., USDA-ARS-SEPRL, In peer review
Infection confirmed by detection of antibody

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<th># pos/total</th>
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White Leghorns (light breed, egg layers)

White rocks (heavy breed, meat birds)

- BID$_{50}$ for chickens $\approx 10^6$ EID$_{50}$ (Quail $\approx 10^2$, Duck $>10^6$ and pigeons $>10^6$ EID$_{50}$)
- In efficient contact transmission in chickens as compared with efficient between Japanese Quail

Erica Spackman, et al., USDA-ARS-SEPRL, In peer review
H7N9 transmission

• Efficient transmission to contact controls was seen with quail but not with chickens, Pekin ducks or pigeons
  • K. B. Ku et al. Virology, 2014. The Anhui/1/2013 virus replicated poorly in chickens and did not transmit to naive chickens and ferrets
  • J.C. Jones et al. , EID 2014. Songbirds and parakeets supported virus replication, shed high titers through the OP route, and showed few disease signs. Virus was shed into water troughs, and several contact animals seroconverted, although they shed little virus

• Possible explanations:
  • Studies done with a human isolate
  • Different experimental settings
  • Differences in susceptibility in chickens (breed, physiology, immunosuppression, stress, effect of other infectious and non-infectious agents)
National Analysis

Risk Factors for Delayed Eradication of HPAI

- No significant association between economic indicators GDP, AGDP, %AGDP, GDP/capita, GNI and HDI with HPAI outbreak data
- OECD membership (high-income economies, transparency and good governance), had shorter and significantly fewer HPAI outbreaks, quicker eradication times, lower mortality rates and higher culling rates than non-OECD

OIE PVS tool: Higher critical competencies associated with better HPAI control (shorter HPAI eradication time, lower mortality rate, lower culling rate, and fewer HPAI outbreaks):

- 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
Summary

• Inoculation with the H7N9 virus resulted in infection of all bird species examined, but no clinical signs

• Strong tropism for upper respiratory tract with little evidence of systemic spread
  In all species, virus was detected at much higher levels from oral swabs when compared to cloacal swabs

• There was a difference among species in the amount of virus shed and length of shed
  Virus shedding in quail, chickens and Muscovy ducks was much higher and prolonged (≥ 10 dpi) than in the rest of the species (≤ 6 dpi).

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<th>High</th>
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<tbody>
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<td>Quail</td>
<td>Pekin ducks</td>
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<td>Chickens</td>
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• Efficient transmission to contact controls was seen with quail but not with Pekin ducks or pigeons
Acknowledgements

• Funding
  • CEIRS
  • USDA
• CDC for providing the virus
• The Southeast Poultry Research Team
• Leadership of CEIRS and MCEIRS
Merci Beaucoup!