Ducks and transmission of H5N1 highly pathogenic avian influenza: innocent or guilty?

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H5N1 highly pathogenic avian influenza (HPAI)  
What was all the fuss about?

1. Risk to poultry production, particularly in Asia
2. Risk to human health
H5N1: 1. Risk to poultry production

• Importance of poultry to Asian people
  – Poultry meat and eggs = major source of animal protein
  – Millions of livelihoods depend on the poultry industries
  – Many rural poor households rely on small flocks of scavenging chickens, ducks, other poultry for nutrition and income

• Since 2003, > 400 million domestic poultry killed or culled because of H5N1

• Estimated economic loss = US$20 billion
H5N1: 2. Risk to human health

• Immediate threat of bird-to-human transmission
• Potential threat of human-to-human transmission (‘end of the human race’ scenario)
Potential threat: human-to-human transmission

- Feared repeat of the 1918 Spanish flu epidemic
- Would the virus mutate/reassort to allow human-to-human transmission?

1918 influenza: makeshift hospital wards and digging mass graves
H5N1: Facts

- In 1997, H5N1 virus caused disease outbreak in poultry in Hong Kong, 18 people infected, serious disease, 6 deaths
- Re-emerged in 2003, spread throughout SE Asia, then to Europe & Africa
- Since 2003, 63 countries have reported outbreaks of H5N1 in domestic or wild birds
- By end of 2012, 620 confirmed human infections, 367 deaths (case-fatality rate ~ 60%)
- Outbreaks of disease in birds and human cases continue to be reported from small number of countries (esp. Indonesia, Egypt, China)
Avian Influenza: the virus

- 8 segments of single stranded, negative sense RNA
- 2 different peplomers:
  - H (haemagglutinin)
  - N (neuraminidase)
- 16 types of H, 9 types of N
- all subtypes have been found in birds, especially ducks

new human influenza viruses arise from antigenic shift (reassortment of RNA segments in cell co-infected with 2 different viruses)

this process likely occurs in pigs:
  - the human virus provides the replication genes
  - the avian virus provides new H or N genes
  - the pig provides the “mixing pot”
H5N1 virus: what’s different?

- H5N1 has ability to infect humans directly
- Thus, could humans act as the ‘mixing vessel’ for genetic re-assortment?
- If H5N1 virus gained human influenza virus replication genes, a human influenza pandemic could occur
H5N1 : Ducks

• Ducks and other waterfowl play central role in maintenance and evolution of all known avian influenza subtypes

• Thus, it was considered likely that ducks play a similar role with H5N1 viruses

• However, there was limited field or experimental data to support this view
ACIAR project

• We conducted a research project funded by the Australian Centre for International Agricultural Research (ACIAR) on H5N1 virus in smallholder duck farms

• This project aimed to:
  – better understand the role of ducks in the transmission and maintenance of H5N1 viruses
  – investigate the effects of the virus in ducks and the response of ducks to vaccination against H5N1 virus

• A combination of field and experimental studies, with activities in 3 countries: Indonesia, Viet Nam & Australia
ACIAR project: Field studies

• Longitudinal studies of both ‘stationary’ duck flocks (12-months) and ‘moving’ duck flocks (6-months)
  – Questionnaire survey
  – Antibody and virus prevalence/incidence
Results: seroprevalence, Indonesia
Results: H5N1 disease outbreaks, Indonesia

- 34 of 96 monitored (35%) farms reported HPAI outbreaks over study period
Incidence (rate of new infections in seronegative birds) & risk factors - Indonesia

- Large increase in the incidence rate during July-September 2007
- Risk factors identified:
  - Ducks scavenging around neighbouring houses - ↑ risk
  - Ducks confined overnight on farm - ↓ risk
H5N1 virus isolations - Indonesia

- 132 H5N1 viruses isolated from 46 of 96 farms in longitudinal study in Indonesia (from bi-monthly survey samples or from disease investigations on the farm)
- Of 61 duck-derived viruses, 80% were isolated from live birds, whereas only 14% of 71 chicken-derived isolates were from live birds
H5N1 virus isolations - Indonesia

- On 17 farms, virus was isolated on >1 occasion, either from single species (chicken or duck) or both species
- Was this the result of persistence of a single genetic variant or introduction of new variants?
- HA gene of 84 virus isolates were sequenced, comprising:
  - 28 from live ducks
  - 8 from dead ducks,
  - 2 from live chickens
  - 46 from dead chickens
### H5N1 virus isolations - Indonesia

- Different genetic variants were detected on the same farm at the same time

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<th>Farm No (Farm ID)</th>
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<th>Date</th>
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• Identical virus variants were found at different farms in the same village

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Experimental studies

• Objective: To investigate the pathogenesis and transmission dynamics of H5N1 infection in ducks and chickens
• Conducted at Australian Animal Health Laboratory (AAHL), Geelong
Clinical signs and tissue tropism in chicken

Strain | Dk/211
---|---
**Clinical signs**
Lung
Heart
Brain

Al antigen in vital tissues

Strain | Dk/213
---|---
**Clinical signs**
Lung
Heart
Brain

Al antigen in vital tissues
Tissue tropism in ducks

<table>
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<tr>
<th>Strain</th>
<th>Small amount of antigen detected in the epithelium of air sacs and paranasal sinus</th>
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<td>Dk/213</td>
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<td><img src="image3.png" alt="Image of Air sac" /> <img src="image4.png" alt="Image of Paranasal sinus" /></td>
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Means of detectable virus titres in selected tissues (brain, heart, lung, spleen, pancreas, skeletal muscle) of H5N1-infected chickens and ducks. Two different virus isolates.
Virus shedding in chickens

- Shedding of virus was detected in oral and cloacal swabs either by virus isolation in eggs or virus titration in Vero cells.
Virus shedding in ducks

Virus isolation from oral (O) and cloacal (C) swabs of ducks infected with Dk/211 and Dk/213

Dk/211

Dk/213

Low virus titre was detected in both of two groups with the range between: $10^{-0.7} - 10^{1.2}$ TCID$_{50}$/0.1 ml
Challenge trials: summary

Pathogenicity of virus:
- Although the 2 virus isolates were isolated from different disease status in ducks in the field (one dead, one healthy), they were highly pathogenic in chickens, but not able to cause apparent disease in ducks

Replication and shedding of virus:
- In chickens, both viruses replicated in most tissues and were shed orally and cloacally at high titres
- In ducks, virus replication in ducks was limited to a few tissues and virus was shed intermittently from oral route (not cloacal) at very low concentration
Transmission & persistence trials

- A further series of challenge trials was conducted to investigate the persistence of viral infection in ducks
- Two groups of ducks were experimentally infected with H5N1 virus, and another 2 groups were introduced at the acute stage (1 dpi) and post-acute (10 dpi)
- All in-contact ducks at the acute stage became infected
- None of the 9 contact ducks at 10 dpi became infected (i.e. did not seroconvert, did not shed virus), although viral RNA was detected at very low copy number in oral and cloacal swabs of 5 ducks (3 oral, 2 cloacal)
- Thus, no evidence of persistent viral infection in ducks was demonstrated
Conclusions

• Most ducks survive H5N1 virus infection, showing minimal clinical signs
• Excretion of virus from ducks is at low levels, is intermittent, and does not persist
• In contrast, chickens are severely affected, and shed very high titres of virus, but die quickly
• Ducks are likely to play an important role in maintenance of the virus in nature
• However, ducks probably pose a lesser risk of virus transmission to humans than chickens
ACKNOWLEDGEMENTS

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