Case Study:
H5N1 avian influenza

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### Influenza A Virus Host Range

<table>
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<tr>
<th>H1</th>
<th>H2</th>
<th>H3</th>
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- H1: Humans
- H2: Pigs
- H3: Horses
- H4: Birds
- H5: Cattle
- H6: Cattle
- H7: Cattle
- H8: Cattle
- H9: Cattle
Migratory Bird Reservoirs of all Influenza A Viruses

All 16 HA and 9 NA subtypes
Ecology of avian influenza in wild birds

- AI viruses replicate primarily in the intestinal tract.
- Limited overt disease signs (low path)
  - There can be a “cost”.
- Only LPAI are perpetuated in wild birds
  - Including H5 and H7 subtypes
  - Has H5N1 changed this rule?
- LPAI in wild birds are the reservoir of all influenza A viruses in other species
The H5 and H7 subtypes are unique

- Low pathogenic in wild bird reservoir
  - Largely intestinal replication
- Evolve rapidly after transmission to domestic poultry
  - Host response varies
  - Generalized infection, systemic spread-poultry, tigers
  - Replication and spread-ducks, pigs, horses

| LETHAL INFECTION | INAPPARENT INFECTION |
A child dies in Hong Kong..

1997-1998 Hong Kong H5N1 Avian Influenza Outbreak in Humans

Number of cases

0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

May June July Aug Sept Oct Nov Dec Jan

diagnoses
 deaths
H5N1 – The Starting Point?

Culling of all poultry in Hong Kong
-No more human infections
<table>
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<tr>
<th>S.E. China</th>
<th>Hong Kong</th>
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<th>Fujian Hong Kong</th>
<th>S. Korea Japan</th>
<th>Vietnam Thailand Laos Cambodia</th>
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<tbody>
<tr>
<td>Index Case</td>
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<td>Jan. 2003</td>
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<td>Jan. 2004 Clade 1</td>
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<td>Index Cluster</td>
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Initial Spread of H5N1: 2004

Current status:
- Poultry: +500 million
- Human Cases: 602
- Human Deaths: 355
Continuing evolution of H5 hemagglutinin
Control Strategies

- Stamping out-compensation - successful

- Vaccination, reduces disease signs - fails to eradicate
Ongoing outbreaks of highly pathogenic H5N1 in domestic poultry & wild birds

Bangladesh
Hong Kong
Israel
Nepal
Egypt
Bhutan
India
Indonesia
Myanmar
Vietnam
China

Humans: 24 cases/15 deaths
Pandemic Preparedness
The Spread of Pandemic H1N1
April - November 2009

- At least 6,000 deaths.
- Modest severity except in young adults
- Decreased relevance of H5N1
Rationale for research on pathogenicity and transmissibility of H5N1

"...the genetic processes and external factors leading to the emergence of pandemic influenza viruses remain incompletely understood (e.g. the potentials for mutation and genetic reassortment, as do factors associated with infectivity, transmissibility and pathogenicity"

“Research Recommendations:

1.1.1 Investigate virus-specific factors associated with zoonotic and pandemic potential (e.g. infectivity, transmissibility and pathogenicity)"

"Learning more about how influenza viruses circulate between animal reservoirs and about the evolutionary pressures that lead to the emergence and spread of new viral sub-types—especially the factors that favor transmission from animals to humans—are urgent research priorities."
Reverse genetics for influenza viruses

- Luytjes..., Palese
- Neumann...Kawaoka
- Hoffmann et al

A/HongKong/213/03 (H5N1)

- Luytjes..., Palese

A/Puerto Rico/8/34 (H1N1)

- Neumann...Kawaoka
- Hoffmann et al

Cell 1989
PNAS 1999
PNAS 2000
Approval processes for a “typical” H5N1 transmission experiment

**Approvals based on “science”**
- Peer review of grant funding
- NIH Office of Biotechnology Activities (for some experiments)
- Institutional Biosafety Committee
- Institutional Animal Care and Use Committee

**Approvals based on “facilities”**
- Select Agent registration
  - Requires
  - Specific HPAI enhancements to standard BSL3
  - USDA and/or CDC registration paperwork
  - Multiple inspections for facilities, security, inventory, etc.
  - Renewed every three yrs
- Personnel requirements
  - Dept. Justice personnel clearance
  - Documented personnel training and proficiency

**Approvals based on “material transfer”**
- Importation permits
  - Requires
  - USDA permit
  - CDC permit
  - USDA facility inspections
- Export permits
  - Requires
  - Dept. of Commerce License
Biosecurity -

*to prevent loss, theft, or misuse of microorganisms and biological material*

Accomplished by limiting access to facilities, research materials, and information

- All personnel undergo FBI Security Risk Assessment
- Access granted through biometric reader
- Inventory and tracking of virus strains is strictly controlled
- Inventory is inspected/verified biannually by USDA
Biosafety
- to reduce or eliminate exposure of individuals and the environment to potentially hazardous biological agents

Accomplished by four primary controls

1. **PPE:**
   Powered-Air Respirator (PAPR), Scrub Suit, Tyvek Coverall, Dedicated Shoes, Booties, Disposable Gown, 2 Pair Nitrile Gloves

2. **Work Place Practices:**
   Entry and Exit Process, Decontamination, Emergency Procedures, Handling Sharps, Working in Class II Biosafety Cabinets

3. **Administrative:**
   Immunizations, Security Clearance, Training, Supervision

4. **Engineering:**
   Directional Air Flow, HEPA Filtration, BSCs, Entry and Access Point Control
The Fouchier/Kawaoka reports

- Avian HP H5N1 viruses have the potential to become mammalian transmitted
- Identification of specific markers in HA
  - Receptor binding
  - Stability
  - Glycosylation
- Multiple strategies to become mammalian transmissible
- Confusing information provided to NSABB
An Engineered Doomsday
Scientists have long worried that an influenza virus that has ravaged poultry and wild birds in Asia might evolve to pose a threat to humans. Now scientists financed by the National Institutes of Health have shown in a laboratory how that could happen...

The Latest on the Doomsday Virus
We can worry less that a newly created bird flu virus might kill tens or hundreds of millions of people if it escaped from the laboratory. But there is still some residual danger....
Global influenza program for influenza
WHO/Indonesia/Vietnam

Sharing of influenza viruses and knowledge
**H5N1: The continuing global threat**

**Indonesia**
- Humans: 188 cases/156 deaths
- Endemic in poultry

**Egypt**
- Humans: 167 cases/60 deaths
- Endemic in poultry

**Bangladesh**
- Humans: 6 cases/0 deaths
- Poultry: over 2 million dead

**Wild birds: Clade 2.3.2.1**
- Whooper swan, Grebes, Tufted duck, passerines
- China, Japan, Mongolia, Israel, Vietnam, South Korea

*Nature is the greatest bioterrorist threat*
Benefits

Public Health:
- Knowledge that H5N1 virus can adapt to become efficiently transmissible in mammals/humans.
  - Pandemic preparedness for H5 is needed: viruses persistently endemic in poultry in many countries, such as China, Indonesia, Egypt and Vietnam.
  - Continued monitoring of H5N1 antigenic changes in the field.
  - Control measures for blocking transmission from avian species to mammalian species (such as swine) to prevent further adaptation. (> 500 millions pigs in China)
- Provide the knowledge to determine which of the multitude of influenza viruses have pandemic potential – risk assessment
  - Which clade of H5N1 is acquiring the necessary mutations for transmissibility?
  - Vaccine seed stock preparation - need to keep updating the vaccine seed.
  - Stamping out – with international assistance.

Science:
- Look for the mutations that would confer such functionality (they may already exist!!!):
  - Need for increased surveillance and rapid sequencing and sharing.
  - Importance of deep sequencing of all human H5N1 original isolates.
More Science to be addressed...

- Is there a required sequence of events for the transmission to occur; i.e. are the receptor binding mutations a critical first step during the adaptation?
- Does the virulence of transmissible H5N1 increase or decrease for mammals (as a result of tissue tropism change)?
- Do the changes in the receptor binding domain alter the antigenicity and immunogenicity of H5N1 viruses?
- Does the transmissible H5N1 possess the capacity to spread in wild birds or in swine?
- Do the transmissible H5N1 viruses have a higher propensity to reassort with the circulating pandemic H1N1? (it most likely occurs in China, Thailand, Vietnam)
Risks

- High lethality in humans?

- Laboratory escape: intentional or accidental

- Development of bioterrorist agent – possible but less likely
  - H5N1 continues to evolve and spread, including in countries with high terrorist threat.
    - Shutting down research in US will not necessarily be followed globally.

- Pandemic influenza will definitely emerge again. Will it be H5? (Nature poses the greatest bioterrorist threat.)

- Life is not risk free.
The future... Risk management

- “The cat is out of the bag.” Things cannot be undone.
- Managing the risk instead of avoiding the risks
  - Managing the risk instead of thinking that stopping US research will make the risk disappear
- Are the risks manageable?
  - Assess the risk of research projects and receive pre-approval
    - Dual Use Research of Concern (DURC)
  - Inspections, inspections...
  - “Real-time” communication between principle investigators with funding agency; avoid surprises...
- Can the questions be addressed using low-path viruses?
  - Yes partially but not the high pathogenic aspects
- Education of the scientists and younger generations.
“Risk free?”
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