The A(H7N9) influenza outbreak in China

Anne Kelso
Director
WHO Collaborating Centre for Reference and Research on Influenza
Melbourne
Influenza in the 21st century: why do we worry?
Influenza in the 21st century: population density

Influenza in the 21st century: connectedness

Influenza in the 21st century: animal husbandry and trade

China has ~12 billion poultry.
“Habitat” of influenza A viruses

Water birds are their natural host.

Viruses may cause sporadic infection and become adapted to new avian or mammalian hosts.
Influenza A viruses

8 RNA strands

NA (neuraminidase)

HA (haemagglutinin)

Basis of nomenclature: H1N1, H3N2 etc

Adapted from De Jong et al, J Infect 40:218-228 (2000)

Linda M. Stannard, University of Cape Town
Influenza A viruses: subtypes, immunity and variation

- **8 RNA strands**
- **NA (neuraminidase)**
- **HA (haemagglutinin)**

Major sites of variation due to immune pressure (antigenic drift)

10^-4 mutation rate due to lack of proof-reading mechanism

Segmented genome allows reassortment during co-infection (antigenic shift)

Major targets of human antibodies that protect against infection
Development of new influenza A viruses

Mutation

Drift: Random genetic changes, immune selected

Reassortment

Shift: Genome shuffling when two viruses infect one cell
The unpredictability of influenza

- Influenza viruses are highly mutable; human population immunity drives the emergence of “antigenic drift” variants.

- Co-infection with different influenza viruses can lead to the formation of new “reassorted” viruses which may cause pandemics.

- Avian and other animal reservoirs provide a perpetual supply of influenza A viruses which can adapt or reassort to infect humans.

- Virulence and transmissibility of new influenza viruses are unpredictable.

- There is a small but real risk of a catastrophic pandemic.
## Human infection with influenza A viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Year</th>
<th>Pathogenicity</th>
<th>Human-human transmission</th>
<th>Pandemic</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(H1N1)</td>
<td>1918</td>
<td>+++</td>
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<td>yes</td>
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<td>A(H1N1)pdm09</td>
<td>2009</td>
<td>+</td>
<td>++</td>
<td>yes</td>
</tr>
<tr>
<td>HPAI A(H5N1)</td>
<td>2003 – now</td>
<td>+++</td>
<td>+/-</td>
<td>no</td>
</tr>
</tbody>
</table>
The event
### The first week of the outbreak

<table>
<thead>
<tr>
<th>Date</th>
<th>Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunday 31 March</td>
<td>- China notified WHO of 3 cases of H7N9 infection: severe pneumonia, 2 deaths (Shanghai), one critical (Anhui)</td>
</tr>
<tr>
<td></td>
<td>- China WHO CC deposited full genome sequences in GISAID database</td>
</tr>
<tr>
<td>Monday 1 April</td>
<td>- Report that samples from some of thousands of dead pigs in river were negative for virus</td>
</tr>
<tr>
<td>Tuesday 2 April</td>
<td>- Report of 4 new cases from different parts of Jiangsu province: all critical</td>
</tr>
<tr>
<td>Wednesday 3 April</td>
<td>- Report of 2 new cases in Zhejiang province: one death, one critical</td>
</tr>
<tr>
<td>Thursday 4 April</td>
<td>- Report of one death in Shanghai, one new case in Zhejiang province</td>
</tr>
<tr>
<td></td>
<td>- Report of virus detection in pigeon sample from Shanghai market</td>
</tr>
<tr>
<td></td>
<td>- Report of 4 more cases in Shanghai: 2 deaths, one critical, one recovering (4 year old)</td>
</tr>
<tr>
<td>Friday 5 April</td>
<td>- Report of 2 new cases in Jiangsu: critical</td>
</tr>
<tr>
<td>Saturday 6 April</td>
<td>- Report of 2 new cases in Shanghai: under treatment</td>
</tr>
<tr>
<td></td>
<td>- Reports of further connections with market poultry (chickens, quail)</td>
</tr>
<tr>
<td></td>
<td>- Closure of Shanghai live poultry markets</td>
</tr>
<tr>
<td>Sunday 7 April</td>
<td>- Report of 2 new cases in Shanghai and Anhui</td>
</tr>
</tbody>
</table>
Date of onset of illness of first 82 confirmed patients

Figure 1. Date of Onset of Illness in First 82 Patients with Confirmed H7N9 Virus Infection, According to Province in China. CDC denotes Chinese Center for Disease Control and Prevention, ILI influenza-like illness, and NIC National Influenza Center.
Location of H7N9 Influenza in China (5/7/13)*

*132 total cases/31 deaths

<table>
<thead>
<tr>
<th>Province/City</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anhui</td>
<td>4</td>
</tr>
<tr>
<td>Beijing</td>
<td>2</td>
</tr>
<tr>
<td>Fujian</td>
<td>5</td>
</tr>
<tr>
<td>Henan</td>
<td>4</td>
</tr>
<tr>
<td>Hunan</td>
<td>2</td>
</tr>
<tr>
<td>Jiangsu(^\alpha)</td>
<td>27</td>
</tr>
<tr>
<td>Jiangxi</td>
<td>6</td>
</tr>
<tr>
<td>Shandong</td>
<td>2</td>
</tr>
<tr>
<td>Shanghai</td>
<td>34</td>
</tr>
<tr>
<td>Zhejiang</td>
<td>46</td>
</tr>
</tbody>
</table>

\(^\alpha\) includes a case hospitalized in Taiwan
A(H7N9) viruses
A novel reassortant virus of avian origin

The NEW ENGLAND JOURNAL OF MEDICINE

ORIGINAL ARTICLE

Human Infection with a Novel Avian-Origin Influenza A (H7N9) Virus

Rongbao Gao, M.D., Bin Cao, M.D., Yunwen Hu, M.D., Zijian Feng, M.D., M.P.H., Dayan Wang, M.D., Wanfu Hu, M.D., Jian Chen, M.D., Zhijun Jie, M.D., Haibo Qiu, M.D., Ph.D., Ke Xu, M.D., Xuwei Xu, M.D., Hongzhou Lu, M.D., Ph.D., Wenfei Zhu, M.D., Zhancheng Gao, M.D., Nijuan Xiang, M.D., Yinzhong Shen, M.D., Zebao He, M.D., Yong Gu, M.D., Zhanyong Zhang, M.D., Yi Yang, M.D., Ph.D., Xiang Zhao, M.D., Lei Zhou, M.D., Xiaodan Li, M.D., Shumei Zou, M.D., Ye Zhang, M.D., Xiyan Li, M.D., Lei Yang, M.D., Junfeng Guo, M.D., Jie Dong, M.D., Qun Li, M.D., Libo Dong, M.D., Yun Zhu, M.D., Tian Bai, M.D., Shiwen Wang, M.D., Pei Hao, M.D., Weizhong Yang, M.D., Yanping Zhang, M.D., Jun Han, M.D., Hongjie Yu, M.D., Desin Li, M.D., George F. Gao, Ph.D., Guizhen Wu, M.D., Yu Wang, M.D., Zhenghong Yuan, Ph.D., and Yuelong Shu, Ph.D.

NEJM, 11 April 2013

Figure 3. Hypothetical Host and Lineage Origins of the Gene Segments of the Novel Reassortant Human Influenza A (H7N9) Viruses. The colors of the gene segments in the ovals indicate their origin. BJ16 denotes A/brambling/Beijing/16/2012, KO14 A/wild bird/Korea/Al4/2011, and ZJ12 A/duck/Zhejiang/12/2011.
What do we know about human H7N9 viruses?

Based on first three viral isolates – A/Shanghai/1/2013, A/Shanghai/2/2013, A/Anhui/1/2013:

- New mixture of influenza genes not previously detected in humans or other species
- Most closely related to Eurasian H7, N9 and H9N2 viruses from birds (95–99% similar):

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nearest known relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>2011 duck virus from Zhejiang (H7N3)</td>
</tr>
<tr>
<td>NA</td>
<td>2010 mallard virus from Czech Republic (H11N9)</td>
</tr>
<tr>
<td></td>
<td>Wild bird viruses from E/SE Asia (H11N9)</td>
</tr>
<tr>
<td>M</td>
<td>2011 chicken virus from Zhejiang (H9N2)</td>
</tr>
<tr>
<td>PB2</td>
<td>2012 brambling virus from Beijing (H9N2)</td>
</tr>
<tr>
<td>PB1</td>
<td>2010 chicken virus from Jiangsu (H9N2)</td>
</tr>
<tr>
<td>PA</td>
<td>2012 brambling virus from Beijing (H9N2)</td>
</tr>
<tr>
<td>NP</td>
<td>2011 chicken virus from Zhejiang (H9N2)</td>
</tr>
<tr>
<td>NS</td>
<td>2011 chicken virus from Dawang (H9N2)</td>
</tr>
</tbody>
</table>

- A/Shanghai/1/2013 is different from the other two viruses, especially in NP gene
- H7 and H9 viruses considered a pandemic threat; H9N2 viruses known to be prone to reassortment
Phylogenetic analyses of human H7N9 viruses

Eurasian H7 viruses with various N subtypes

Various Asian H9N2 viruses

http://epidemic.bio.ed.ac.uk/node/23
Phylogenetic analyses of human H7N9 viruses: HA and NA

Figure 3: Phylogenetic trees for the haemagglutinin (HA1) (A) and neuraminidase (N) (B) genes of H7N9 viruses isolated from a patient and a chicken in Zhejiang, China.

Chen et al., Lancet, 25 April 2013
Important features of human H7N9 virus sequences

Haemagglutinin
• lacks multi-basic cleavage site needed for high pathogenicity in birds
• Q226L mutation allowing strong binding to α2,6-linked sialic acid receptors (mammalian)
• some have V186G mutation which increases α2,6-SA affinity
• T156A mutation causing loss of glycosylation site

Neuraminidase
• lacks H275Y mutation which confers Tamiflu resistance

PB2
• in some cases, E627K mutation associated with replication in mammalian respiratory tract (low temp)

M, PB1 and NS1
• several mutations associated with virulence in mice
• PB1 has 368V mutation associated with H5 transmission in ferrets
• M gene has S31N mutation which confers resistance to adamantane class of antiviral drugs

Characteristics of H7N9 viruses

- Novel reassortants of avian H7, N9 and H9N2 (internal genes) viruses, probably in eastern China
- Differences between several isolates suggest different ancestry
- May have circulated for several months before detection
- Human and poultry isolates closely related (99%+)
- Features of a low-pathogenic avian virus adapted to poultry
- Genetic signatures of adaptation to infection of mammalian hosts
- Increased $\alpha$-2,6- and decreased $\alpha$-2,3-linked sialic acid binding (glycan array binding)
- Sensitive to oseltamivir (Tamiflu), zanamivir (Relenza), peramivir and laninamivir
- Grow well in embryonated eggs and mammalian cell lines (MDCK with trypsin)
- Agglutinate a variety of avian and mammalian red blood cells
- Detected by real-time RT-PCR with primers and probes for Eurasian H7 viruses
Human H7N9 cases
Current status of cases by date of symptom onset (9 May)

Figure 1. Current health status of H7N9 cases in China and Taiwan by Date of Symptom Onset, as of 9 May 2013. Seven cases have not been included due to incomplete data.
Median age ~61 years
Male:female ratio 2.4
Case fatality rate ~20%
Mostly urban

Underlying medical conditions:
- hypertension
- heart disease
- diabetes
- chronic bronchitis....
Comparison of China's Population Distribution with the Number of H7N9 Cases by Age Groups, April 22, 2013

FluTrackers.com
Family Cluster 1

Index case patient
(Suspected case)

Shanghai family cluster

Case patient 2
(Confirmed case 1)

Case patient 3
(Confirmed case 2)
What is the true severity of H7N9 infection?

- Deaths
- Hospitalisations
- Confirmed
- Exposed
- Immune
- Asymptomatic

- Waiting on seroprevalence studies and serological testing of close contacts to distinguish these scenarios
Characteristics of human H7N9 cases

- Apparently high rates of severe and fatal disease, especially in older age groups
- Strong male bias, mainly urban, often with co-morbidities
- Mild cases mainly in the young, including one 4 year-old asymptomatic case
- Cases generally not epidemiologically linked
- Testing of large number of close contacts: little ILI, very few PCR-positive
- Possible limited person-to-person transmission in a few family clusters
- Early symptoms often fever and cough but upper respiratory tract symptoms short-lived
- Rapid progression to pneumonia, ARDS, multi-organ failure
- Deep lung involvement consistent with dual binding to α-2,3- and α-2,6-linked sialic acid receptors in respiratory tract
The source
Possible sources of H7N9 viruses infecting humans

- Poultry: live bird markets, farms
- Other domestic birds: homing pigeons, songbirds
- Other domestic animals: pigs, cats, dogs
- Wild birds
Closed live poultry wholesale market in Shanghai

All live bird markets in Shanghai were closed on 6 April after detection of H7N9 by PCR in market poultry.
Potential spread of highly pathogenic avian influenza H5N1 by wildfowl: dispersal ranges and rates determined from large-scale satellite telemetry
Sources of human H7N9 infection

• Hundreds of thousands of poultry, wild bird and environmental samples tested

• About 50 positives, mostly from chickens, poultry pigeons, ducks or environment in live poultry wholesale or trading markets

• No positives detected at poultry farms within or outside the affected provinces

• Positive markets linked to human cases

• Poultry isolates genetically closely related to human isolates

• One positive wild bird in Nanjing

• No positives among thousands of swine, dog and cat samples

• A high proportion of human cases had poultry contact

➢ Exposure to poultry in live bird markets is the major identified risk factor

➢ Consistent with amplification of virus in live markets
Public health interventions
Public health interventions

• Enhanced and continuing human and animal surveillance throughout China

• Closure of live poultry markets in some cities (for how long?)
Cases of H7N9 Influenza in China by Province/City (5/7/13)*

*4 cases from Jiangsu, 4 cases from Shanghai, 1 case from Beijing, 1 case From Jiangxi and 1 case from Henan are missing date of onset. Case hospitalized in Taiwan is not shown.

Total cases = 132
Cases of H7N9 Influenza in China by Province/City (5/7/13)*

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Total cases = 132
Public health interventions

• Enhanced and continuing human and animal surveillance throughout China

• Closure of live poultry markets in some cities (for how long?)

• Other containment?

• Clinical guidelines for patient care (including antiviral drug use) and HCW protection

• Development and validation of RT-PCR-based diagnostic tests:
  Sharing of protocols and primer/probe sequences (CNIC, WHO, CDC, Melb etc)
  Distribution of H7N9 RNA as reference material (WHO labs including Melb)
  Distribution of PCR kits (CNIC, CDC)

• Initiation of vaccine development in readiness – earlier H7 candidate vaccine viruses likely to be poorly matched
Public health interventions: vaccine development

- Vaccine must be produced at BSL3 or attenuated for safety reasons.
- WHO is developing guidance on essential safety testing before vaccine viruses can be used at BSL2.
- Several WHO labs are developing reverse genetics viruses or conventional reassortant viruses (H7, N9 + different internal genes).
- Others are developing live-attenuated vaccines.
- “Potential” candidate vaccine viruses will be available to vaccine manufacturers before full testing.
- WHO will recommend a vaccine strain once antigenic data are available (pending).
- Individual countries and manufacturers will decide whether to produce/purchase vaccine but might not do so unless there is evidence of sustained person-to-person transmission.
- Vaccines normally take several months to develop, trial and produce in bulk.
Risk assessment
## Risk assessment of human H7N9 viruses

<table>
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<tr>
<th>Parameter</th>
<th>Characteristics</th>
</tr>
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<tbody>
<tr>
<td><strong>Source of infection</strong></td>
<td>• domestic poultry, especially in live poultry markets</td>
</tr>
<tr>
<td></td>
<td>• low pathogenicity in poultry</td>
</tr>
<tr>
<td></td>
<td>• origin of virus contaminating markets still unknown</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td>• now detected over a large area of China</td>
</tr>
<tr>
<td></td>
<td>• high population and animal density</td>
</tr>
<tr>
<td></td>
<td>• Shanghai is major travel and business hub</td>
</tr>
<tr>
<td><strong>Severity in humans</strong></td>
<td>• severe pneumonia with high mortality</td>
</tr>
<tr>
<td></td>
<td>• very few mild cases detected</td>
</tr>
<tr>
<td></td>
<td>• seropositivity in contacts unknown</td>
</tr>
<tr>
<td></td>
<td>• general population expected to lack pre-existing antibodies</td>
</tr>
<tr>
<td><strong>Human-human transmission</strong></td>
<td>• a few suspect family clusters</td>
</tr>
<tr>
<td></td>
<td>• disease not generally detected in close contacts</td>
</tr>
<tr>
<td><strong>Genetic signals</strong></td>
<td>• reassortant of avian viruses</td>
</tr>
<tr>
<td></td>
<td>• several adaptations to mammals</td>
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➢ Pandemic risk difficult to assess but greater than for highly pathogenic avian H5N1
# Human infection with influenza A viruses

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<td>2013</td>
<td>+++?</td>
<td>+/-?</td>
<td>?</td>
</tr>
</tbody>
</table>
H7N9: a cause for continuing concern

- Apparently severe disease in humans with high mortality
- Poultry likely to be the vector but source of infected poultry unknown (low-pathogenic)
- The virus is partially adapted to mammalian hosts = elevated pandemic risk
- Early success by closing live poultry markets
- Long-term changes to poultry production and trade likely to be beneficial
Thank you

Special thanks to the WHO Collaborating Centre for Reference and Research on Influenza, China CDC, Beijing, and other members of the WHO Global Influenza Surveillance and Response System.

The Melbourne WHO Collaborating Centre for Reference and Research on Influenza is supported by the Australian Government Department of Health and Ageing.