Lessons Learnt from Emerging Viruses: (Coronavirus and Influenza A Virus)

Outline

• Coronavirus Background
• SARS Outbreak
  – Cause of the 2002-2003 SARS Outbreak
    • SARS-CoV
      – discovery, diagnostics, transmission
    – Origin of SARS-CoV In Humans
• Influenza A virus background
  – Evolution and ecology
  – Pandemics
    • Past, present
Coronavirus

Enveloped
Large mRNA genome
N, S, (HE), M, E
Respiratory
Pandemic or epidemic
Zoonotic and epizootic

K.V. Holmes, 2003
Coronaviruses Naturally Infect Many Species
<table>
<thead>
<tr>
<th>Virus</th>
<th>Host</th>
<th>Major Disease</th>
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<tbody>
<tr>
<td>HCoV-229E</td>
<td>human</td>
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<td>HCoV-NL63</td>
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<td>enteritis and respiratory infection</td>
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<td>peritonitis, systemic infection</td>
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<td>SARS-CoV</td>
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<td>BCoV</td>
<td>cattle</td>
<td>enteritis</td>
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<td>TCoV</td>
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<td>RCoV</td>
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<tr>
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<td>IBV</td>
<td>chicken</td>
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Coronavirus Spike

Corona" appearance
- Host range
Membrane fusion
Neutralizing antibodies
Severe Acute Respiratory Syndrome (SARS)

Outbreak of “atypical pneumonia” 2002-2003
Rapid progression to ARD
Ventilator required
Alarming death rate
Person-to-person transmission
Emergence of SARS

November 2002

Guangdong Province
Southern China
Outbreak of Severe Acute Respiratory Syndrome

2002

Nov. 16

SARS-CoV

2003

Feb. 14

Hotel (HK)

Feb. 28

WHO alerted

Mar. 12

Global alert

4 Months

July

Human chain of transmission broken

Total of 8,098 cases of SARS and 774 deaths in 30 countries

Dr. Carlo Urbani
(SARS-CoV/Urbani)
SARS-The Disease

Symptoms
- Fever
- Dry cough
- Diarrhea
- Acute interstitial pneumonia
2-10 day incubation period
20% intensive care
10% case fatality rate

Seroconversion
- 2-4 weeks post-onset

Transmission
- respiratory droplets
Symptoms of SARS

- Fever
- Production Cough
- Myalgia
- Dyspnea
- Headache
- Malaise
- Chills
- Diarrhea
- N/V
- Sore Throat
- Arthralgia
- Chest pain
- Production Cough
- Rhinorrhea

JAMA, June 4, 2003 – Vol. 289, No. 21
Clinical Outcome

20% admitted to ICU
5% required mechanical ventilation
~10% died

Increased risk of death or ICU admission if:

- Increased age
  - < 60 yo: 6.8%
  - > 60 yo: 55.0%
- Co-morbidity
- High LDH
- High neutrophil count

Tsui et al. EID 2003; 9: 1064-1069
Fowler et al. JAMA 2003; 290: 367-373
Lew et al. JAMA 2003; 290: 374-380
Chan et al. Thorax 2003; 58: 686-689
Choi et al. Ann Int Med 2003; 139: 715-723
The Hunt for the *Causative Agent of SARS*

1. Ruled out known respiratory viruses
2. Inoculated cells with clinical specimen

+ clinical specimen: oropharyngeal washes
  - respiratory specimen
  - sputum
  - lung biopsy
  - kidney biopsy

*CPE seen only in Vero and FRhK-4*

Figure 1. Ksiazek et al. 2003 NEJM 348:1953-1966
SARS-CoV isolated from SARS patients’
- Lungs, sputum, feces, etc.
- Grown in monkey kidney
  - Vero and pRHK

Serological cross reaction
SARS-CoV not previously circulated in humans
Some distantly related to known RSVs.
Roux’s postulates fulfilled
In a short period of time….

- Virus detected
- Virus amplified
- Virus identified
- Sequenced
- Koch’s postulates
Laboratory diagnosis:

First Generation tests: March 28

- RT-PCR for P gene
- Indirect immuno-fluorescence serology using virus infected cells

positive  negative H₂O

courtesy of J. M. Peiris
Problems Providing Laboratory Diagnostics During an Outbreak

no time to evaluate tests in advance; evaluate “on the run”

avalanche of specimens: 200/day

no/little clinical information available in real time” to help evaluate results

unrealistic expectations

unknown risk to laboratory workers

courtesy of J. M. Peiris
Figure 4: Sequential quantitative RT-PCR for SARS-associated coronavirus in nasopharyngeal aspirates of 14 SARS patients.
Transmission of SARS

Positive samples: respiratory samples, stool, blood, urine, conjunctival secretions

Droplet transmission
- Respiratory-droplet
- Fecal-droplet (?)

Contact transmission
- Direct > indirect

Period of transmissibility
- Symptom onset to at least 10 days after fever resolution*
  - *virus detectable but no reported transmission
Generation of Secondary Cases

- Number of cases
- Number of secondary cases
- Average/case

Days from Onset to Isolation:

- 0
- 1
- 2
- 3
- 4
- 5-6
- 7-8
- 9+

Secondary cases / case:

- 0
- 1
- 2
- 3
- 4

Spread of SARS

ospitals
or amplifiers of virus
% of people infected were HCW

Travel
International flights
37 positive individuals
ights led to spread
>>60 cases exported by air
Transmission in the Face of Precautions

Circumstances:
- Severely ill patients
  - Shedding high titer
- Close contact
- Aerosol generating procedures

Potential Exposures:
- High level of airborne virus
  - Improper N95 respirators (non fit-tested)
- Break in containment
  - For example, improper removal of PPE
SARS-CoV- Group 2 CoV
ARS coronavirus: Where did it come from?

Zoonotic transmission: Viruses genetically related to SARS-CoV isolated from Himalayan palm civet cats.

In live animal markets
SARS-CoV-like virus concurrently identified
Not found in wild civet
Changes in Spike Important in Emergence of SARS-CoV?
Chinese horseshoe bats: Viruses genetically related to SARS-CoV have been isolated from animals.

SARS-CoV
89% RT-PCR
84% N antibody
Possible Genesis of SARS Outbreak

Horseshoe bat

Direct zoonosis

? Intermediate

Palm civet

- SARS-CoV antibodies
  - 40% of wild animal traders
  - 20% of slaughterers
  - 5% of vegetable traders

et al., Science 370: 297

Science 370: 297
Influenza A Virus

- 16 distinct HA’s +1
  - (H1-H16)
- 9 distinct NA’s +1
  - (N1-N9)
- Nomenclature
  - A/Chicken/WI/5/78 (H7N7)

Importance of a Segmented RNA Genome

Parental

H5N1  H3N2

256 Possible Combinations
Influenza A Ecology and Emergence of Strains with Pandemic Potential
### Natural Distribution of HA and NA

#### Hemagglutinin (HA)

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<th>Human Beings</th>
<th>Other Mammals</th>
<th>Aquatic Birds</th>
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<td><img src="human.png" alt="Human Beings" /></td>
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- Zoonotic

#### Neuraminidase (NA)

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<th>N4</th>
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Human Influenza A Virus Evolution

**Genetic Drift**

H3N2 1968 → H3N2 1975 → H3N2 1993 → H3N2 1997 → H3N2 2009
Hong Kong → Victoria → Beijing → Sydney → Albany

**Genetic Shift**

H1N1 1918 ("Spanish") → H2N2 1957 ("Asian") → H3N2 1968 ("Hong Kong") → H1N1 2009 ("SOIV")
Antigenic Drift Important in 2007/08 Season

Pneumonia and Influenza Mortality for 122 U.S. Cities
Week Ending 01/31/2009

- A/Wisconsin/67/2005-like viruses (22%)
- A/Brisbane/10/2007-like (H3N2) (71%)

Epidemic Threshold
Seasonal Baseline
Drift Important In 2007/08 Season

Pneumonia and Influenza Mortality

for 122 U.S. Cities

Week Ending 04/05/2008

A/Wisconsin/67/2005-like viruses (22%)
A/Brisbane/10/2007-like (H3N2) (71%)
Antigenic Shift and Previous Human Pandemics

1918 H1N1 → Entirely Avian
1957 H2N2 → H2 HA HN2 NA PB1
1968 H3N2 → H3 HA PB1
1977 H1N1

- Direct interspecies transmission
- Reassortment between 2 coinfecting strains
- reappearance of a previous strain
Pigs Are "Mixing Vessels" for Influenza A Viruses
Molecular Mechanism of Mixing Vessel

SA\(\alpha\)2,6Gal

SA\(\alpha\)2,3Gal

SA\(\alpha\)2,3Gal

And

SA \(\alpha\)2,6Gal
Emergence of Novel H1N1 Causes Pandemic

Influenza Positive Tests Reported to CDC by U.S. WHO/NREVSS Collaborating Laboratories, National Summary, 2008-09
New H1N1 Causes Out of Season Pediatric Deaths

Number of Influenza-Associated Pediatric Deaths by Week of Death:
2005-06 season to present

As of 4 October 2009, more than 375,000 laboratory confirmed cases of pandemic influenza H1N1 2009 and over 500 deaths reported to WHO.
Where did the 2009 H1N1 pandemic virus come from?
Swine Influenza A Virus in North America

1930 Shope (1931)

Classical Swine Lineage (Little drift)

1997 H3N2 (Human) (A/Sw/Ont/97)
1998 H3N2 (Human X Swine)
2000 H3N2 (Human X Swine X avian)
2001 H1N2 H1N1 rH1N1 huH1N1 huH1N2
2009 cH1N1 H3N2

Triple Reassortant Viruses
Evolution of Human 2009 H1N1 Virus


Figure 4 | Genesis of swine-origin H1N1 influenza viruses. In the late 1990s, reassortment between human H3N2, North American avian, and classical swine viruses resulted in triple reassortant H3N2 and H1N2 swine viruses that have since circulated in North American pig populations. A triple reassortant H1N1 swine virus was identified in Mexican pigs in 2009, which served as the pandemic seed virus that led to the 2009 H1N1 pandemic.
Emerging and Re-emerging Diseases (NIAID)

Group I—Pathogens Newly Recognized in the Past Two Decades
- Anthamebiasis
- Australian bat lyssavirus
- Borna disease virus
- Bartonella henselae
- Brucellosis
- Ehrlichiosis
- Ehrlichia chaffeensis
- Ehrlichia risticii
- Ehrlichia ewingii
- Ehrlichia canis
- Ehrlichia risticii
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Group II—Re-emerging Pathogens
- Enterovirus 71
- Clostridium difficile
- Mumps virus
- Streptococcus, Group A
- Staphylococcus aureus

Group III—Agents with Bioterrorism Potential
See the full list of NIAID Category A, B, and C Priority Pathogens.

Most Recent
- Middle East Respiratory Syndrome-Coronavirus (MERS-CoV)
- H7N9-Influenza A Virus
MERS Epidemiology

By Sex
- 24 female
- 59 male

By Outcome
- 47 alive
- 38 dead

85 selected out of 85 cases | reset all
Clinical Disease Course

SARS

1. Transmission
   - aerosol, fomites
2. Acute Infection
   - virus replication in lung
   - "cytokine storm"
   - mixed inflammatory infiltrates
   - developing lung damage (viral and host)
   - SARS-CoV specific IgG
3. Non-Severe cases (70-80%)
   - decrease titer
   - improved chest x-ray
4. Severe cases (20-30%)
   - intubation
   - ARDS
   - hypoxemia
   - mortality (10% total)

MERS

1. Transmission
   - aerosol, fomites?
2. Acute Infection
   - virus replication in lung (and kidney?)
   - probably "cytokine storm"
   - mixed inflammatory infiltrates
   - developing lung damage (viral and host)
   - MERS-CoV specific IgG
3. Non-Severe cases (50%?)
   - decrease titer
   - improved chest x-ray
4. Severe cases (50%?)
   - intubation
   - ARDS
   - hypoxemia
   - mortality (50% total)
MERS Clinical spread

- Spread from some unknown reservoir to people (Camels/Bats suspected)
- Spread from person to person in hospital settings
- Spread by travel, either vacation or for treatment
MERS-CoV replicates efficiently and causes MERS-like clinical and pathological symptoms in Rhesus Macaques.
Coronavirus Phylogeny

(partial NSP12) (Partial RdRp)
Coronavirus Phylogeny

Chan et al. MBIO 2013
(Partial RdRp)
Zoonotic H7N9 Influenza Outbreak

- Identified unique H7N9 virus in people in late March
- Novel subtype for humans
- Antigenic shift -> Pandemic potential
- Sequence of first viruses available April 1
- 135 cases to date
evolution of H7N9
Mutations In H7 HA Important in Zoonosis/Transmission

Deep sequencing shows mixed population of virus genome in human isolate
R. Fouchier and J. Richt collaboration
Discussion Questions

disease of animals important to humans?

Why/Why not

Examples

do we know SARS-CoV was new to humans?

What profession was dramatically affected by SARS?

What are mechanisms of evolution of influenza A virus?

Is the new human H1N1 pandemic virus composed entirely of swine influenza A virus gene segments?

MERS-CoV and H7N9 serious threats?
Koch’s Postulates

1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
2. The microorganism must be isolated from a diseased organism and grown in pure culture.
3. The cultured microorganism should cause disease when introduced into a healthy organism.
4. The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.