Influenza and Other Viral/Bacterial Infections and Bioterrorism Agents
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Modes of Respiratory Infection Transmission

- **Direct Contact**
  - Inoculation from hands or fomites (objects)
- **Large droplet**
  - Particles >10 μm land on mucosa of a susceptible individual in close proximity
- **Airborne Transmission**
  - Fine particles (droplet nuclei or residue) <5 μm are inhaled & deposited in the respiratory tract, initiating infection.
Classification of Aerosol Transmission of Disease

- Obligate
- Preferential
- Opportunistic

Obligate Airborne Transmission

- Target cells only present in distal lung
  - Infection must initiate in alveoli and/or small airways
  - Fine particle aerosols required to reach target

- Agents
  - *Mycobacterium tuberculosis*
  - H5N1 Influenza A?
Preferential Airborne Transmission

• Target cells present in upper and lower airways
  – Infection can initiate anywhere in respiratory tract.
  – Fine particles, larger droplets, and fingers can reach susceptible tissues
• Modified or attenuated disease if not via lower airways
  – Variolation
    • Skin inoculation or nasal insufflation → milder disease ↓ mortality
  – Inhalation versus Cutaneous Anthrax
  – Experimental influenza:
    • Severity: nose drops << aerosols
    • Infectious dose: nose drops >> aerosols
• Agents:
  – Influenza, measles, smallpox, varicella-zoster, adenovirus, 
    *Bacillus anthracis*

Opportunistic Airborne Transmission

• Target cells present in upper and lower airways and/or GI tract and elsewhere
  – Infection can initiate anywhere in respiratory tract and/or GI tract.
  – Fine particles, larger droplets, and fingers can reach target
  – May require agent to be swallowed after deposition
• Same disease and severity regardless of route
• Often via transmitted via other routes
  – Efficiently transmitted via aerosols in certain environments.
• Agents:
  – Rhinovirus, possibly norovirus and rotavirus
The Aerobiological Pathway for Transmission of Communicable Respiratory Disease

A: Source, B: Transport and Dispersion, C: Deposition

Viral Infectious Diseases Associated with Preferential or Opportunistic Aerosol Transmission

- Influenza virus
  - Seasonal
  - Pandemic
- Corona virus
  - SARS
- Measles virus
- Varicella-zoster
- Variola major
- Rhinovirus

New diseases are emerging at the unprecedented rate of one a year for the last two decades, and this trend seems certain to continue.

SARS??

Amoy Gardens
Model of the Movement of the Virus-Laden Plume

Airborne Transmission: Boeing 737-300 Flight 2 from Hong Kong to Beijing
Probable cases of SARS by reported source of infection, Singapore, Feb 25 – Apr 30 (MMWR)

Superspreading

SARS and Masks

- N95 and surgical mask protective in ICU RR = 0.23 (p=0.02) compared with neither
- N95 compared with mask RR = 0.5 (ns)

Table 3. Nurses’ risk of acquiring SARS based on use of personal protective equipment

<table>
<thead>
<tr>
<th>Type of personal protective equipment</th>
<th>Consistent</th>
<th>Inconsistent</th>
<th>Relative risk (95% CI)</th>
<th>2-Tailed Fisher exact p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gloves</td>
<td>4.2 (18)</td>
<td>5.12 (42)</td>
<td>0.36 (0.10 to 1.24)</td>
<td>0.12</td>
</tr>
<tr>
<td>Gloves</td>
<td>4.22 (13)</td>
<td>4.10 (40)</td>
<td>0.45 (0.14 to 1.46)</td>
<td>0.22</td>
</tr>
<tr>
<td>N95 or surgical mask</td>
<td>4.28 (13)</td>
<td>5.99 (50)</td>
<td>0.22 (0.07 to 0.70)</td>
<td>0.02</td>
</tr>
<tr>
<td>N95*</td>
<td>2.26 (19)</td>
<td>5.99 (50)</td>
<td>0.22 (0.07 to 0.70)</td>
<td>0.06</td>
</tr>
<tr>
<td>Surgical mask*</td>
<td>3.4 (25)</td>
<td>5.99 (50)</td>
<td>0.45 (0.07 to 2.71)</td>
<td>0.56</td>
</tr>
<tr>
<td>N95 versus surgical mask*</td>
<td>2.16 (13)</td>
<td>1.4 (25)</td>
<td>0.40 (0.06 to 4.19)</td>
<td>0.51</td>
</tr>
</tbody>
</table>

*The comparison is use of mask. The denominator is (total-1). Changes for these comparisons are in the rows who consistently used the indicated personal protective equipment were compared to nurses who wore no masks.

*Consistent use of the N95 mask versus consistent use of a surgical mask. The denominator is (total-1). Changes for these comparisons in the nurses who consistently used the indicated personal protective equipment were compared to the indicated unique group, rather than to the row of the masks.

Loeb M et al. EID 2004;10:251
Cutaneous Anthrax 2001

Inhalation Anthrax 2001
Lethality of Anthrax: US Biowarfare studies

- Cynomolgus monkeys
  - Aerosol of Volum 1B strain, 1,236 animals
  - Median lethal dose ($LD_{50}$) 4,100 spore (95% confidence 1,980 – 8,630)
  - Probit slope = 0.67 (95% confidence 0.52 – 0.82)
- Rhesus monkeys
  - $LD_{50}$ 2,500 spores
1,236 Cynomolgus Monkeys Exposed to Airborne Spores < 5 \mu m in Diameter

Smallpox

- Viral illness
- Communicable by airborne route and by contact with contaminated materials (fomites)
Is Smallpox Airborne?

• Extensive face-to-face contact required to transmit (in most cases).
• Cases most contagious when oropharyngeal lesions are present.
• Large quantities of virus released from skin lesions including scabs after period of maximum infectiousness is over.
• Some outbreaks show clear airborne pattern.

Airborne Dispersal of Smallpox

• 1971 outbreak in German hospital
• 1 smallpox patient with cough
• 19 secondary cases over 3 floors

Mod. from Maj M.R. Bell, USUHS, Bethesda, MD
Historical Observations

- Variolation (vaccination with variola major) produced modified disease with reduced fatality rate (but oral lesions present).
- 90% of household contacts had positive throat cultures – but only 5-10% developed clinical disease.
- Virus was isolated from the exhaled breath of cases – but at very low concentrations.
- **Meets criteria for Preferential Aerosol Transmission**

Influenza

- Influenza: viral respiratory infection characterized by upper and lower respiratory symptoms, fever, generalized malaises and myalgias.
- Complications include viral pneumonia, bacterial superinfection, cytokine storm.
- Wide range of clinical outcome of infection, from asymptomatic to fatal.


Genetic Relationships among Human and Relevant Swine Influenza Viruses, 1918-2009

Figure 2: Influenza A viruses circulating in the human population. Viruses with three different hemagglutinin subtypes (H1, H2 or H3) and two neuraminidase subtypes (N1 or N2) have been identified in humans. Solid squares indicate the introduction of the pandemic H1N1, H2N2 and H3N2 strains in 1918, 1957 and 1968, respectively. In 1977, H1N1 viruses similar to those of 1950 were reintroduced. Broken lines indicate the absence of virus isolates and only indirect evidence for circulating strains based on serologic data.

Palese P. Nat Med 2004; 12: S82-S87

Emergence of Influenza A (H1N1) Viruses from Birds and Swine into Humans

Influenza Global Impacts

- Human influenza: 500,000 deaths /y globally in a “good” year.
- Three pandemics in 20th century
  - Deaths ranged from ~2 million (1968) to 50 -100 million (1918)
  - Another pandemic expected eventually
- Avian flu (H5N1)
  - 243 deaths / 385 cases, 16 countries, 2003-08 (19 June)

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<table>
<thead>
<tr>
<th>Years</th>
<th>Circulating Virus (Genetic Mechanism)</th>
<th>Excess Deaths from Any Cause No. per 100,000 Persons/yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1918–19</td>
<td>H1N1 (viral introduction), pandemic</td>
<td>598.0</td>
</tr>
<tr>
<td>1928–29</td>
<td>H1N1 (drift)</td>
<td>96.7</td>
</tr>
<tr>
<td>1934–36</td>
<td>H1N1 (drift)</td>
<td>52.0</td>
</tr>
<tr>
<td>1947–48</td>
<td>H1N1 A* (intrahostic reassortment)</td>
<td>8.9</td>
</tr>
<tr>
<td>1951–53</td>
<td>H1N1 (intrahostic reassortment)</td>
<td>34.1</td>
</tr>
<tr>
<td>1957–58</td>
<td>H2N2 (antigenic shift), pandemic</td>
<td>40.6</td>
</tr>
<tr>
<td>1968–69</td>
<td>H3N2 (antigenic shift), pandemic</td>
<td>16.9</td>
</tr>
<tr>
<td>1972–73</td>
<td>H3N2 A Port Chalmers (drift)</td>
<td>11.8</td>
</tr>
<tr>
<td>1975–76</td>
<td>H3N2 (drift) and H1N1 (“swine flu” outbreak)</td>
<td>12.4</td>
</tr>
<tr>
<td>1977–78</td>
<td>H3N2 (drift) and H1N1 (viral return)</td>
<td>21.0</td>
</tr>
<tr>
<td>1997–99</td>
<td>H3N2 A Sydney (intrahostic reassortment) and H1N1 (drift)</td>
<td>49.5</td>
</tr>
<tr>
<td>2003–04</td>
<td>H3N2 A Fujian (intrahostic reassortment) and H1N1 (drift)</td>
<td>17.1</td>
</tr>
<tr>
<td>2009</td>
<td>H3N2 and H1N1 (drift) and swine-origin H1N1 (viral introduction), pandemic</td>
<td>?</td>
</tr>
</tbody>
</table>

* Mortality data include deaths associated with all influenza A and B viruses combined. Many of these data have been calculated with the use of differing methods and may not be strictly comparable.\(^*\) The 1934, 1951, and 1997 data span 2 years.

Sources of Evidence Regarding Role of Aerosols in Transmission of Influenza A Virus

- Outbreak reports
- Experimental infection of humans
- Animal experimentation
- Zanamivir trials
- Livermore Veterans Study
- New data on exhaled breath
An Airplane Outbreak Report

– Airplane passengers: plane grounded 4 hr, 1 source case, 72% passengers became ill within 72 hours. Lower attack rate among persons who got off during delay. (Moser MR et al. 1979)

Experimental Infection of Human “Volunteers”

• Permit clear separation of aerosol route from transmission by large droplets
  – Homogeneous small particle aerosols without large droplets
  – Large droplets transmission by intranasal drops (no accompanying aerosols)
Experimental Design

- Human “volunteers”
  - Antibody levels measured
- Aim at establishing Human infectious Dose 50% (HID$_{50}$); very low doses of virus
- Aerosolized virus from culture;
  - 1-3 $\mu$m, RH 45-55%
- 10 liters of aerosol administered by mask

Alford et al
Results

• Neutralizing antibodies are protective
• 1 HID\textsubscript{50} = 0.6 - 3 TCID\textsubscript{50}

Comparison of Human Infectious Dose of Influenza A Virus by Aerosol or Intranasal Route

• Aerosol (airborne):
  HID\textsubscript{50} = 0.6 to 3 TCID\textsubscript{50}
• Intranasal (large droplet)
  HID\textsubscript{50} = 127 to 320 TCID\textsubscript{50}

Couch et al J Infect Dis 1971; 124: 473-80
Couch et al J infect Dis 1974; 129: 411-420
“When the infectious dose deposited by aerosol in the nose is smaller than the infectious dose by nasal drops, it is probable that the lower respiratory tract is the site of initiation of infection” (V. Knight)

Animal Models

- Ferrets: transmission of influenza A from sick to healthy ferrets through straight, S or U-shaped ducts (Andrewes and Glover 1941)
- Mice: infected via influenza aerosol generator (Edward D et al., 1943)
- Mice: inverse correlation between air exchange and infection rate regardless of mice proximity, infectious particles found in air (Schulman JL, 1967; Schulman JL, 1968)
- Guinea pig: transmission over distance of 91 cm only in direction of airflow and affected by RH and temperature (Lowen et al, 2006, 2007)
Animal Model Take Home

- At low RH (and lower temperatures)
  - Transmitted effectively by fine particle aerosols
- At high RH (and higher temperatures)
  - Transmitted only by direct contact

Hemmes et al 1962; Antonie Van Leeuwenhoek 1962; 28: 221-233

Median diameter:

5-6 μm

Fig. 6. Survival curves of poliomyelitis (CoL) and influenza (PR8) virus aerosolized in mixed suspensions at 37°C.
Short-Term Treatment with Zanamivir to Prevent Influenza: Results of a Placebo-Controlled Study

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Oliver Keene,* and Frederick G. Hayden†

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Table 2. Incidence of symptomatic influenza (S) or asymptomatic influenza (AS) after initiation of prophylaxis, by treatment group.

<table>
<thead>
<tr>
<th>Proven influenza</th>
<th>Zanamivir</th>
<th>Placebo</th>
<th>Total no. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intestinal (n = 144)</td>
<td>Inhaled (n = 144)</td>
<td></td>
</tr>
<tr>
<td>S or AS during 21 d after initiation</td>
<td>28 (20)</td>
<td>16 (13)</td>
<td>44</td>
</tr>
<tr>
<td>S during 10 d after initiation</td>
<td>9 (6)</td>
<td>4 (3)</td>
<td>13</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>0.81 (0.30-2.22)</td>
<td>0.21 (0.05-1.04)</td>
<td></td>
</tr>
<tr>
<td>S during 5 d of prophylaxis</td>
<td>4 (3)</td>
<td>2 (1)</td>
<td>6</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>0.30 (0.02-2.77)</td>
<td>0.52 (0.07-4.55)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Data are no. % of subjects, except as indicated. ORs and 95% CIs stratified by center were calculated by use of Mantel-Haenszel estimates with test-based CIs.
Exhalair Breath Sampler

- Tidal Breathing into CPAP Mask
- Records:
  - Airflow
  - Particle counts 0.3- >5μm (optical)
- Collects filter sample

Manufacturer:
Pulmatrix, Inc, Lexington, MA & PMS, Inc, Boulder, CO

Influenza Virus in Human Exhaled Breath: An Observational Study
Hong Kong 2007

- Recruited subjects from 3 clinics in HK
  - Rapid test positive for influenza virus A or B
  - Within 3 days of disease onset
- Answered health questionnaire
- Collected a 3 minute exhaled breath sample for particle counts
- Collected a 15 minute exhaled breath sample for influenza virus

Hong Kong 2007
Total Virus Detection by qPCR

• Virus detection in exhaled breath:
  – Four (33%) of the 12 studied patients.
    • Three of five (60%) subjects with influenza A
    • One of seven (14%) subjects with influenza B
  – No age, sex, vaccination, duration, or symptom differences between + and – cases.

• Copy Number
  – <48 /filter (3 cases) and 300 (1 case) per filter
  – <3.2 and 20 copies per minute.
  – LOQ = 6 /PCR well = 48 /filter = 3.2 /min


Hong Kong: Total Exhaled Particles

• Total particles = 67 to 8,500 / liter of air.
• > 87% of particles < 1 µm
• < 0.1% of particles > 5 µm

• Suggests that virus (detected by qPCR) was present in fine particles generated during tidal breathing.

• All large droplets excluded by collection device. 97% of particles <0.5 µm

<table>
<thead>
<tr>
<th></th>
<th>All influenza positive subjects</th>
<th>Exhaled breath positive</th>
<th>Cough positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>28</td>
<td>4 (14%)</td>
<td>18 (64%)</td>
</tr>
<tr>
<td>Male</td>
<td>54%</td>
<td>75%</td>
<td>61%</td>
</tr>
<tr>
<td>Average age (years)</td>
<td>22.6 (7.2)</td>
<td>29 (18)</td>
<td>22 (8.5)</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>18-56</td>
<td>19-56</td>
<td>18-56</td>
</tr>
<tr>
<td>Body temperature (°F)</td>
<td>100 (1.2)</td>
<td>100 (0.8)</td>
<td>100 (1.2)</td>
</tr>
</tbody>
</table>

Original Gesundheit Machine
1960s Ft. Detrick, MD

Gesundheit Machine II

- Testing ear-loop surgical mask with coughing
- 41 Cases tested
- Lab analysis ongoing
  - Preliminary results: about 50% reduction in virus release with mask use.

UML 2009
The Big Gap: Evidence Regarding Primary Mode of Infection Transmission

- Demonstration of infectious virus in fine particles does not prove that they transmit infection.

- “What is essential and necessary evidence is the demonstration that the elimination of one or more of the means of spread, keeping the remaining ones constant, radically and consistently reduces the incidence of actual disease.” AD Langmuir Bacteriological Reviews 1966;30(3):672-4

The 1957-58 Livermore Veterans Pandemic Influenza Study

- Livermore, CA Veterans Hospital July 1957 – March 1958
  - Upper Room UVC
    - Used in Building 62 but not Building 2
    - Long-term TB patients
    - Patients restricted to assigned building
    - Serology July, November, March

The 1957-58 Livermore Study Results

- Health Care Workers
  - 18% attack rate
  - Equivalent exposure to patients in both buildings
- **UV was 90% effective in patients**
  - (95% CI: 73% to 96%)

<table>
<thead>
<tr>
<th>UV</th>
<th>At Risk</th>
<th>+Flu</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>209</td>
<td>4</td>
<td></td>
<td>1.9%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No UV</th>
<th>At Risk</th>
<th>+Flu</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>395</td>
<td>75</td>
<td></td>
<td>18.9%</td>
</tr>
</tbody>
</table>


The 1957-58 Livermore Study Weaknesses

- **Publication**
  - Discussion in proceedings of a conference on the 1957 pandemic
  - Mention in Riley’s 1961 book
  - Never published in a peer reviewed journal article.
- **Repetition**
  - Not attempted
Summary

• Influenza probably preferentially aerosol transmitted:
  – Lower infectious dose via aerosol
  – More severe symptoms after aerosol dose
  – Protection by inhaled but not nasal prophylaxis
  – Evidence for protection by upper-room UVC
  – Evidence for presence in exhaled fine particles
• Still controversial

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