Paediatric Virology: Gastroenteritis Respiratory Disease

Michael Nissen
Departments of Infectious Diseases & Microbiology
Royal Brisbane & Children’s Hospitals
Herston, Queensland.
Childhood Gastroenteritis

- Greatest cause of death in the developing world.
- Up to 10 million children die p.a.
  - mainly due to rotavirus infections in malnourished infants.
- An increasing range of viruses are identified as true GE pathogens
- A diagnostic revolution targeting viral GE pathogens is essential
  - In USA aetiology of GE known in <10% of cases
- A safe, cheap and effective rotavirus vaccine is urgently required

- Rotavirus
- Norovirus
- Astrovirus
- Adenoviruses
- Others
  - Sapovirus
- Co-infections not unusual
<table>
<thead>
<tr>
<th>Causative agent</th>
<th>Patient age groupings</th>
<th>Selected symptoms</th>
<th>Incubation period</th>
<th>Duration of illness</th>
<th>Mode of transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus, group A</td>
<td>Infants and toddlers</td>
<td>Vomiting: Common</td>
<td>1–3 days</td>
<td>5–7 days</td>
<td>Water, PTP, ?food, ?air, nosocomial, fecal–oral</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fever: Common</td>
<td></td>
<td></td>
<td>Water, PTP, fecal–oral</td>
</tr>
<tr>
<td>Rotavirus, group B</td>
<td>Children and adults</td>
<td>Vomiting: Variable</td>
<td>56 hours</td>
<td>3–7 days</td>
<td>Fecal–oral</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fever: Rare</td>
<td>(average)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotavirus, group C</td>
<td>Infants, children, and adults</td>
<td>Vomiting: Unknown</td>
<td>24–48 hours</td>
<td>3–7 days</td>
<td>Fecal–oral</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fever: Unknown</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenovirus (enteric)</td>
<td>Young children</td>
<td>Vomiting: Common</td>
<td>7–8 days</td>
<td>8–12 days</td>
<td>Nosocomial, fecal–oral</td>
</tr>
<tr>
<td>Calicivirus</td>
<td>Infants, young children, and</td>
<td>Fever: Common</td>
<td>1–3 days</td>
<td>1–3 days</td>
<td>Food, water, nosocomial, fecal–oral</td>
</tr>
<tr>
<td></td>
<td>adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calicivirus (Norwalk</td>
<td>Older children and adults</td>
<td>Vomiting: Common</td>
<td>18–48 hours</td>
<td>12–48 hours</td>
<td>Food, water, PTP, ?air, fecal–oral</td>
</tr>
<tr>
<td>virus)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrovirus</td>
<td>Young children and elderly</td>
<td>Vomiting: Occasional</td>
<td>1–4 days</td>
<td>2–3 days; occasionally 1–4 days</td>
<td>Food, water, fecal–oral</td>
</tr>
<tr>
<td></td>
<td>people</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


* Diarrhea is common and is usually loose, watery, and nonbloody when associated with gastroenteritis.

* PTP, Person-to-person; ?, not confirmed.
Rotavirus

- **Properties** *(Reoviridae)*
  - dsDNA, 18 Kb, 11 segments
  - 6 serogroups A-F (VP6)

- **Epidemiology**
  - Ages 6-24 mths (12 mths)
  - Asymptomatic infections
  - Faecal oral & water spread
  - Seasonality
  - Nosocomial outbreaks

- **Pathogenesis**
  - Incubation 1-3 days
  - Shed for 3-7 days
  - $10^{10}$ particles/g/faeces

- **Clinical Features**
  - VD 4-5 days, fever
  - Death rare in well child

- **Diagnosis**
  - EIA, Latex agglutination
  - IEM
  - PCR
## Current Status of Rotavirus Vaccines

<table>
<thead>
<tr>
<th>Licensed vaccines</th>
<th>Company</th>
<th>Concept</th>
<th>Status of vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLR</td>
<td>Lanzhou Institute of Biological Products, China</td>
<td>Monovalent lamb strain (P[12].G10)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Late-stage development</th>
<th>Company</th>
<th>Concept</th>
<th>Status of vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotaleq</td>
<td>Merck, USA</td>
<td>WC-3-based pentavalent bovine-human reassortants</td>
<td>Phase III</td>
</tr>
<tr>
<td>Rotarix</td>
<td>GlaxoSmithKline, Belgium</td>
<td>Monovalent human strain (P1A[8].G1)</td>
<td>Phase III</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Early-stage development</th>
<th>Company</th>
<th>Concept</th>
<th>Status of vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV3</td>
<td>University of Melbourne, Australia</td>
<td>Neonatal strain (P2A[6].G3)</td>
<td>Phase II</td>
</tr>
<tr>
<td>UK-reassortant strain</td>
<td>US National Institutes of Health (pending)</td>
<td>Tetravalent bovine-human reassortants</td>
<td>Phase II</td>
</tr>
<tr>
<td>Rhesus tetravalent</td>
<td>BIOVIRx, USA</td>
<td>Tetravalent rhesus-human reassortants</td>
<td></td>
</tr>
</tbody>
</table>

**Vaccines currently licensed or in clinical trials**

- Rotashield
- Rotaleq
- Rotarix
Norovirus

- **Properties** (*Caliciviridae*)
  - +ssRNA 7.5 kb, 3 ORF
  - 3 major antigenic groups

- **Epidemiology**
  - Ubiquitous & year round with winter peak
  - 19-42% of non-bacterial GE outbreaks
  - 5-17% community incidence
  - 5-7% requiring medical Rx

- **Pathogenesis**
  - SI villi tips infected
  - Immunity short-lived

- **Clinical Features**
  - Incubation 18-36 hrs
  - NVD, abdo cramps, headache, myalgia, fever, “winter vomiting”

- **Diagnosis**
  - Non-culturable
  - EIA, RT-PCR, IEM
Astrovirus

- Properties (*Astroviridae*)
  - +ssRNA, 7.2 kb, 8 serotypes

- Epidemiology
  - 2-17% infantile GE
  - 2% asymptomatic carriage
  - Year-round with winter peak
  - Community epidemics
  - Co-infections; rotavirus (3-19%), adenoviruses (2-4%)

- Pathogenesis
  - Cytoplasmic replication
  - [High particle] faeces $\sim 10^8$/g

- Clinical Features
  - Mild GE, incubation 1-4 days,
  - Long term immunity

- Diagnosis
  - EIA, PCR
  - Culture (trypsin)-HEK, CaCo2
Properties (Adenoviridae)
- Genus: Mastadenovirus
- dsDNA, 38 kb, 12 s. proteins

Epidemiology
- GE (serotypes 31, 40, 41)
- Infantile diarrhoea (~10%)

Pathogenesis
- Incubation 3-10 days
- Prolonged excretion (wk-mth)

Clinical Features
- Variable
- Diarrhoea 6-9 days

Diagnosis
- EIA, IEM
- Cultivable (Graham-293)
Sapovirus

Properties

- Family: *Calciviridae*, +ssRNA
- 3 major genomic & antigenic diverse groups (genogroups)
  - I, II, III (GI, GII, GIII)
- 3 ORF but genomic organisation differs from Norovirus
  - Polyprotein and capsid genes are fused into a single ORF (ORF1), open reading frame (3’-ORF), ORF overlapping capsid gene (capsid overlap, only in the GI strains) and 3’ untranslated region (3’-UTR)

Epidemiology

- Causative agent of GE in children & adults worldwide
- Infection occurs less frequently than Norovirus
- Appears in sporadic cases but also in outbreaks
- No seasonality reported
- Transmission: person-to-person, foodborne

Diagnosis

- IEM, RT-PCR (Non-culturable)
**Diagnostic Revolution?**

- PCR for viral GIT pathogens
  - Han, Y et al. (2003). Detection of norovirus (GI, GII), Sapovirus and astrovirus in fecal samples using reverse transcription single-round multiplex PCR. *J. Virol. Methods* 114:37–44
Childhood Respiratory Disease

- Respiratory infections are the most common afflictions of humans, and most are caused by viruses
- Estimated 5 million deaths from respiratory infections in children p.a. worldwide, at least 1 million are viral in origin
- Children, on average, contract 6-9 respiratory illnesses p.a.
  - significant proportion of all health care visits, and
  - unnecessary antibiotic use within the community.
- Serious viral lower respiratory tract infections due to RSV and influenza occur in the very young and elderly
  - increasing recognition of their role in immunocompromised individuals
- Respiratory viral disease has been revolutionised by;
  - molecular diagnostics, and
  - discovery and emergence of several “new” pathogens such as avian influenza, metapneumovirus, and the coronaviruses (HCoV-SARS, HCoV-NL63).
Childhood Respiratory Disease

- hRSV
- Influenza A & B
- Parainfluenza 1,2,3,4
- Adenoviruses
- Rhinoviruses
- Coronaviruses
- Enteroviruses
  - Coxsackie
  - Echo
- HSV/VZV
- EBV/CMV
- Measles
- Emerging Viruses
  - hMPV
  - HCoV-SARS
  - H5N1 influenza
  - HCoV-NL63

14/10/2005       Viruses in July       12
PAEDIATRIC VIRAL RESPIRATORY DISEASE

- hRSV: 63%
- FluA: 3%
- FluB: 2%
- AdV: 7%
- PIV: 23%
- Others: 2%


14/10/2005 Viruses in July
VIRAL INFECTIONS RCH 2001

Total Specimens

Month

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

VIRAL INFECTIONS RCH 2001

14/10/2005 Viruses in July

16
Paramyxoviridae-Classification

Paramyxovirinae
- Pneumovirinae
  - Pneumo
    - hRSV
    - bRSV
  - Henipah
    - HeV
    - NiV
  - Respiro
    - CDV
    - PDV
    - RPV
    - MV
    - hPIV1
    - bPIV3
    - SeV
    - hPIV3

Morbilli
- Rubula
  - NDV
  - LPMV
  - MuV
  - hPIV2
  - SV41
  - SV5

Metapneumo

Viruses in July
Paramyxoviridae
General Morphology

- Fusion protein (F)
- SH protein
- Attachment protein (G)
- Matrix protein (M)
- Polymerase (L)
- Nucleoprotein (N)
- Phosphoprotein (P)
- Lipid bilayer
Epidemiology
- Principal cause of LRTI in infants worldwide
- Annual seasonal variation
- 2 major antigenic lineages: types A & B

Pathogenesis
- RT incubation 4-5 days

Clinical Features
- Acute bronchiolitis
- Infections continue to occur throughout life
- ↑ recognition in elderly & immunocompromised
hRSV Australia 2002
Distribution of hRSV A and B in Australia

% positive

NSW | NT | QLD | SA | TAS | TOTAL

RSV A | RSV B

14/10/2005 Viruses in July
hRSV

- **Diagnosis**
  - DFA
  - Culture
  - RT-PCR

- **Treatment**
  - Supportive
  - ? Ribavirin

- **Prevention**
  - IVIG
  - Monoclonal IG (Palivizumab)
  - ? vaccine

14/10/2005  Viruses in July
Gel Electrophoresis

Subjective reading of band size

Non-specific bands

Primer-dimer
Respiratory Multiplex

PROBES  

↓  

RSV  

PIV 1  

PIV 2  

PIV 3  

Flu A  

Flu B  

ADV  

SAMPLES/CONTROLS  

1 2 3 4 5 6 7  

1: RSV + Control  
2: PIV 1 + Control  
3: PIV 2 + Control  
4: PIV 3 + Control  
5: Flu A + Control  
6: Flu B + Control  
7: ADV + Control
## Respiratory Multiplex PCR

<table>
<thead>
<tr>
<th></th>
<th>DFA/CADFA Positive</th>
<th>DFA/CADFA Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiplex Positive</td>
<td>179</td>
<td>23</td>
</tr>
<tr>
<td>Multiplex Negative</td>
<td>0</td>
<td>396</td>
</tr>
</tbody>
</table>

CA-DFA: Culture Amplified- Direct Fluorescent Antibody
- **Sensitivity** = 89%, **Specificity** = 100%
- **PPV** = 100%, **NPV** = 95%

14/10/2005 Viruses in July
## Respiratory Multiplex PCR

<table>
<thead>
<tr>
<th></th>
<th>DFA +</th>
<th>DFA -/CADFA +</th>
<th>DFA/CADFA -</th>
<th>m-RT-PCR-ELAHA +</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>(%)</td>
<td>No.</td>
<td>(%)</td>
</tr>
<tr>
<td><strong>n=598</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADV</td>
<td>11</td>
<td>(65)</td>
<td>3</td>
<td>(18)</td>
</tr>
<tr>
<td>Flu A</td>
<td>5</td>
<td>(36)</td>
<td>4</td>
<td>(29)</td>
</tr>
<tr>
<td>Flu B</td>
<td>2</td>
<td>(100)</td>
<td>0</td>
<td>(0)</td>
</tr>
<tr>
<td>PIV 1</td>
<td>1</td>
<td>(17)</td>
<td>4</td>
<td>(67)</td>
</tr>
<tr>
<td>PIV 2</td>
<td>5</td>
<td>(71)</td>
<td>1</td>
<td>(14)</td>
</tr>
<tr>
<td>PIV 3</td>
<td>29</td>
<td>(88)</td>
<td>0</td>
<td>(0)</td>
</tr>
<tr>
<td>RSV</td>
<td>108</td>
<td>(88)</td>
<td>6</td>
<td>(5)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>161</td>
<td>(80)</td>
<td>18</td>
<td>(9)</td>
</tr>
</tbody>
</table>

CA-DFA: Culture Amplified- Direct Fluorescent Antibody
Advantages of multiplex PCR

- Increased sensitivity over DFA and CA-DFA.
- High specificity equaling DFA and CA-DFA.
- Same day result turnaround time (~5 hrs)
- Reduced cost of diagnosis (PCR $18 v/s CA-DFA $35)
- Improved patient management & limit unnecessary antibiotic use
- Cost-effective
  - Reduced hands on time
  - Reduced use of consumables
  - Can detect up to seven virus types in one reaction
Influenza

- Properties (Orthomyxoviridae)
  - 3 genera A, B, C
  - -ssRNA, 13.6 kb, 8 segments

- Epidemiology
  - Antigenic “drift” & “shift”
  - Seasonality
  - B less pathogenic

- Pathogenesis
  - Transcription/replication in cell nucleus
  - Incubation 1-4 days
  - Large no. of virions shed
Influenza Epidemics

- Spanish flu (H1N1) 1918-1920
- Asian flu (H2N2) 1956-1958
- Hong Kong flu (H3N2) 1968-1970
- Russian flu (H1N1) 1977-1979
- Avian flu (H5N1) 1997-2003
Influenza

Clinical Features

- Abrupt onset of fever, sore throat, cough, myalgia, headache, malaise.
- Duration 3-7 days
- Complications vary with age: croup, pneumonia, OM
- 20 bacterial pathogens: *S. aureus*, *S. pneumoniae*, *H. influenzae*.
Control
- Surveillance
- Vaccination

Influenza trivalent vaccine (2004)
- A/New Caledonia/20/99 H1N1
- A/Fujian/411/2002 H3N2
- B/Hong Kong/330/2001

Is it time to immunise kids?
Influenza

**Treatment**

- Neuramidase inhibitors
- Commence within 24-48 hrs of symptoms
- Oseltamivir (Tamiflu™)
  - Capsules/powder
  - ≥ 1 yoa
- Zanamivir (Relenza™)
  - Rotadisc powder
  - ≥ 5 yoa
- ? Prophylactic use
Parainfluenza Viruses

- **Properties** (*Paramyxoviridae*)
  - ssRNA, 15 kb, 6-7 genes encoding 10-12 proteins
  - Types 1 & 3 - *Respirovirus*
  - Types 2, 4a & 4b - *Rubulavirus*

- **Epidemiology**
  - Types 1 & 2 - “croup”
  - Type 3 infects majority by 2 yo

- **Pathogenesis**
  - Incubation 2-6 days
  - Shedding ~7 days

- **Clinical Features**
  - <6 mth - acute bronchiolitis
  - 6mth-5 yo: croup

- **Diagnosis**
  - DFA, Culture, RT-PCR

- **Treatment**
  - Symptomatic, PNSL
### Diseases Caused by Human Adenoviruses

<table>
<thead>
<tr>
<th>Disease</th>
<th>Age</th>
<th>Common serotypes&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Major subgenus</th>
<th>Major source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory infections</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pharyngitis</td>
<td>Young children</td>
<td>1, 2, 3, 5, 6, 7</td>
<td>B, C</td>
<td>Throat</td>
</tr>
<tr>
<td>Acute respiratory disease</td>
<td>Military recruits</td>
<td>3, 4, 7, 14, 21</td>
<td>B, E</td>
<td>Throat</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>Young children</td>
<td>1, 2, 3, 4, 5, 7, 21</td>
<td>B, C</td>
<td>Throat</td>
</tr>
<tr>
<td></td>
<td>Military recruits</td>
<td>4, 7</td>
<td>B, E</td>
<td>Throat</td>
</tr>
<tr>
<td>Ocular infections</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pharyngoconjunctival fever</td>
<td>Children</td>
<td>1, 2, 3, 4, 6, 7</td>
<td>B, C, E</td>
<td>Throat, eye</td>
</tr>
<tr>
<td>Epidemic keratoconjunctivitis</td>
<td>Any age</td>
<td>8, 19, 37</td>
<td>D</td>
<td>Eye</td>
</tr>
<tr>
<td>Genitourinary infections</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervicitis, urethritis</td>
<td>Adults</td>
<td>19, 37</td>
<td>D</td>
<td>Genital secretions</td>
</tr>
<tr>
<td>Hemorrhagic cystitis</td>
<td>Young children</td>
<td>11, 21</td>
<td>B</td>
<td>Urine</td>
</tr>
<tr>
<td>Enteric infections</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>Young children</td>
<td>31, 40, 41</td>
<td>A, F</td>
<td>Feces</td>
</tr>
<tr>
<td>Infections in immunocompromised individuals</td>
<td>Any age, including AIDS patients</td>
<td>7, 11, 34, 35</td>
<td>B</td>
<td>Urine, lung</td>
</tr>
<tr>
<td>Encephalitis, pneumonia,</td>
<td>AIDS patients</td>
<td>Many D including 43–47</td>
<td>D</td>
<td>Feces</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>AIDS patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generalized</td>
<td>AIDS patients</td>
<td>2, 5</td>
<td>C</td>
<td>Blood</td>
</tr>
</tbody>
</table>

<sup>a</sup> Only the commonly occurring serotypes are listed; those most commonly associated with particular syndromes are in bold type.

Adenoviruses

- **Clinical syndromes**
  - Cold-like syndrome
  - Pharyngo-conjunctival fever
  - Pertussis-like illness
  - Acute respiratory distress
  - Rapidly fatal haemorrhagic pneumonia in immunocompromised

- **Diagnosis**
  - DFA, Culture, PCR, Serology

- **Treatment**
  - ? Ribavirin

- **Control**
  - Vaccination is available to certain populations for ARD
  - live & virulent virus
  - serotypes 4 & 7
Properties (Picornaviridae)
- +ssRNA, 7-8 kb
- Virion acts as mRNA, transcribed as a polyprotein and cleaved progressively to yield S & NS proteins
- Acid-labile (ph<5)
- Rhinoviruses 1-100

Epidemiology
- Year-round infection
- Peaks in autumn & spring
- 3-4 serotypes circulate simultaneously

Pathogenesis
- Predilection to replicate @ 33°C
- Acquired immunity type specific and correlates with locally synthesized IgA antibodies

Clinical Features
- “common cold”
- ↑ recognition in LRTI & wheezing/asthma

Diagnosis
- EIA, PCR, culture

Rhinovirus 14
Coronaviruses

- Properties *(Coronaviridae)*
  - +ssRNA, 30 kb
  - 3-4 struct. proteins (N, S, M, HE)
  - 4 serotypes:
    - HCoV-229E, OC43, SARS, NL63

- Epidemiology
  - Incubation of 2-5 days
  - Viral shedding ~ 1 week
  - Peaks in winter & early spring
  - Outbreaks ~ every 2-4 years

- Pathogenesis
  - Unique replication strategy

- Clinical Features
  - “common cold” (~15%)
  - Nosocomial infections

- Diagnosis
  - EIA
  - PCR
  - Difficult to grow (organ cultures)
Measles pneumonia with lymphocytic infiltration and large multinucleated cells

High power of measles pneumonia. Note the large multinucleated giant cells, named Warthin-Finkeldey cells.
Human metapneumovirus (hMPV) is a novel respiratory tract pathogen

First known mammalian MPV

Avian metapneumovirus (APV) is the only other known MPV

hMPV is now considered ubiquitous

- PCR detection studies
- Seroprevalence studies

Features are thought to be similar to human respiratory syncytial virus (hRSV)

Nucleocapsid
  - single stranded negative sense RNA

Viral particles as seen by EM
  - spherical, pleomorphic & filamentous
  - 150 – 600 nm diameter
  - short envelope projections 13-17 nm

Nucleocapsid
  - average diameter 17 nm
  - length range <200 - >1000 nm
  - filamentous particles average 282 x 62 nm
hMPV Seasonality

Nature Medicine 2001 7:719
### Qld hMPV Testing

<table>
<thead>
<tr>
<th>Year</th>
<th>Tested</th>
<th>hMPV PCR positive</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>915</td>
<td>58</td>
<td>6.3</td>
</tr>
<tr>
<td>2002</td>
<td>2121</td>
<td>82</td>
<td>3.9</td>
</tr>
<tr>
<td>2003</td>
<td>1972</td>
<td>73</td>
<td>3.7</td>
</tr>
<tr>
<td>TOTALS</td>
<td>5008</td>
<td>213</td>
<td>4.2</td>
</tr>
</tbody>
</table>

*Mackay et al. J. Infect. Diseases (in press)*
### Qld hMPV Seasonal Incidence

<table>
<thead>
<tr>
<th>Year</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
<th>Spring</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>2.9</td>
<td>34.3</td>
<td>17.1</td>
<td><strong>45.7</strong></td>
</tr>
<tr>
<td>2002</td>
<td>9.8</td>
<td>1.2</td>
<td>15.9</td>
<td><strong>73.2</strong></td>
</tr>
<tr>
<td>2003</td>
<td>1.4</td>
<td>5.5</td>
<td><strong>80.8</strong></td>
<td>12.3</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>5.7</strong></td>
<td><strong>9.7</strong></td>
<td><strong>30.3</strong></td>
<td><strong>54.3</strong></td>
</tr>
</tbody>
</table>

*Mackay et al. J. Infect. Diseases (in press)*
hMPV Heterogeneity

- 2 hMPV lineages
- 4 hMPV sub-lineages
- F protein (95% conserved) between lineages
- G protein (30% conserved)

Overall 80-81% whole genome nt identity between lineages
- 92-93% identity between strains belonging to same lineage

van den Hoogen BG, PhD thesis, 2004
2 virus types
4 virus subtypes
exist in Australia

Phosphoprotein (P) gene

Mackay et al. J. Infect. Diseases (in press)
### Coinfections with hMPV

<table>
<thead>
<tr>
<th>2001 (n=58)</th>
<th>Number</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A</td>
<td>5</td>
<td>8.6</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>4</td>
<td>6.9</td>
</tr>
<tr>
<td>PIV 3</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>RSV</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Total virus</strong></td>
<td><strong>11</strong></td>
<td><strong>19</strong></td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td><em>B. pertussis</em></td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Total bacteria</strong></td>
<td><strong>2</strong></td>
<td><strong>3.4</strong></td>
</tr>
<tr>
<td><strong>Total pathogens</strong></td>
<td><strong>13</strong></td>
<td><strong>22.4</strong></td>
</tr>
</tbody>
</table>
HCoV-SARS

- Newly recognised and highly contagious respiratory infection by a novel coronavirus
  - Progressive respiratory failure in adults
  - Mortality rate 8–15%
  - Droplet & faecal transmission
  - Role of hMPV?
- Children acquire SARS through close household contact exposure with adults...less infective?
- Disease severity is milder in children
  - No case fatalities recorded
  - Air space consolidation is commonly seen though chest radiographs are normal in 50% of the cases
  - Neonates of infected mothers not affected....reasons?
Recent outbreaks of avian influenza A (H5N1) in poultry throughout Asia have had major economic and health repercussions.

10 Human infections identified in Vietnam in January 2004
- Mean age, 13.7 years (range
- None had preexisting medical conditions

Clear history of direct contact with poultry
- Median time before onset of illness = 3 days

All presented with;
- Fever (T 38.5-40.0°C)
- Respiratory symptoms,
- Marked abnormalities on CXR, and
- Clinically significant lymphopenia (median count 700)

Nine (9) thrombocytopenic (median count 75,500)
Seven (7) patients had diarrhoea

No definitive evidence of human-to-human transmission
Eight (8) patients died
Identification and characterization of a novel coronavirus
- Fouchier RM et al. (2004) PNAS 101(16):6212

Isolation from 2 children with LRTI (7 & 8 mths) then further 11 cases
- Distinctive full genome sequence revealed a new Group 1 HCoV (43-67% id)
  - Unique N-terminal fragment within spike protein
  - Closest relatives: HCoV-229E & porcine epidemic diarrhea virus

Replication in-vitro: tertiary MK & LLC-MK2

Table 3. Patients suffering from RTI associated with HCoV-NL63 infection

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Sample date</th>
<th>Symptoms</th>
<th>Underlying disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mo</td>
<td>Male</td>
<td>January 12, 1988</td>
<td>Pneumonia</td>
<td>Unknown</td>
</tr>
<tr>
<td>3 mo</td>
<td>Female</td>
<td>November 1, 2000</td>
<td>Fever (39.4°C)</td>
<td>Giant cell hepatitis</td>
</tr>
<tr>
<td>4 mo</td>
<td>Female</td>
<td>December 19, 2000</td>
<td>Subfebrile (37.6°C)</td>
<td>Trisomy-21</td>
</tr>
<tr>
<td>4 mo</td>
<td>Female</td>
<td>January 18, 2001</td>
<td>Runny nose</td>
<td>AVSD</td>
</tr>
<tr>
<td>10 yr</td>
<td>Female</td>
<td>January 18, 2001</td>
<td>Subfebrile (37.8°C)</td>
<td>Pertussis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sever cough</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fever (38.6°C)</td>
<td>Rett syndrome</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Runny nose</td>
<td>Epilepsia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dry cough</td>
<td></td>
</tr>
</tbody>
</table>

AVSD, atrioventricular septum defect.

Fouchier RM et al. (2004) PNAS 101(16):6212
Acknowledgements to CVRU & MMRU, SASVRC, RCH
Unofficially sponsored by Lowes
Thank you Questions?
Table 1. Hospitalisation rates due to acute respiratory disease in children without high-risk conditions

<table>
<thead>
<tr>
<th>Period and age group</th>
<th>Rate/100 000 person-months (95% CI)</th>
<th>Northern California Kaiser, 1993-1997</th>
<th>Group Health Cooperative, 1992-1997</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period when influenza virus predominated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1 year</td>
<td>231 (197-271)</td>
<td>193 (154-238)</td>
<td></td>
</tr>
<tr>
<td>2-4 years</td>
<td>53 (38-72)</td>
<td>21 (11-38)</td>
<td></td>
</tr>
<tr>
<td>5-17 years</td>
<td>19 (15-24)</td>
<td>16 (12-22)</td>
<td></td>
</tr>
<tr>
<td>Period when respiratory syncytial virus predominated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1 year</td>
<td>309 (278-343)</td>
<td>372 (321-426)</td>
<td></td>
</tr>
<tr>
<td>2-4 years</td>
<td>51 (40-64)</td>
<td>44 (29-65)</td>
<td></td>
</tr>
<tr>
<td>5-17 years</td>
<td>23 (19-27)</td>
<td>10 (7-13)</td>
<td></td>
</tr>
<tr>
<td>Preseasonal baseline period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1 year</td>
<td>120 (108-133)</td>
<td>107 (85-133)</td>
<td></td>
</tr>
<tr>
<td>2-4 years</td>
<td>38 (32-44)</td>
<td>24 (14-37)</td>
<td></td>
</tr>
<tr>
<td>5-17 years</td>
<td>14 (12-16)</td>
<td>10 (7-13)</td>
<td></td>
</tr>
<tr>
<td>Summer baseline period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1 year</td>
<td>81 (72-90)</td>
<td>66 (49-87)</td>
<td></td>
</tr>
<tr>
<td>2-4 years</td>
<td>27 (23-32)</td>
<td>16 (8-25)</td>
<td></td>
</tr>
<tr>
<td>5-17 years</td>
<td>19 (17-21)</td>
<td>12 (9-14)</td>
<td></td>
</tr>
</tbody>
</table>

Cl=confidence interval. Adapted from reference 10.
Table 2. Effect of illness episodes on school and family in 313 schoolchildren monitored during an influenza season

<table>
<thead>
<tr>
<th>Variable</th>
<th>Influenza-attributable events per 100 children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illness episodes</td>
<td>27.8</td>
</tr>
<tr>
<td>Missed school days</td>
<td>62.9</td>
</tr>
<tr>
<td>Febrile illnesses</td>
<td>26.1</td>
</tr>
<tr>
<td>Antibiotic courses</td>
<td>-0.64</td>
</tr>
<tr>
<td>Analgesics used</td>
<td>24.0</td>
</tr>
<tr>
<td>Health-care visits</td>
<td>4.2</td>
</tr>
<tr>
<td>Working days missed by parent</td>
<td>19.8</td>
</tr>
<tr>
<td>Household members ill in the 3 days after absence</td>
<td>21.7</td>
</tr>
</tbody>
</table>

Values calculated by subtracting expected from reported outcomes during an influenza season. An excess event rate per 100 children was generated by dividing by the number of children in the cohort (n=313) and multiplying by 100. Adapted from reference 14.
<table>
<thead>
<tr>
<th></th>
<th>Household contacts of children with influenza diagnosis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (n=915)</td>
<td>Negative (n=9128)</td>
</tr>
<tr>
<td>Hospitalisation (%)</td>
<td>3 (0.3)</td>
<td>11 (0.1)</td>
</tr>
<tr>
<td>Parents (%)</td>
<td>2/704 (0.3)</td>
<td>7/6638 (0.1)</td>
</tr>
<tr>
<td>Siblings (%)</td>
<td>1/211 (0.5)</td>
<td>4/2290 (0.2)</td>
</tr>
<tr>
<td>Additional medical visits, mean (SD)</td>
<td>0.39 (0.76)*</td>
<td>0.14 (0.47)</td>
</tr>
<tr>
<td>Parents (%)</td>
<td>0.28 (0.55)*</td>
<td>0.07 (0.25)</td>
</tr>
<tr>
<td>Siblings (%)</td>
<td>0.48 (0.98)*</td>
<td>0.22 (0.73)</td>
</tr>
<tr>
<td>Lost working days (parents), mean (SD)</td>
<td>1.39 (3.09)*</td>
<td>0.59 (2.02)</td>
</tr>
<tr>
<td>Lost school days (siblings), mean (SD)</td>
<td>1.27 (2.47)*</td>
<td>0.49 (2.33)</td>
</tr>
<tr>
<td>No of days help was needed to care for ill children, mean (SD)</td>
<td>1.10 (1.76)*</td>
<td>0.85 (1.63)</td>
</tr>
</tbody>
</table>

*p<0.0001 vs influenza-negative children; no other significant differences. Adapted from reference 12.
Table 4. Estimated rates of influenza-associated hospitalisation by age group and risk group from selected studies*

<table>
<thead>
<tr>
<th>Study years</th>
<th>Population</th>
<th>Age group</th>
<th>Hospitalisations/100,000 people with high-risk conditions</th>
<th>Hospitalisations/100,000 people without high-risk conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1973–1993</td>
<td>Tennessee</td>
<td>0–11 mo</td>
<td>1900</td>
<td>496–1038</td>
</tr>
<tr>
<td>1973–1993</td>
<td>Medicaid</td>
<td>1–2 yr</td>
<td>800</td>
<td>186</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3–4 yr</td>
<td>320</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5–14 yr</td>
<td>92</td>
<td>41</td>
</tr>
<tr>
<td>1992–1997</td>
<td>Two health</td>
<td>0–23 mo</td>
<td>144–187</td>
<td></td>
</tr>
<tr>
<td></td>
<td>maintenance</td>
<td>2–4 yr</td>
<td>0–25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>organisations</td>
<td>5–17 yr</td>
<td>8–12</td>
<td></td>
</tr>
</tbody>
</table>

*Rates estimated in years and populations with low vaccination rates. Hospitalisation rates can be expected to decrease as vaccination rates increase. Adapted from reference 1.
Table 5. Reactogenicity rates in studies of T-CAIV in healthy children aged 1–8 years

<table>
<thead>
<tr>
<th>Events*</th>
<th>After dose 1 T-CAIV (%)</th>
<th>Placebo (%)</th>
<th>After dose 2 T-CAIV (%)</th>
<th>Placebo (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>26-9</td>
<td>28-7</td>
<td>27-4</td>
<td>29-0</td>
</tr>
<tr>
<td>Runny nose/nasal discharge</td>
<td>57-6</td>
<td>48-0</td>
<td>42-9</td>
<td>42-2</td>
</tr>
<tr>
<td>Congestion</td>
<td>10-0</td>
<td>8-6</td>
<td>6-6</td>
<td>7-4</td>
</tr>
<tr>
<td>Sore throat</td>
<td>9-5</td>
<td>7-1</td>
<td>6-1</td>
<td>6-4</td>
</tr>
<tr>
<td>Headache</td>
<td>4-2</td>
<td>4-1</td>
<td>3-6</td>
<td>2-3</td>
</tr>
<tr>
<td>Chills</td>
<td>6-8</td>
<td>4-4</td>
<td>6-0</td>
<td>4-4</td>
</tr>
<tr>
<td>Vomiting</td>
<td>16-1</td>
<td>13-1</td>
<td>12-5</td>
<td>11-8</td>
</tr>
<tr>
<td>Fever:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1†</td>
<td>16-4</td>
<td>12-3</td>
<td>11-3</td>
<td>10-1</td>
</tr>
<tr>
<td>Grade 2‡</td>
<td>2-9</td>
<td>3-5</td>
<td>2-3</td>
<td>3-5</td>
</tr>
<tr>
<td>Grade 3§</td>
<td>0</td>
<td>0-1</td>
<td>0-3</td>
<td>0-5</td>
</tr>
</tbody>
</table>

*Days 0–10 after immunisation. †Oral temperature >37-8°C, rectal or aural temperature >38°C, or axillary temperature >37-6°C. ‡Oral temperature >38-4°C, rectal or aural temperature >39-2°C, or axillary temperature >38-7°C. §Oral temperature >40°C, rectal, or aural temperature >38-7°C, or axillary temperature >39-8°C. Adapted from reference 3.
### Table 6. Effectiveness of influenza vaccine among household contacts of influenza vaccinated healthy children and unvaccinated controls

<table>
<thead>
<tr>
<th>Event</th>
<th>Vaccine effectiveness</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory tract infections</td>
<td>30</td>
<td>0.0005</td>
</tr>
<tr>
<td>Medical visits for respiratory illness</td>
<td>32</td>
<td>0.002</td>
</tr>
<tr>
<td>Lost maternal working days</td>
<td>33</td>
<td>0.001</td>
</tr>
<tr>
<td>Lost paternal working days</td>
<td>43</td>
<td>0.001</td>
</tr>
<tr>
<td>Days at home to care for ill children</td>
<td>83</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Mean values (SD). *Vaccine effectiveness = 1 minus attack rate (defined as rate of illness divided by the total population) among household contacts of vaccinated children divided by attack rate among household contacts of controls. Adapted from reference 12.
Table 7. Pharmacoeconomic studies of influenza vaccination in children

<table>
<thead>
<tr>
<th>Country/author</th>
<th>Population</th>
<th>Conclusions regarding influenza vaccination</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>US/OTA</td>
<td>Children aged &lt;3 and 3–14 years</td>
<td>Cost effective</td>
<td>Net costs per year of healthy life gained were US$1122 and 853 ($2000) for children aged &lt;3 and 3–14 yr, respectively</td>
</tr>
<tr>
<td>US/Cohen and Nettlemann</td>
<td>Preschool children</td>
<td>Cost saving</td>
<td></td>
</tr>
<tr>
<td>US/White et al</td>
<td>School-aged children</td>
<td>Cost saving</td>
<td></td>
</tr>
<tr>
<td>US/Luce et al</td>
<td>Children aged 15–71 months</td>
<td>Probably cost effective</td>
<td>Break-even costs for vaccination were US$5–28</td>
</tr>
<tr>
<td>US/Meltzer et al</td>
<td>Children aged 0–19 years</td>
<td>Cost saving (for vaccination cost of US$21)</td>
<td>Model pandemic influenza</td>
</tr>
<tr>
<td>Hong Kong/Fitzner et al</td>
<td>Children aged 0–19 years</td>
<td>Not cost saving</td>
<td>Cost benefit ratio for vaccination of children was HK$3.81 in costs for every HK$1 saved</td>
</tr>
<tr>
<td>Argentina/Dayan et al</td>
<td>High-risk children aged 6 months–15 years</td>
<td>Cost saving</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from reference 80.