Emerging virus infections
“in a changing world”

Animals as reservoirs of human viruses

Albert Osterhaus
Virus threats worldwide
- 1996-2003, WHO outbreak reports -

- West Nile
- Yellow fever
- Dengue
- Avian influenza
- Lassa
- Meningitis

- Japanese encephalitis
- Ebola/Marburg
- Rift valley
- Polio
- Nipah
- SARS

- Venez. Eq. encephalitis
- Crimean-Congo HF
- VHF
- Monkeypox
- Hanta
- Enterovirus
Emerging virus infections in the last decennia
- Part 1 -

Facilitated by changes in:

<table>
<thead>
<tr>
<th>Social environment</th>
<th>Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Behaviour</td>
<td>- Medical</td>
</tr>
<tr>
<td>(taboos, mores, i.v. drug abuse)</td>
<td>(transfusion, transplantation, vaccination)</td>
</tr>
<tr>
<td>- Mobility</td>
<td>- Food production</td>
</tr>
<tr>
<td>(air travel, wars …)</td>
<td>(recycling…)</td>
</tr>
<tr>
<td>- Demography</td>
<td></td>
</tr>
<tr>
<td>(population density, urbanisation…)</td>
<td></td>
</tr>
<tr>
<td>- Public health measures</td>
<td></td>
</tr>
<tr>
<td>(breakdown…)</td>
<td></td>
</tr>
</tbody>
</table>
Emerging virus infections in the last decennia
- Part 2 -

Facilitated by changes in:

**Ecology:**
- Animal contacts (wildlife, zoo, pets…)
- Agriculture (deforestation…)
- Fisheries (predator migration…)
- Environmental pollution (immune suppression…)
- Global warming (flora and fauna…)

**Virus:**
- Mutation (host range, virulence…)
- Recombination/reassortment

**Bioterrorism?**
Lentiviruses
- Host range -
Influenza pandemics of the 20th century

<table>
<thead>
<tr>
<th>Virus</th>
<th>Subtype</th>
<th>Name</th>
<th>Est. # deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1918</td>
<td>H1N1</td>
<td>“Spanish Flu”</td>
<td>&gt; 40 million</td>
</tr>
<tr>
<td>1957</td>
<td>H2N2</td>
<td>“Asian Flu”</td>
<td>&gt; 1 million</td>
</tr>
<tr>
<td>1968</td>
<td>H3N2</td>
<td>“Hong Kong Flu”</td>
<td>&gt; 1 million</td>
</tr>
</tbody>
</table>
Bird markets
- Asia -
Influenza A virus
- Man as a “mixing vessel” -

Highly pathogenic avian influenza (HPAI) seriously suspected clinically: February 28th 2003
Reports of conjunctivitis
- By date of onset of symptoms -

Koopmans et al., Lancet 2004
X-ray fatal H7N7 infection day of admission
### H7N7 in The Netherlands

- Sequence analysis of full-length genomes -

**Fouchier et al., PNAS, 2004**

<table>
<thead>
<tr>
<th>Virus</th>
<th># substitutions in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A/Netherlands/33/03 (conj.)</td>
</tr>
<tr>
<td></td>
<td>Nucleotide</td>
</tr>
<tr>
<td>Segment</td>
<td>1 (PB2)</td>
</tr>
<tr>
<td></td>
<td>2 (PB1)</td>
</tr>
<tr>
<td></td>
<td>3 (PA)</td>
</tr>
<tr>
<td></td>
<td>4 (HA)</td>
</tr>
<tr>
<td></td>
<td>5 (NP)</td>
</tr>
<tr>
<td></td>
<td>6 (NA)</td>
</tr>
<tr>
<td></td>
<td>7 (MA)</td>
</tr>
<tr>
<td></td>
<td>8 (NS)</td>
</tr>
</tbody>
</table>

1. Sequences were compared to those of A/Chicken/Netherlands/1/03
2. Both mutations in NS1
H7N7 in The Netherlands
- Chain of transmission -
Order Mononegavirales, family Paramyxoviridae

Paramyxovirinae
- Henipavirus
- Morbillivirus

Pneumovirinae
- Pneumovirus
- Metapneumovirus
  - hRSV
  - bRSV
  - APV
  - hMPV

- Respirovirus
  - NDV
  - Avulavirus
  - Rubulavirus

DNA Maximum likelihood, Polymerase ORF
Newly discovered paramyxovirus
- Electron microscopy -

- Pleiomorph
- Average size: 100-600 nm
- Envelope projections: 13-17 nm
- Paramyxovirus

B.G. van den Hoogen et al.
Nat. Med. 7:719-724, 2001
Human metapneumovirus
- Risk groups -

- (Young) children
  > 7-8% of children with RTI?

- Immunocompromised individuals

- Elderly

- Normal individuals
  > 2-3% of RTI in community surveillance studies

Osterhaus and Fouchier, Lancet 2004
Severe acute respiratory syndrome (SARS) - A world-wide threat -
The same week: Hong Kong researchers announce findings of Paramyxoviruses in SARS patients. Similar findings communicated from Canada through SARS etiology network.
Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome

Christian Drosten, M.D., Stephan Günther, M.D., Wolfgang Preiser, M.D., Sylvie van der Werf, Ph.D., Hans-Reinhard Brodt, M.D., Stephan Becker, Ph.D., Holger Rabenau, Ph.D., Marcus Panning, M.D., Larissa Kolesnikova, Ph.D., Ron A.M. Fouchier, Ph.D., Annemarie Berger, Ph.D., Ana-Maria Burguière, Ph.D., Jindrich Cinatl, Ph.D., Markus Eickmann, Ph.D., Nicolas Escrivà, Ph.D., Klaus Grywna, M.Sc., Stefanie Kramme, M.D., Jean-Claude Manuguerra, Ph.D., Stefanie Müller, M.Sc., Volker Rickerts, M.D., Martin Stürmer, Ph.D., Simon Vieth, Hans-Dieter Klenk, M.D., Albert D.M.E. Osterhaus, Ph.D., Herbert Schmitz, M.D., and Hans Wilhelm Duerr, M.D.

A Novel Coronavirus Associated with Severe Acute Respiratory Syndrome

Thomas G. Ksiazek, D.V.M., Ph.D., Dean Erdman, Dr. P.H., Cynthia Goldsmith, M.S., Sherif A. Zaki, M.D., Ph.D., Teresa Peret, Ph.D., Shannon Emery, Suhai-Tong, Ph.D., Carlo Urbani, M.D., James A. Comer, Ph.D., M.P.H., Wilma Lim, Pierre E. Rollin, M.D., Scott Dowell, M.D., M.P.H., Al-Ee Ling, M.D., Charles Humphrey, Ph.D., Wun-Ju Shieh, M.D., Jeannette Guarnieri, M.D., Christopher D. Paddock, M.D., Paul Rota, Ph.D., Barry Fields, Ph.D., Joseph DeRisi, Ph.D., Jyh-Yuan Yang, Ph.D., Nancy Cox, Ph.D., James Hughes, M.D., James W. LeDuc, Ph.D., William Bellini, Ph.D., Larry J. Anderson, M.D., and the SARS Working Group

Coronavirus as a possible cause of severe acute respiratory syndrome

HCoV-NL: a fourth coronavirus of humans
- phylogeny -

Fouchier et al., PNAS 2004
SARS and the Koch’s postulates

- Two viruses have been isolated from SARS patients: SCV and hMPV

- Koch’s postulates modified for viruses by Rivers (1937):
  1. Isolation from diseased hosts
  2. Cultivation in host cells
  3. Proof of filterability
  4. Production of comparable disease in host or related species
  5. Reisolation of the virus
  6. Detection of specific immune response
Cynomolgus monkeys 
(*Macaca fascicularis*)

*Simultaneous SCV-hMPV infection experiments:*

- SCV alone
- hMPV alone
- SCV followed by hMPV

Fouchier et al., Nature 2003
SARS coronavirus (SCV) in macaques

Histological lesions in lungs from cynomolgus macaques infected with SCV

A

B

C

D

Kuiken et al.
The Lancet 2003
## SARS coronavirus in macaques

<table>
<thead>
<tr>
<th>Time after inoculation (days)</th>
<th>Macaque # 3</th>
<th></th>
<th></th>
<th></th>
<th>Macaque # 4</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Throat</td>
<td>Nose</td>
<td>Faeces</td>
<td>IF</td>
<td>Throat</td>
<td>Nose</td>
<td>Faeces</td>
<td>IF</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>N.T.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>N.T.</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>N.T.</td>
<td>N.T.</td>
<td>+</td>
<td>+</td>
<td>N.T.</td>
<td>N.T.</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>N.T.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>N.T.</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>N.T.</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>N.T.</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>N.T.</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>N.T.</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>N.T.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>N.T.</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>N.T.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>N.T.</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Exclusion of hMPV as the primary cause of SARS by macaque infection experiment

- Koch’s postulates modified for viruses by Rivers:
  1. Isolation from diseased hosts
  2. Cultivation in host cells
  3. Proof of filterability
  4. **No production of comparable disease in related species**
  5. Reisolation of the virus
  6. Detection of specific immune response

- SCV infected macaques subsequently inoculated with hMPV did not show disease exacerbation
Search for natural reservoir of SARS-CoV

(Guan et al. 2003 Science)

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>PCR/isolation</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Himalayan palm civet</td>
<td>6</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Chinese ferret badger</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Raccoon dog</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Hog badgers (3), beavers (3), domestic cats (3), Chinese hares (4), and Chinese muntjacs (2) all tested negative.
Excretion of SCV in domestic cats and ferrets

Treatment of SARS-CoV infected macaques with pegylated interferon-α (Haagmans et al. 2004, Nature Medicine)

- 3 groups of cynomolgus macaques
  - Control (n = 4)
  - Prophylactic (n = 4)
  - Post-exposure (n = 4)

- Treated with 3 mg/kg IFN, at
  - -3, -1, +1, +3 dpi (prophylactic)
  - +1, +3 (post-exposure)

- Same infection protocol

- Euthanasia at 4 dpi

- Examination by:
  - Pathology (gross, histology)
  - Immunohistochemistry
  - RT-PCR
  - Virus isolation
SARS-CoV excretion from pharynx

Haagmans et al., Nature Med. 2004
SARS-CoV replication, Ag-expression and histopathological lesions in lungs

Haagmans et al., Nature Med. 2004
Emerging virus infections
- Conclusions 1 -

- A complex mix of **social, technological, ecological, and viral** changes
  
  - Predisposes for the **emergence** of virus infections
  
  - Originating from **animal reservoirs**
Emerging virus infections
- Conclusions 2 -

- Extensive diagnostic and surveillance networks

- As well as novel vaccine- and antiviral development strategies

- Should provide the safeguards to limit their impact
Acknowledgements

Dept. Virology, Erasmus MC
Theo Bestebroer
Bé Niemeyer
Georgina Aron
Robert Dias-d’Ullois
Geert van Amerongen
Gerard van Doornum
Martin Schutten
Bert Niesters
Bart Haagmans
Byron Martina
Guus Rimmelzwaan
Thijs Kuiken
Ron Fouchier
Ab Osterhaus

WHO & Members of the WHO SARS aetiology team

Centers for Disease Control & Prevention, Atlanta, USA
Central Public Health Laboratory, London, UK
Public Health Laboratory Centre, Hongkong, SAR China
Prince of Wales Hospital, SAR China
Queen Mary Hospital, SAR China
Singapore General Hospital, Singapore
Federal Laboratories for Health Canada, Winnipeg, Canada
Health Canada, Ottawa, Canada
Bernhard-Nocht Institute, Hamburg, Germany
Institut Pasteur, Paris, France
National Institute of Infectious Disease, Tokyo, Japan