SARS: Aetiology

- JSM Peiris
  The University of Hong Kong & Queen Mary Hospital
- WHO SARS Laboratory Network
- Hospital Authority and Department of Health, HK
Early February 2003

- Surveillance of severe atypical pneumonia in Hospital Authority in Hong Kong
- Initiate contacts in Guangdong
Severe atypical pneumonia admitted to ICU in Hospital Authority Hong Kong

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of Episodes</th>
<th>Total IP &amp; DP Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td></td>
<td></td>
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<tr>
<td>2003</td>
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H5N1
Aetiology

- March 17: WHO Network of SARS Labs
- Influenza and other conventional respiratory pathogens ruled-out
- Strategy:
  - unconventional cell lines to grow the virus
  - consensus primer / low stringency PCR
  - random primer RT-PCR / differential display
  - Electron microscopy (on lung biopsy)
- Paramyxovirus / human metapneumovirus detected
A Novel coronavirus is associated with SARS

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. Fauchier, Ph.D., Annemarie Berger, Ph.D., Ana Maria Burguiere, Ph.D., Jindrich Cintal, Ph.D., Markus Eckmann, Ph.D., Nicolas Escriou, Ph.D., Klaus Grywna, M.Sc., Stefanie Krammke, 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 Doerr, 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 R. Zaki, M.D., Ph.D., Teresa Peret, Ph.D., Shannon Emery, Suxiang Tong, Ph.D., Carlo Urbani, M.D.,* James A. Comer, Ph.D., M.P.H., Willina Lim, Pierre E. Rollin, M.D., Scott Dowell, M.D., M.P.H., Ai-Ee Ling, M.D., Charles Humphrey, Ph.D., Wun-Ju Shieh, M.D., Jeannette Guarner, 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

J S M Peiris, S T Lai, L L M Poon, Y Guan, L Y C Yam, W Lim, J Nicholls, W K S Yee, W W Yan, M T Cheung, V C C Cheng, K H Chan, D N C Tsang, R W H Yung, T K Ng, K Y Yuen, and members of the SARS study group*
Two patients:

*Cytopathic effect in FRhK-4 cells*

Tested negative with reagents / PCR / RT/PCR for influenza A/B, adenovirus, RSV, parainfluenza, human metapneumovirus, **enterovirus**, rhinovirus, mycoplasma, chlamydia
Confirm the link between virus isolate and other patients with SARS

- Acute and convalescent sera from patients with suspected SARS
- Tested by indirect immunofluorescence on cells infected with the suspect virus

- Seroconversion in 8/8 patients
Coronavirus-like agent is isolated
Detection of SARS sequence by random RT-PCR

• A 646 nt sequence of coronavirus origin

- Murine hepatitis virus strain ML-11
- Murine hepatitis virus
- Murine hepatitis virus strain 2
- Murine hepatitis virus
- Murine hepatitis virus (strain JHM)
- Bovine coronavirus
- Bovine coronavirus
- **SARS virus, Hong Kong isolate**
- Avian infectious bronchitis virus
- Avian infectious bronchitis virus (strain Beaudette CK)
- Transmissible gastroenteritis virus
- Human coronavirus 229E
- Porcine epidemic diarrhea virus
Koch’s postulates:
Association of microbe and disease

SEROLOGY:

• 107 patients with clinically defined SARS
  – Rising titre to coronavirus 104 / 107 (97%)
  – Rising IFA titre to human metapneumovirus 0 / 50 (0%)

• 45 paired sera from non-SARS patients: no antibody to CV

• 200 blood donors: no antibody
## Sero-prevalence in blood donors

**Indirect immunofluorescence**

<table>
<thead>
<tr>
<th>Date tested</th>
<th>No Positive / No Tested</th>
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<tr>
<td>May 2003:</td>
<td>0 / 1,800</td>
</tr>
<tr>
<td>March 2003:</td>
<td>0 / 200</td>
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SARS-coronavirus in macaques

Macaque # 4:
Severe multifocal pulmonary consolidation
Coronavirus detected in lung tissue
Severe interstitial pneumonia

Fouchier et al -
Dual infections with SARS Coronavirus

Human metapneumovirus

J Tam et al
F Plummer et al.
Dual infections with SARS Coronavirus

From ~ 800 SARS seroconverisons

<table>
<thead>
<tr>
<th>Virus</th>
<th>Count (+)</th>
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<tbody>
<tr>
<td>Mycoplasma</td>
<td>4 (+3)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>5 (+5)</td>
</tr>
<tr>
<td>Flu A</td>
<td>8 (+4)</td>
</tr>
<tr>
<td>Flu B</td>
<td>3 (+3)</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>7 (+3)</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>1 (+1)</td>
</tr>
<tr>
<td>HSV</td>
<td>7</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>1</td>
</tr>
<tr>
<td>Norwalk</td>
<td>2</td>
</tr>
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- W Lim, Department of Health
Aetiology of SARS:

- SARS coronavirus (+/- host response) is necessary and sufficient to cause SARS
- We should now focus on SARS coronavirus rather than on SARS
- Co-factors (viruses, microbes or other) may play a role in explaining
  - Severity
  - “Super-spreading incidents”
Genome of the SARS-associated coronavirus

The Genome Sequence of the SARS-Associated Coronavirus


Robert C. Brunham, Mal Krajden, Martin Petrie, Dunuya M. Skowronska

Chris Upton, Rachel L. Roper

Characterization of a Novel Coronavirus Associated with Severe Acute Respiratory Syndrome

Viral load in Nasopharyngeal Aspirate

Figure 4: Sequential quantitative RT-PCR for SARS-associated coronavirus in nasopharyngeal aspirates of 14 SARS patients
Subsequent findings

- Laboratory diagnosis: Serology and RT-PCR
- Virus excretion in the faces
- Transmission: more likely in later phase of illness?
- Virus stability in environment
WHO Network of Laboratories

- Federal Laboratories for Health Canada, Winnipeg, Canada
- Health Canada, Ottawa, Canada
- Public Health Laboratory Centre, Hongkong SAR China
- Prince of Wales Hospital, Hongkong SAR China
- The University of Hongkong, Hong Kong SAR, China
- Institut Pasteur, Paris, France
- Bernhard-Nocht Institute, Hamburg and Johann Wolfgang Goethe Universitat, Frankfurt, Germany
- National Institute of Infectious Disease, Tokyo, Japan
- Erasmus MC, Rotterdam, The Netherlands
- Singapore General Hospital, Singapore
- Central Public Health Laboratory, London, UK
- Centers for Disease Control & Prevention, Atlanta, USA
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