

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

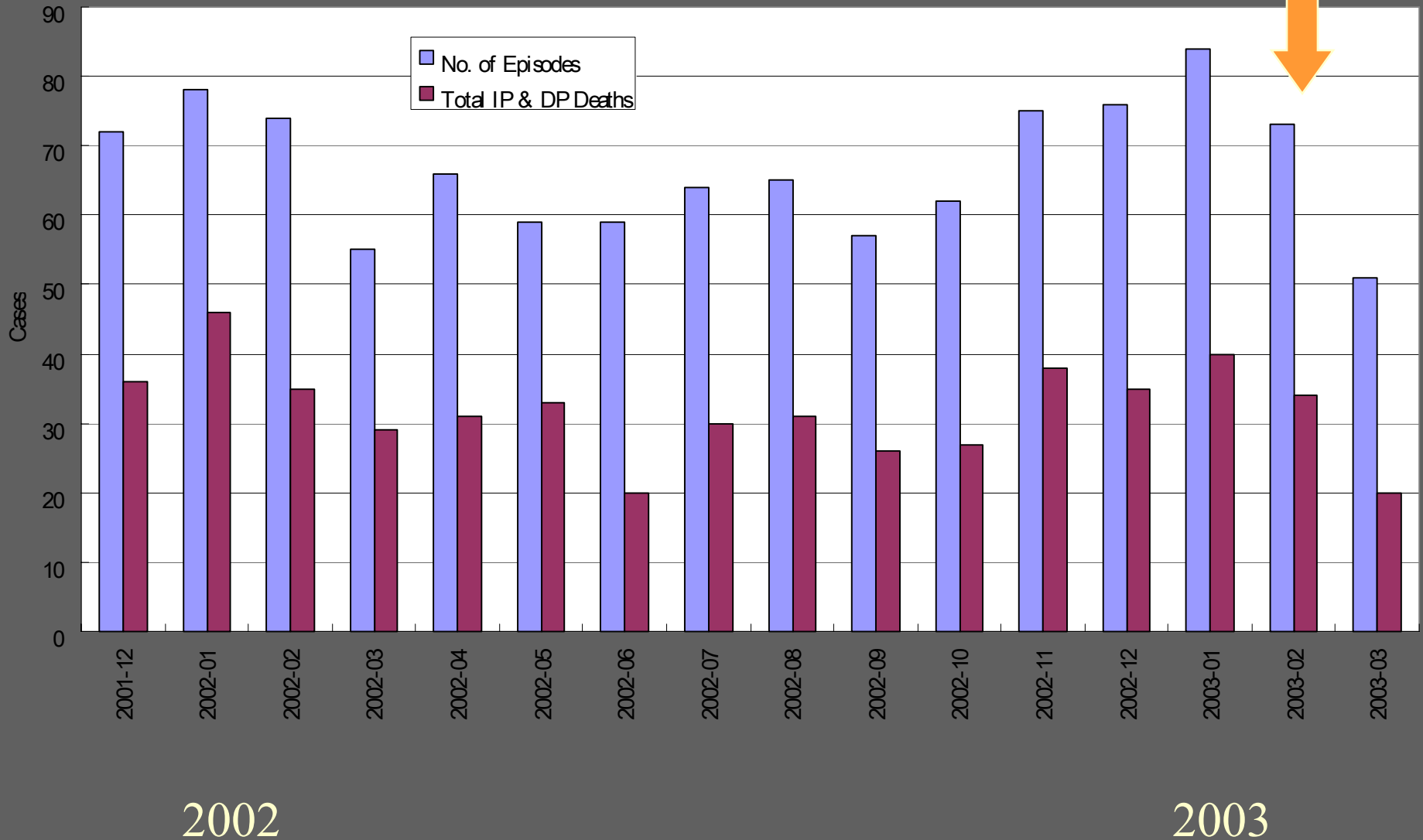
- Surveillance of severe atypical pneumonia in Hospital Authority in Hong Kong
- Initiate contacts in Guangdong



Severe atypical pneumonia admitted to ICU in Hospital Authority Hong Kong

Severe Atypical CAP in ICU

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

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

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 Escriou, 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 Doerr, M.D.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

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., Wilina 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†

ARTICLES

Articles

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*

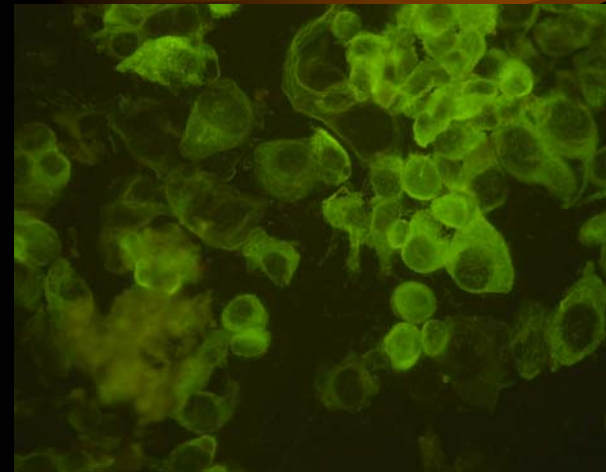
Two patients: Cytopathic effect in FRhK-4 cells



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



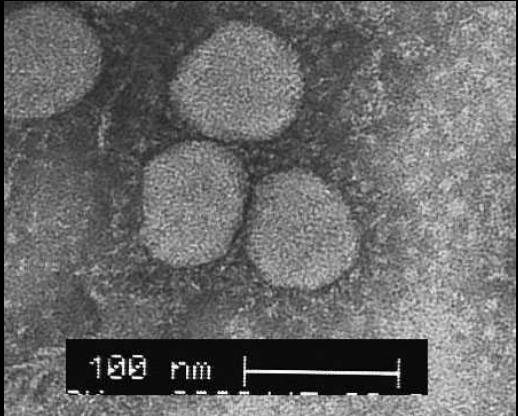
- Seroconversion in 8/8 patients



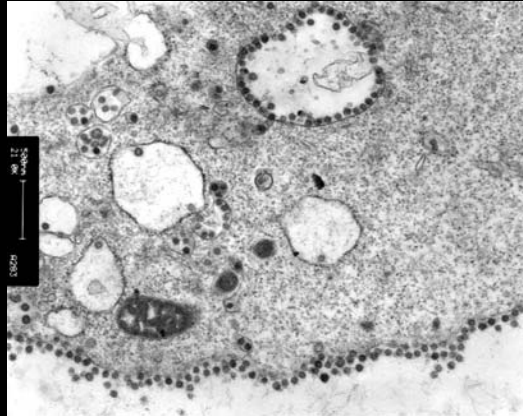
Control cells



Infected cells



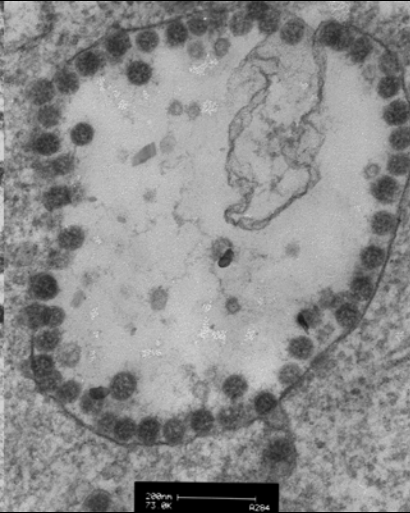
Negative stain
Cultured virus



Thin sect
Cultured virus



Direct EM
Lung Bx virus

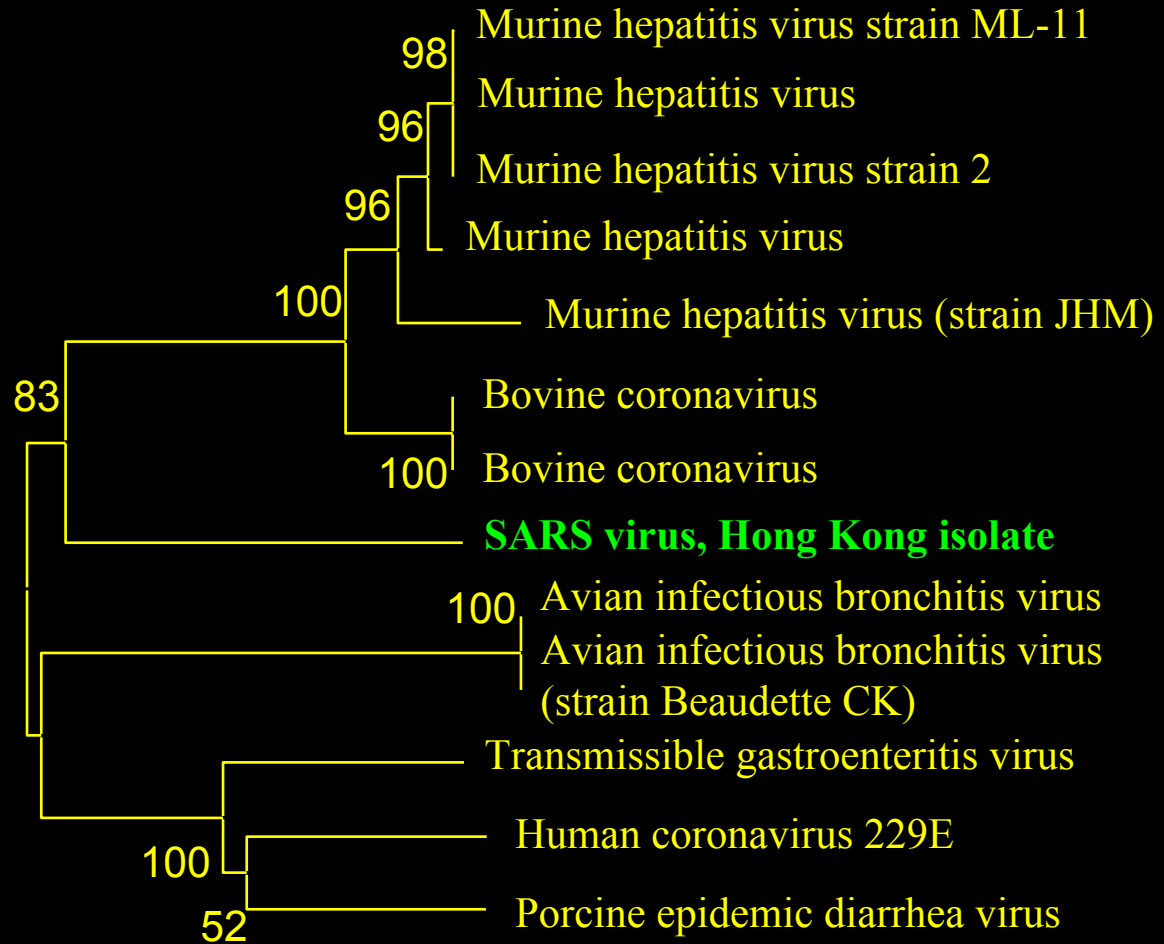
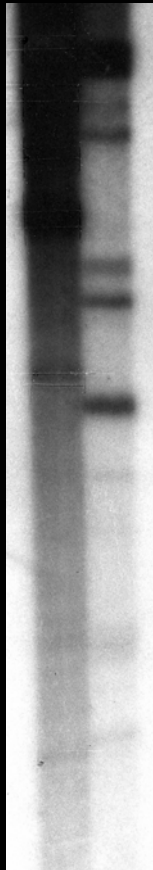


Thin sect
Cultured virus

Coronavirus-like agent is isolated

Detection of SARS sequence by random RT-PCR

- A 646 nt sequence of coronavirus origin



10

*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

- Date tested No Positive / No Tested
- May 2003: 0 / 1,800
- March 2003: 0 / 200

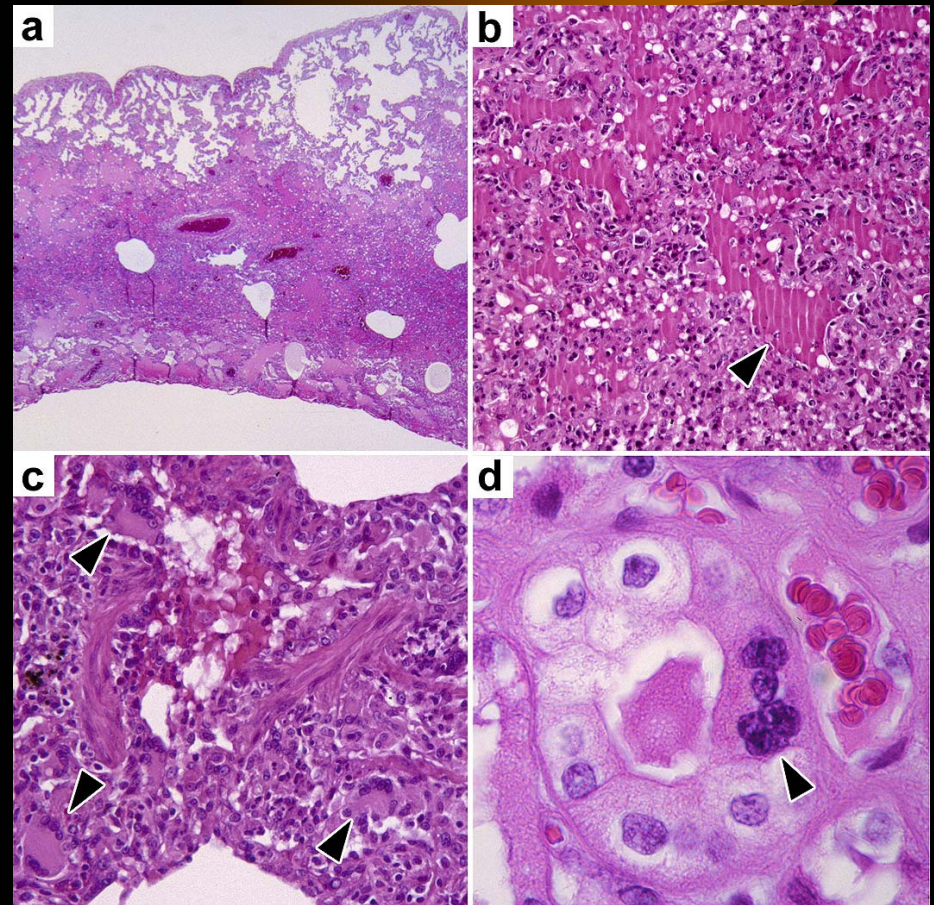
SARS-coronavirus in macaques

Macaque # 4:

Severe multifocal pulmonary consolidation

Coronavirus detected in lung tissue

Severe interstitial pneumonia



*Fouchier et al -
Nature 2003, 423: 240.*

Dual infections with SARS Coronavirus



Human metapneumovirus

J Tam et al

F Plummer et al.

Dual infections with SARS Coronavirus

Human metapneumovirus

J Tam et al

F Plummer et al.

From ~ 800 SARS seroconversions

Mycoplasma 4 (+3)

Adenovirus 5 (+5)

Flu A 8 (+4)

Flu B 3 (+3)

Parainfluenza 7 (+3)

Chlamydia 1 (+1)

HSV 7

Rotavirus 1

Norwalk 2

• *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

Scienceexpress

Research Article

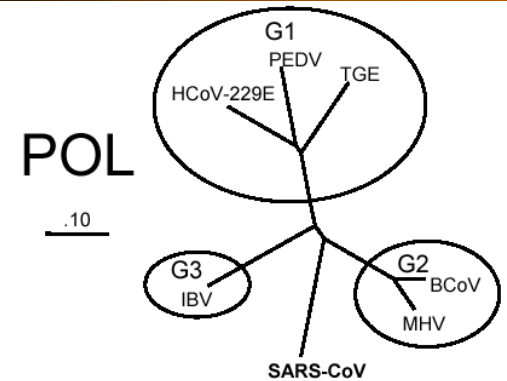
The Genome Sequence of the SARS-Associated Coronavirus

Marco A. Marra,^{1*} Steven J. M. Jones,¹ Caroline R. Astell,¹ Robert A. Holt,¹ Angela Brooks-Wilson,¹ Yaron S. N. Butterfield,¹ Jaswinder Khattri,¹ Jennifer K. Asano,¹ Sarah A. Barber,¹ Susanna Y. Chan,¹ Alison Cloutier,¹ Shaun M. Coughlin,¹ Doug Freeman,¹ Noreen Girn,¹ Obi L. Griffith,¹ Stephen R. Leach,¹ Michael Mayo,¹ Helen McDonald,¹ Stephen B. Montgomery,¹ Pawan K. Pandoh,¹ Anca S. Petrescu,¹ A. Gordon Robertson,¹ Jacqueline E. Schein,¹ Asim Siddiqui,¹ Duane E. Smailus,¹ Jeff M. Stott,¹ George S. Yang¹

Francis Plummer,² Anton Andonov,² Harvey Artsob,² Nathalie Bastien,² Kathy Bernard,² Timothy F. Booth,² Donnie Bowness,² Michael Drebot,² Lisa Fernando,² Ramon Flick,² Michael Garbutt,² Michael Gray,² Allen Grolla,² Steven Jones,² Heinz Feldmann,² Adrienne Meyers,² Amin Kabani,² Yan Li,² Susan Normand,² Ute Stroher,² Graham A. Tipples,² Shaun Tyler,² Robert Vogrig,² Diane Ward,² Brynn Watson²

Robert C. Brunham,³ Mel Krajden,³ Martin Petric,³ Danuta M. Skowronski³

Chris Upton,⁴ Rachel L. Roper⁴

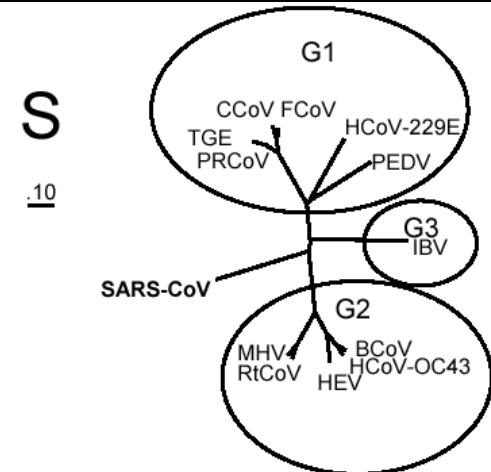


Scienceexpress

Research Article

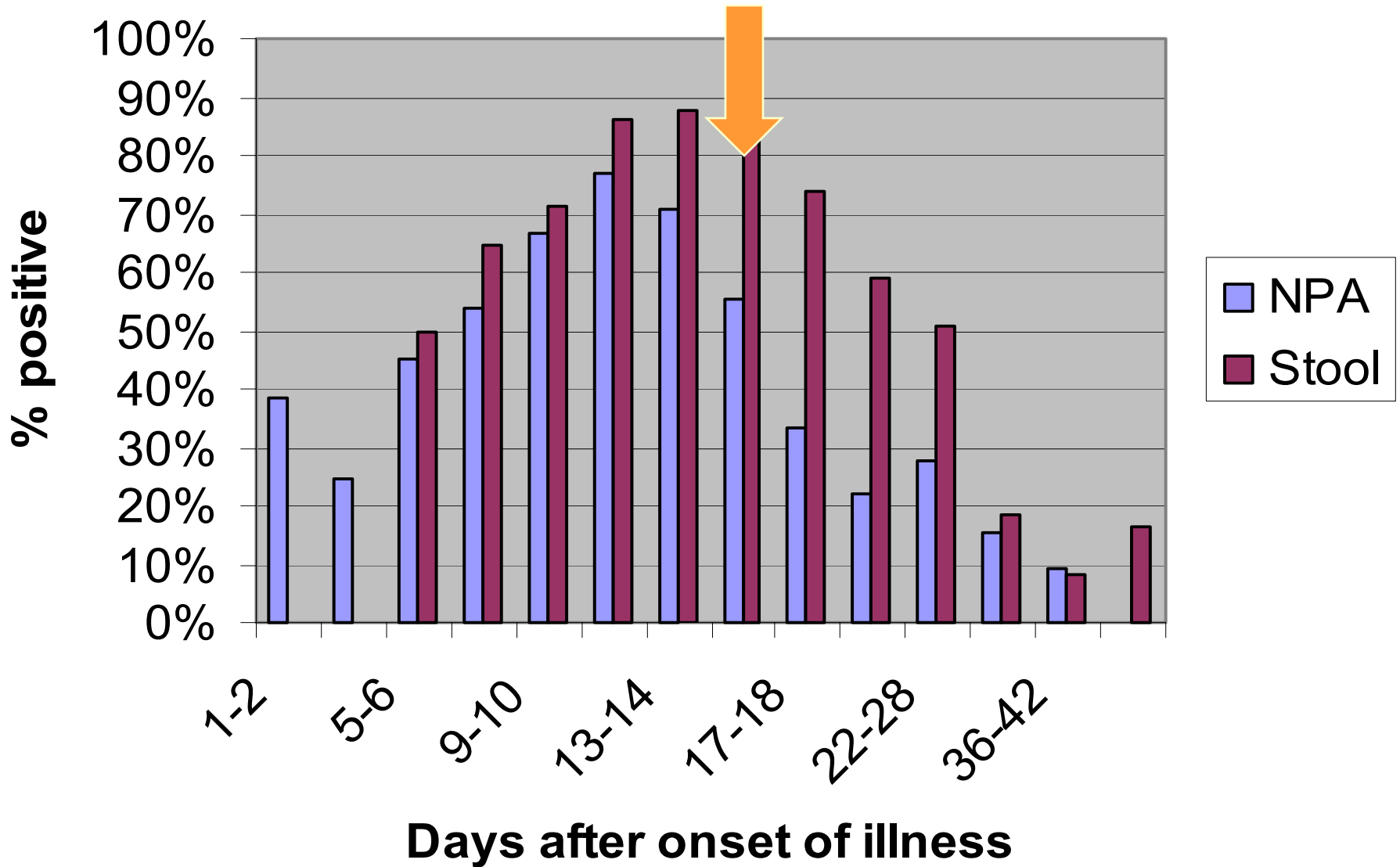
Characterization of a Novel Coronavirus Associated with Severe Acute Respiratory Syndrome

Paul A. Rota,^{1*} M. Steven Oberste,¹ Stephan S. Monroe,¹ W. Allan Nix,¹ Ray Campagnoli,¹ Joseph P. Icenogle,¹ Silvia Peñaranda,¹ Bettina Bankamp,¹ Kaija Maher,¹ Min-hsin Chen,¹ Suxiong Tong,¹ Azaibi Tamin,¹ Luis Lowe,¹ Michael Frace,¹ Joseph L. DeRisi,² Qi Chen,¹ David Wang,² Dean D. Erdman,¹ Teresa C. T. Peret,¹ Cara Burns,¹ Thomas G. Ksiazek,¹ Pierre E. Rollin,¹ Anthony Sanchez,¹ Stephanie Liffick,¹ Brian Holloway,¹ Josef Limor,¹ Karen McCaustland,¹ Melissa Olsen-Rasmussen,¹ Ron Fouchier,³ Stephan Günther,⁴ Albert D. M. E. Osterhaus,³ Christian Drosten,⁴ Mark A. Pallansch,¹ Larry J. Anderson,¹ William J. Bellini¹



Clinical SARS: % positive by RT-PCR

Faeces	9	10	10	17	21	29	64	42	19	34	67	38	12	6
NPA	39	57	62	41	42	26	34	27	9	9	18	13	11	



Viral load in Nasopharyngeal Aspirate

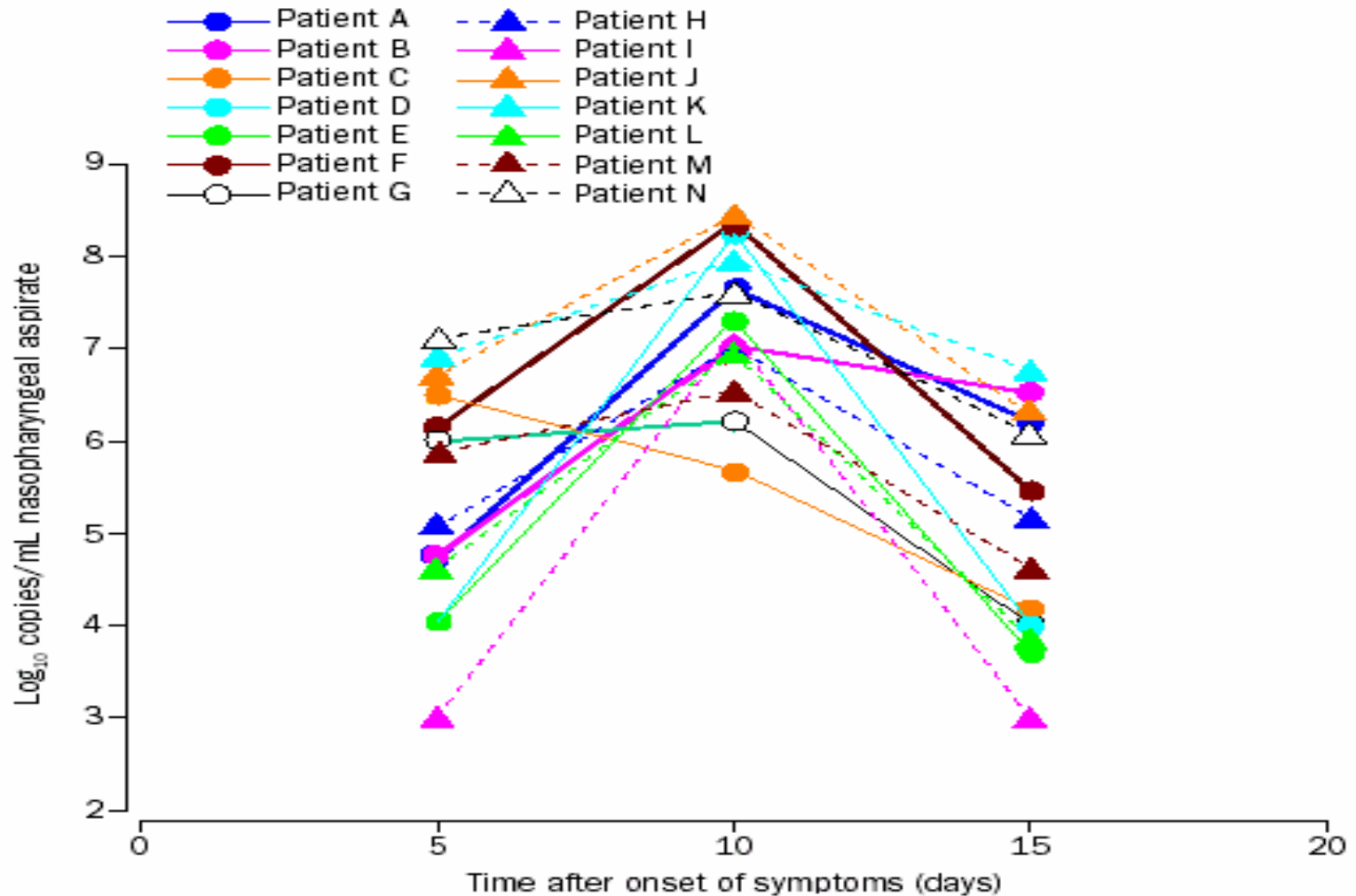


Figure 4: **Sequential quantitative RT-PCR for SARS-associated coronavirus in nasopharyngeal aspirates of 14 SARS patients**

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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