

Severe acute respiratory syndrome (SARS)—paradigm of an emerging viral infection[☆]

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Abstract

An acute and often severe respiratory illness emerged in southern China in late 2002 and rapidly spread to different areas of the Far East as well as several countries around the globe. When the outbreak of this apparently novel infectious disease termed severe acute respiratory syndrome (SARS) came to an end in July 2003, it had caused over 8000 probable cases worldwide and more than 700 deaths.

Starting in March 2003, the World Health Organization (WHO) organised an unprecedented international effort by leading laboratories working together to find the causative agent. Little more than one week later, three research groups from this WHO-coordinated network simultaneously found evidence of a hitherto unknown coronavirus in SARS patients, using different approaches. After Koch's postulates had been fulfilled, WHO officially declared on 16 April 2003 that this virus never before seen in humans is the cause of SARS.

Ever since, progress around SARS-associated coronavirus (SARS-CoV) has been swift. Within weeks of the first isolate being obtained, its complete genome was sequenced. Diagnostic tests based on the detection of SARS-CoV RNA were developed and made available freely and widely; nevertheless the SARS case definition still remains based on clinical and epidemiological criteria. The agent's environmental stability, methods suitable for inactivation and disinfection, and potential antiviral compounds have been studied, and development of vaccines and immunotherapeutics is ongoing.

Despite its grave consequences in humanitarian, political and economic terms, SARS may serve as an example of how much can be achieved through a well-coordinated international approach, combining the latest technological advances of molecular virology with more "traditional" techniques carried out to an excellent standard.

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1. Introduction

Severe acute respiratory syndrome (SARS) is the latest in a series of emerging infectious diseases, and certainly one of the most widely publicised. This acute and often severe respiratory illness seems to have emerged in southern China in late 2002 (World Health Organization, 2003c). It soon caused considerable international alarm, after several index cases had given rise to outbreaks of sometimes

enormous scales, and when the disease's ability to spread to distant areas within a very short period of time became obvious (World Health Organization, 2003d). A definition was developed for suspected and probable SARS cases, based on clinical and epidemiological criteria; it has since been modified on several occasions.

While SARS demonstrated very vividly that in the modern world with an enormous volume of intercontinental traffic, infectious agents may be spread rapidly across the globe, it also serves as an example of how modern technology—provided there is the necessary will, determination, and coordination to make best use of it—may help in combating such threats with unprecedented speed and enormous success.

SARS is characterized clinically by fever followed by respiratory signs and symptoms which may lead to rapidly progressive respiratory failure. As of September 2003, 8098

[☆] This review is dedicated to all those who were prepared to risk their lives to provide care to SARS patients and control the first pandemic of the 21st century.

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people have been notified to the World Health Organization (WHO) as fulfilling the criteria for “probable SARS”, and of these, 774 have died from SARS (http://www.who.int/csr/sars/country/2003_08_15/en/).

What made SARS—in contrast, e.g. to influenza—notorious is its propensity to cause hospital outbreaks; some of these have affected over 100 people, including health care staff, other patients and visitors (Dwosh et al., 2003). In contrast to many other emerging viral infections such as Ebola, hantavirus pulmonary syndrome, and Nipah, SARS also clearly demonstrated its ability for easy and rapid geographic spread. This is because the SARS agent affected a generally rather mobile population, and because those infected normally remain well enough to travel for several days after onset of infectivity.

2. Search for the causative agent

On 17 March 2003, the WHO set up a worldwide network of virological laboratories investigating SARS cases (World Health Organization, 2003a). The investigations conducted by the members of these networks were coordinated by WHO’s Department of Communicable Disease Surveillance and Response (CSR) through normally daily telephone conferences and a password-protected internet website. Thus results and planned further studies were communicated and views and comments exchanged almost in “real-time” which made possible the rapid progress in elucidating the aetiological agent. In its final form, this network comprised 13 participating laboratories from ten countries (World Health Organization Multicentre Collaborative Network for Severe Acute Respiratory Syndrome Diagnosis, 2003).

Investigations had soon ruled out a novel influenza virus strain, possibly of avian origin, as the cause of SARS, and then focussed on members of the *Paramyxoviridae* family, including human metapneumovirus (hMPV), and *Chlamydia*-like organisms, including *Chlamydia pneumoniae*. However, further investigations did not confirm these findings; the said agents were indeed found in a number of SARS patients but not in all (WHO multicentre collaborative networks for severe acute respiratory syndrome (SARS) diagnosis, 2003). Almost nobody knew at that stage that virologists in Beijing had already discovered a new virus in samples from some of the earliest SARS patients. However, the official line in China at the time was that the novel

“atypical pneumonia” was caused by *Chlamydia* (Enserink, 2003a). Nevertheless, before the end of March, laboratories in Hong Kong, Germany, Canada, and the United States of America found evidence of a novel coronavirus in patients with SARS by cell culture, electron microscopy, and by polymerase chain reaction (PCR) using primers at low stringency designed for other agents followed by sequencing (Peiris et al., 2003b; Drosten et al., 2003a; Ksiazek et al., 2003; Poutanen et al., 2003; Drosten et al., 2003b). These results could not rule out that very thorough and extensive testing had by chance led to the discovery of a novel agent that was not responsible for the new illness but rather an “innocent bystander”. However, the sequences obtained in different parts of the world were shown to belong to the same, previously unrecognised, coronavirus (Ruan et al., 2003). It could also be shown that SARS patients underwent seroconversion against this coronavirus, using cells infected with patient isolates as antigen for indirect immunofluorescent antibody tests (Drosten et al., 2003a; Ksiazek et al., 2003; Fig. 1). Furthermore, no evidence of present or past infection with this agent could be detected in limited surveys of healthy control individuals not suffering from SARS (Ksiazek et al., 2003). This strengthened the case for the novel coronavirus being the cause of SARS, but only after it had been shown to cause a similar illness in artificially infected macaques could it be regarded as fulfilling all four of Koch’s postulates (Fouchier et al., 2003; World Health Organisation Multicentre Collaborative Networks for Severe Acute Respiratory Syndrome Diagnosis, 2003). On April 16, 2003, less than a month after the laboratory network had been brought into existence, WHO officially announced that a new coronavirus, never before seen in humans or animals and now provisionally termed SARS-associated coronavirus (abbreviated as SARS-CoV), was the cause of SARS (Kuiken et al., 2003).

3. Virology

Coronaviruses are large, enveloped, positive-stranded RNA viruses with a diameter of 60–220 nm. Most but not all viral particles display the characteristic appearance of surface projections, giving rise to the virus family’s name (corona, Latin, = crown). They have the largest genomes of all RNA viruses. Based on their unique transcription strategy that involves the formation of “nested” mRNA molecules

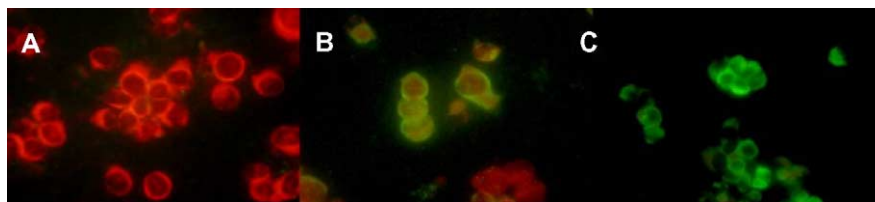


Fig. 1. Seroconversion during the course of SARS demonstrated by IFA using SARS-CoV-infected Vero cells. Serum samples from wife of Frankfurt index patient diluted 1:50. (A) 9 days, (B) 12 days, (C) 14 days after hospitalisation (photographs by G. Bauer, Inst. f. Med. Virology, Frankfurt, Germany).

Table 1
Overview of coronavirus species, group assignment, host species, disease manifestation and availability of a vaccine

Group	Virus		Host	Types of infection	Vaccine available
I	TGEV	Transmissible gastroenteritis virus	Pig	Enteric	(Yes)
	PRCoV	Porcine coronavirus	Pig	Respiratory	No
	PEDV	Porcine epidemic diarrhea virus	Pig	Enteric	No
	FIPV	Feline infectious peritonitis virus	Cat	Systemic, peritonitis	Yes
	FeCoV	Feline enteric coronavirus	Cat	Enteric	No
	CCoV	Canine coronavirus	Dog	Enteric	No
	RbCoV	Rabbit coronavirus	Rabbit	Enteric	No
	HCoV-229E	Human coronavirus strain 229E	Human	Respiratory	No
II	MHV	Mouse hepatitis virus	Mouse	Respiratory, enteric, hepatitis, neurologic	No
	SDAV	Sialodacryoadenitis virus	Rat	Neurologic	No
	RCoV	Rat coronavirus	Rat	Respiratory	No
	HEV	Hemagglutinating encephalitis virus	Pig	Respiratory, enteric, neurologic	No
	BCoV	Bovine coronavirus	Cattle	Enteric	Yes
	HCoV-OC43	Human coronavirus strain OC43	Human	Respiratory	No
III	IBV	Infectious bronchitis virus	Chicken	Respiratory, hepatitis, urologic	Yes
	TCoV	Turkey coronavirus	Turkey	Respiratory, enteric	No
IV ?	SARS-CoV	SARS-associated coronavirus	Human, other?	Respiratory, enteric	No

all sharing the same 3' end, the families Coronaviridae and Arteriviridae (of no significance to human virology) are grouped together in the order Nidovirales (nidus, Latin, = nest) (Cavanagh, 2000). Within the Coronaviridae, the genera Torovirus and Coronavirus (type species: infectious bronchitis virus, IBV) are distinguished. A unique feature of coronavirus genetics is a high frequency of RNA recombination as a result of discontinuous transcription and polymerase “jumping” (Lai and Cavanagh, 1997). One example is the porcine respiratory coronavirus (PRCoV), which evolved in the early 1980s from the enteropathogenic porcine transmissible gastroenteritis coronavirus (TGEV), known since the 1940s (Pensaert et al., 1986). Through a large deletion in the S gene, the virus acquired an altered tissue tropism, causing mild respiratory infections.

Based on homologies on the amino acid sequence level, the known coronaviruses can be divided into three groups. Table 1 gives an overview of coronavirus species, group assignment, host species, disease manifestation and availability of a vaccine. There are more than a dozen known coronaviruses affecting different animal species; whereas group I and II coronaviruses affect various mammals, those in group III infect birds. Some of these cause major problems in the livestock industry or may affect companion animals; therefore, considerable efforts have been devoted to their control, including development of active immunisation.

Negative-stain transmission electron microscopy of respiratory samples from SARS patients and of infected cell culture supernatants reveals pleomorphic, enveloped virus-like particles with diameters of between 60 and 130 nm (Fig. 2). Most but not all viral particles showed the characteristic coronavirus-like surface features (Ksiazek et al., 2003).

In contrast to most coronaviruses, which infect only the cells of their natural host species and a few closely related species, the SARS-CoV is able to infect different

cell cultures, such as African green monkey (*Cercopithecus aethiops*) kidney cells (Vero) and the human colorectal adenocarcinoma cell line (Caco-2), causing a massive cytopathic effect (CPE) after as little as 2 days or 3 days (Fig. 3). It should be mentioned that these cell lines were not commonly used for the isolation of human respiratory viruses. Interestingly during cell culture passages of the Frankfurt isolate a virus variant emerged with a nucleotide deletion of 45 bases in the ORF7b (Thiel et al., 2003). The biological significance of this finding remains to be elucidated.

Complete genome sequences of SARS-CoV were first published by a Canadian laboratory and the Centers for Disease Control (CDC), Atlanta (Marra et al., 2003; Rota et al., 2003). As of end-October 2003, 45 full genome sequences are available on <http://www.ncbi.nlm.nih.gov/genomes/SARS/SARS.html>. The genomic data available so far from several SARS-CoV strains suggest that the novel agent does not belong to any of the known groups of coronaviruses,

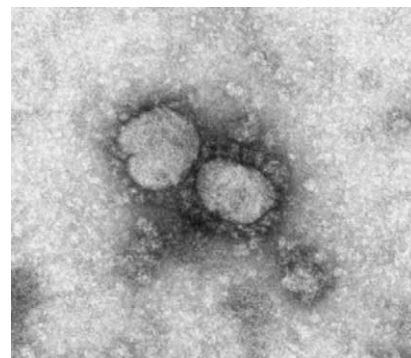


Fig. 2. Electron microscopy image of SARS-CoV particle from infected cell culture supernatant after ultracentrifugation, 2% formalin fixation and negative staining with uranyl acetate (photograph by H. R. Gelderblom, Robert Koch Institute, Berlin, Germany).

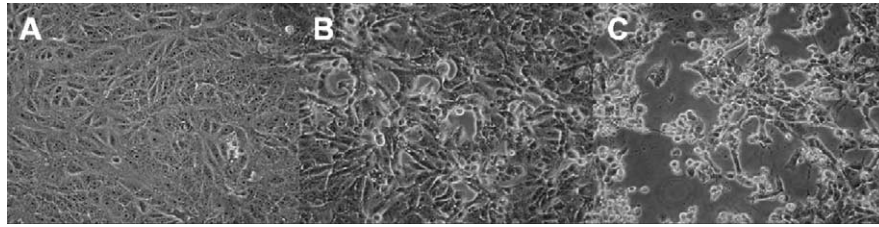


Fig. 3. Cytopathic effect (CPE) caused by SARS-CoV in Vero cell culture (A) 0h, (B) 24h, and (C) 48h after inoculation (photographs by G. Bauer, Inst. f. Med. Virology, Frankfurt, Germany).

including the two human coronaviruses (HCoV) OC43 and 229E (Drosten et al., 2003a; Marra et al., 2003; Peiris et al., 2003b; Rota et al., 2003), to which it is only moderately related (Fig. 4).

The SARS-CoV appears to be neither a mutant of a known coronavirus nor a recombinant between known coronaviruses (Holmes, 2003). It has been proposed that the new virus defines a fourth lineage of coronavirus (Group IV) (Marra et al., 2003) (Fig. 4). However, more recently it was suggested that SARS-CoV may be an early split-off from the group 2 lineage (Snijder et al., 2003). The sequence analysis of SARS-CoV suggests that it is an animal virus with a still unknown natural host species that has recently developed the ability to productively infect humans. A genetically very close but not identical virus was found in wild animals (masked palm civets and a raccoon dog) from a wildlife market in Guangdong (Guan et al., 2003). But uncertainties remain over the exact source of this virus;

the animals sampled could have been infected from humans or another animal species (Cyranoski and Abbott, 2003; Normile and Enserink, 2003).

Sequence analysis of different SARS-CoV isolates reveals two distinct genotypes. One genotype was linked with infections originating from Hotel M in Hong Kong, the other one comprises isolates from Hong Kong, Guangdong and Beijing that had no association with Hotel M (Ruan et al., 2003; Tsui et al., 2003). To date, there is no information as to whether different SARS-CoV strains may have different degrees of virulence.

4. Epidemiology

There is little doubt that SARS originated from Guangdong province of southern China (Breiman et al., 2003). The first cases retrospectively identified as SARS occurred there

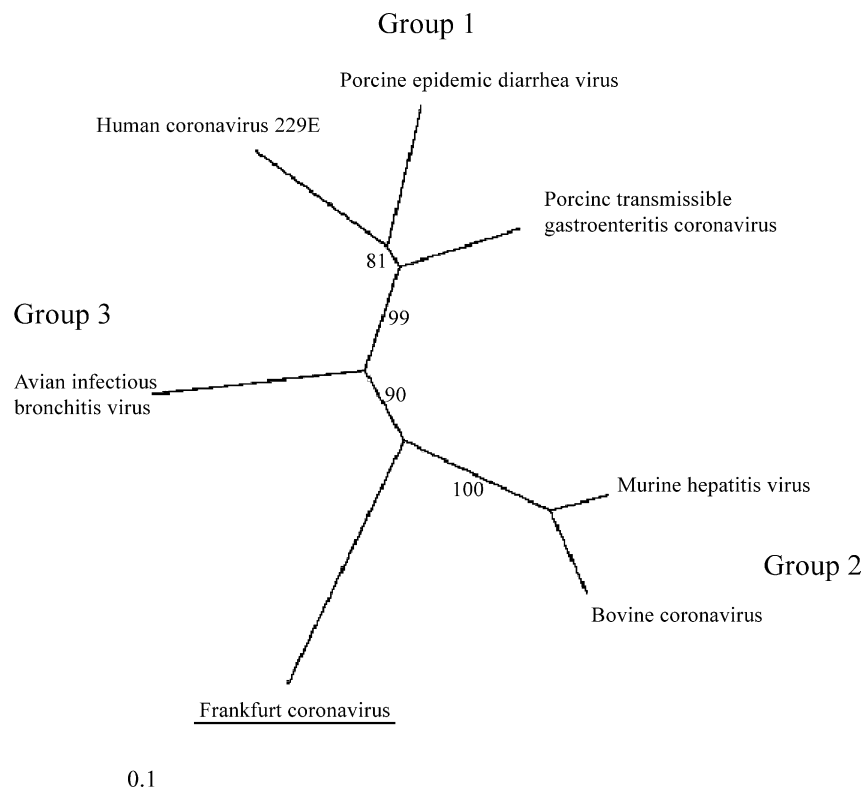


Fig. 4. Phylogenetic tree of known coronaviruses (provided by S. Günther, Bernhard Nocht Institute, Hamburg).

in November 2002. Interestingly, amongst these early cases there seems to have been a significantly higher percentage of food handlers, chefs, etc. than in the general population, lending further support to a zoonotic origin. The worldwide spread of SARS-CoV was triggered through a single infected individual from Guangdong who spent some time in Hong Kong before succumbing to SARS (Chan-Yeung and Yu, 2003). During that time he unwittingly infected several others that in turn gave rise to a series of outbreaks (Centers for Disease Control and Prevention, 2003). Through sometimes several generations of transmissions, this event carried the virus to different Hong Kong hospitals and communities as well as to Vietnam, Singapore, Canada, the United States of America, and beyond to a total of 30 countries and areas of the world (World Health Organization, 2003d). The virus travelled in infected humans and was passed on over several generations, as reflected in the genetic relatedness of isolates from these countries. Although China was late in admitting it, the SARS-CoV had unsurprisingly also been spread within mainland China; in the end, the worst affected area was the capital, Beijing, with 2521 cases in total, which surpasses the count for Guangdong with 1512 by far (World Health Organization Western Pacific Region, country office China: http://www.wpro.who.int/wr/chn/chn_sars.asp).

The incubation period of SARS is short, ranging from 2 to 16 days. Large studies consistently noted a median incubation period of 6 days (Booth et al., 2003; Lee et al., 2003; Tsang et al., 2000). However, the time from exposure to the onset of symptoms may vary considerably (Donnelly et al., 2003). The WHO continues to conclude that the current best estimate of the maximum incubation period is 10 days (WHO Update 49—SARS case fatality ratio, incubation period, http://www.who.int/csr/sars/archive/2003_05_07a/en/).

Based on the latest data, the case fatality ratio is estimated to be <1% in persons aged 24 years or younger, 6% in persons aged 25–44 years, 15% in persons aged 45–64 years, and greater than 50% in persons aged 65 years and older (Donnelly et al., 2003; WHO Update 49—SARS case fatality ratio, incubation period, http://www.who.int/csr/sars/archive/2003_05_07a/en/). Pregnant women with SARS appear to have a worse prognosis and a higher mortality. Therefore, early delivery or termination of pregnancy should be considered in those who are seriously ill with SARS. For women who are relatively well with SARS, however, there seems to be no reason for elective preterm delivery, such as reducing the risk of materno-fetal transmission (Wong et al., 2003a).

Compared with adults and teenagers, SARS seems to take a less aggressive clinical course in younger children (Hon et al., 2003). Multivariable analysis showed that the presence of diabetes, advanced age or other comorbid conditions were independently associated with a poor outcome (Booth et al., 2003; Donnelly et al., 2003; Fowler et al., 2003).

At the present time, with no new cases—apart from the isolated laboratory-acquired one—having been reported since 15 June 2003, SARS-CoV has apparently been driven out of

the human population (World Health Organization, 2003d). In the meantime, WHO has issued a consensus document on SARS epidemiology (WHO Department of Communicable Disease Surveillance and Response, 2003).

5. Transmission

The pattern of geographic spread of SARS was similar across all affected areas: typically, a patient with SARS arrived from a previously affected area, was not identified as such when hospitalised, and thus infected health care workers, other patients and hospital visitors; these then infected their close contacts, and then the disease moved into the larger community (Hawkey et al., 2003).

The virus seems to be spread predominantly by respiratory droplets over a relatively close distance (Dwosh et al., 2003), however, at least under some circumstances direct and indirect contact with respiratory secretions, faeces or animal vectors may also lead to transmission (Hong Kong Department of Health, 2003; WHO Environmental Health Team, 2003; Tsang et al., 2000; Ng, 2003). Shedding of SARS-CoV in faeces and urine also occurs but its significance is unknown. The duration of infectivity is still unclear. Faecal shedding seems to last for several weeks; this however does not necessarily mean that there is sufficient excretion of infectious viral particles to infect other individuals (Peiris et al., 2003a). Practising stringent droplets and contact precaution significantly reduces the risk of infection after exposure to patients with SARS. Therefore, the protective role of the mask suggests that the main route of transmission is by droplets (Seto et al., 2003). SARS-CoV spreads more efficiently in sophisticated hospital settings. Evidence suggests that certain procedures, such as intubation under difficult circumstances and use of nebulizers increase the risk of infection (Chan-Yeung et al., 2003).

The only case of laboratory-acquired SARS-CoV transmission so far occurred in Singapore in September 2003. It involved a postgraduate who worked in a virology laboratory. Subsequent investigation showed inappropriate laboratory standards (WHO Severe acute respiratory syndrome (SARS) in Singapore—update 2, http://www.who.int/csr/don/2003_09_24/en/); no secondary transmission arose from this case. It demonstrates the need for optimal biosafety precautions in laboratories working with SARS-CoV; these constitute the only places on earth where SARS-CoV is currently known to still exist and might be at the source of a re-emergence.

Blood transfusions or administration of blood products have not been implicated in transmission anywhere. This is despite the demonstration of viraemia during the clinical phase of the illness, albeit at low to moderate titres (Drosten et al., 2003a). Nevertheless, the potential of blood-borne transmission led to the early implementation of measures such as exclusion of possibly exposed individuals from the donor pool.

The SARS-CoV is only moderately transmissible. A single infectious case will infect about three secondary cases (Lipsitch et al., 2003; Riley et al., 2003). Nevertheless, the clusters of cases in hotel and apartment buildings in Hong Kong show that transmission of the SARS-CoV can be extremely efficient. Attack rates in excess of 50% have been reported. One common observation in various areas was the occurrence of so-called “super-spreaders”, i.e. individuals that transmit the infection to at least ten others (World Health Organization, 2003b). These “super-spreaders” were mostly very ill and often died from SARS, and invariably serious lapses in infection control precautions had occurred during their management. So far there is no evidence that differences in virus strains may be responsible for the “super-spreader” phenomenon. There is also no firm evidence suggesting that subsequent transmissions led to clinically less severe illness, possibly through attenuation of the virus. It is also unclear why children are relatively under-represented amongst SARS cases, and why on average they seem to suffer less severe SARS illness.

Studies on the stability of the new SARS-CoV demonstrate the virus is more stable at room temperature than the previously known human coronaviruses (Sizun et al., 2000). The virus has been shown to survive for up to 48 hours on plastic surfaces and up to 4 days in diarrhoea. Nevertheless the virus loses infectivity after exposure to different commonly used disinfectants and fixatives. Heat exposure at 56 °C quickly reduces infectivity (World Health Organization (WHO): First data on stability and resistance of SARS-CoV compiled by members of WHO laboratory network available at http://www.who.int/csr/sars/survival_2003_05_04/en/index.html).

6. Diagnosis

As defined by the WHO, a person is suspected to have SARS if she has documented high fever (>38 °C), plus cough or breathing difficulty, and has been in contact with a person believed to have had SARS, or has a history of travel to or stay in a geographic area where documented transmission of the illness has occurred, during the 10 days prior to onset of symptoms (“suspect case”). A suspect case with infiltrates consistent with pneumonia or respiratory distress syndrome (RDS) by chest X-ray is reclassified as a probable case. The revised case definition as of 1 May 2003, (see: <http://www.who.int/csr/sars/casedefinition/en/>) for the first time includes virus-specific laboratory results: a suspect case that tests positive for SARS-CoV in one or more assays should also be reclassified as probable.

While recommendations have been issued for the use of laboratory methods for SARS-CoV (see: <http://www.who.int/csr/sars/labmethods/en/>), there are, however, at present no defined criteria for negative SARS-CoV test results to reject a diagnosis of SARS. Given the rather low shedding of SARS-CoV from the upper respiratory tract (Drosten et al.,

2003a), and the insufficient sensitivity of presently available laboratory methods, premature exclusion on the basis of negative test results may lead to tragic consequences. Positive laboratory test results for other agents able to cause atypical pneumonia may serve as exclusion criteria; according to the case definition, a case should be excluded if an alternative diagnosis can fully explain the illness. Nevertheless, the possibility of dual infection must not be ruled out completely.

The required epidemiological linkage has repeatedly proven to be problematic. Until an area is recognised as being affected, only imported cases fulfil the criteria for SARS but not those who became infected locally through contact with unrecognised cases. Thus, precious time may be lost until cases are recognised and appropriate measures taken. A thorough analysis showed that the existing WHO criteria lack sensitivity in the pre-hospital setting (Rainer et al., 2003). This again may be problematic as it may delay appropriate management of SARS cases.

7. Laboratory methods

The human coronaviruses known prior to March 2003 are difficult to propagate in cell cultures. Their disease associations—generally mild respiratory illness (“common cold”), enteric and rarely possibly neurological disease—led to their widespread neglect in medical virology; only few groups worked on various scientific aspects, and very few laboratories offered routine diagnostic tests, mainly by PCR. SARS-CoV, on the other hand, is readily propagated in vitro and may also be detected by PCR and indirectly through antibody testing. Nevertheless, and despite considerable progress in this field, much remains to be done until laboratory tests become a useful tool for the management of SARS cases (World Health Organization Multicentre Collaborative Network for Severe Acute Respiratory Syndrome Diagnosis, 2003).

The presence of the infectious virus can be detected by inoculating suitable cell cultures (e.g., Vero cells) with patient specimens (such as respiratory secretions, blood or stool) and propagating the virus in vitro. Once isolated, the virus must be identified as SARS-CoV using further tests. According to international consensus, such work has to be performed under biosafety level (BSL) three conditions.

SARS-CoV-specific RNA can be amplified from various clinical specimens, especially in respiratory secretions and in stool, by PCR. High concentrations of viral RNA of up to 100 million molecules per millilitre were found in sputum. Viral RNA was also detected, albeit at extremely low concentrations, in plasma during the acute phase and in faeces during the late convalescent phase, suggesting that virus may be shed in faeces for prolonged periods of time (Drosten et al., 2003a). A commercial real-time RT-PCR test kit containing primers and positive and negative controls developed by the Bernhard Nocht Institute (<http://www.bni-hamburg.de/>) is available (<http://www.artus-biotech.de/>). An

inactivated standard preparation is also available for diagnostic purposes through the European network for imported viral infections (ENIVD; <http://www.enivd.de>). ENIVD is also preparing for an international external quality assessment scheme for SARS-CoV assays. The existing PCR tests cannot rule out, with certainty, the presence of SARS-CoV in patients (Poon et al., 2003). On the other hand, contamination of samples in laboratories performing PCR may lead to false-positive results, unless appropriate precautions are taken.

Various methods were developed for the detection of antibodies produced in response to infection with SARS-CoV by probably virtually all patients. The first type of antibody test to be employed was the immunofluorescence assay (IFA). Using cells infected with the patient's own virus isolate and an antihuman IgG:FITC conjugate, we were able to demonstrate specific seroconversion in the two Frankfurt SARS patients (Drosten et al., 2003a; Fig. 1). An enzyme-linked immunosorbent assay (ELISA) was developed that detects antibodies in the serum of SARS patients and reliably yields positive results at around day 21 after the onset of illness (World Health Organization Multicentre Collaborative Network for Severe Acute Respiratory Syndrome Diagnosis, 2003). The neutralisation test (NT) assesses and quantifies, by means of titration, the ability of patient sera to neutralise the infectivity of SARS-CoV on cell culture; the NT titre may therefore be correlated to clinical immunity although this has yet to be demonstrated. However, NT is limited to institutions with BSL-3 facilities. The only antibody test commercially available so far is an IFA which yields a positive result from about day 10 after the onset of illness (Euroimmun, Lübeck, Germany). As the diagnosis of SARS is based entirely on a set of clinical and epidemiological criteria so far, reported case numbers are likely to include a substantial number of non-SARS patients. Therefore, recovered patients should be tested systematically for specific SARS-CoV antibody reactivity to confirm their diagnoses in retrospect and thus allow a better understanding of epidemiological and other features (Li et al., 2003).

Although electron microscopy has an important role in the rapid diagnosis of infectious agents in emergent situations (Hazelton and Gelderblom, 2003), it has provided only circumstantial evidence in the case of SARS. When a virus-like agent was first visualised in clinical material from SARS cases by electron microscopy, its classification was ambiguous; it later turned out to be human metapneumovirus (Poutanen et al., 2003). Even in cases in whom coronavirus-like particles were detected, these could not be distinguished from the 'classic' human coronaviruses.

8. Antiviral treatment

No specific treatment recommendations can be made at this time. Primary measures include isolation and the implementation of stringent infection control measures to

effectively prevent further transmissions. Empiric therapy should include coverage for organisms associated with any community-acquired pneumonia of unclear aetiology, including agents with activity against both typical and atypical respiratory pathogens. Treatment choices may be influenced by severity of the illness. Oxygen supplementation is often necessary, and severe cases seem to do better if intensive care including artificial respiration is commenced early (So et al., 2003).

Efforts are underway at various institutions to assess potential anti-SARS-CoV agents in vitro. Ribavirin, a "broad spectrum" agent active against various RNA viruses has also been used clinically in SARS patients (Koren et al., 2003), but seems to lack an in vitro effect (Cinatl et al., 2003a).

Corticosteroids were widely used in SARS patients, particularly in China. The rationale for their administration is the observation that tissue changes suggest that part of the lung damage is due to cytokines induced by the virus (Peiris et al., 2003a). Some clinical reports also underline their usefulness (Zhao et al., 2003).

Other therapies are being explored, such as convalescent plasma (Wong et al., 2003b) or normal human immunoglobulin which may be beneficial through an immunomodulatory effect or through acting against agents causing secondary infections. Preliminary clinical data suggest that protease inhibitors used for anti-HIV therapy, lopinavir and nelfinavir (Yamamoto N, personal communication), might have some efficacy, both as initial therapy and in the rescue setting. Hong Kong researchers reported at the WHO Global Conference on SARS in Kuala Lumpur in June 2003 that SARS patients treated with Kaletra (lopinavir with low-dose ritonavir) plus ribavirin experienced a 50% reduction in death rate.

While efforts are underway to develop more targeted anti-SARS-CoV approaches, broad screening of available substances in vitro has led to some potentially important clues. Recently glycyrrhizin, a compound found in liquorice roots (*Glycyrrhiza glabra* L.), was reported to have good in vitro activity against SARS-CoV (Cinatl et al., 2003a). The mechanism of glycyrrhizin's activity against SARS-CoV is unclear. Glycyrrhizin has previously been used to treat patients with HIV-1 and chronic hepatitis C virus (Liu et al., 2001). Interestingly, this compound may be contained in some of the herbal preparations widely used in SARS patients in China as part of traditional Chinese medicine (Lin et al., 2003). Furthermore, interferons inhibit SARS-CoV in vitro. In a recent study (Cinatl et al., 2003b), interferon β was more potent than interferon α or γ . Therefore, it could become a drug of choice in future, alone or in combination with other antiviral drugs.

9. Conclusions and outlook

The rapid success in identifying the causative agent of SARS results from a collaborative effort—rather than a

competitive approach—by high-level laboratory investigators making use of all available techniques, from cell culture through electron microscopy (Hazelton and Gelderblom, 2003) to molecular techniques, in order to identify a novel agent. Hopefully this approach, coordinated by WHO, will serve as a model for future instances of emerging infections that will undoubtedly take place (Ludwig et al., 2003).

Despite the exemplary efforts that led to the identification of the causative novel coronavirus and allowed enormous knowledge about it to be accumulated within only a few months, it is maybe surprising that this success in terminating the outbreak has to be attributed to “old-fashioned” measures such as rapid and strict isolation of suspect cases and thorough contact tracing (World Health Organization, 2003c); one is left wondering whether the same might also have been achieved without knowledge of the aetiology. Thanks to an internationally well-coordinated and in most cases timely and determined response no new cases of SARS have been notified since 15 June 2003. Several countries reported SARS cases imported from areas reporting outbreaks but did not experience secondary transmission; likewise, Vietnam was the first country to demonstrate that—through a combination of early detection and public alert followed by decisive public health action and often heroic efforts by individuals—further transmission could be curtailed (Reilley et al., 2003).

The absence of new clinical cases worldwide suggests that SARS-CoV no longer circulates within the human population; however, the possibility of clinically “silent” infections or of long-term virus carriers cannot be ruled out completely. Furthermore, the origin of the agent remains obscure; SARS-CoV or a closely related virus persisting in a hitherto unidentified animal reservoir may yet again cross the species barrier and lead to human outbreaks. Numerous questions relating to the epidemiology of SARS have yet to be answered (Normile and Enserink, 2003; Breiman et al., 2003).

At the time of writing (October 2003) it is completely uncertain whether SARS will ever reappear. It is unclear whether seasonal recurrences may occur. In southern China, unlike Europe and North America, the annual influenza peak incidence is from March to July (Huang et al., 2001); thus, it shows a similar epidemic curve as the SARS outbreak in 2003 (Enserink, 2003b).

The advent of the next ‘flu season will pose considerable problems, given the lack of reliable laboratory methods for the early diagnosis of SARS. The case definitions, too, will need to be adjusted to a world without SARS; in theory, new cases are “impossible” as the criterion of an epidemiological link cannot be fulfilled. Precious time may therefore be lost before a reappearance is detected. Vigilance for SARS must clearly be maintained (see: Alert, verification and public health management of SARS in the post-outbreak period—14 August 2003—rationale for continued vigilance for SARS; <http://www.who.int/csr/sars/postoutbreak/en/>).

For this purpose, WHO has defined three geographical zones according to their presumed risk for a SARS recurrence: a potential zone of re-emergence, comprising Guangdong and other areas where animal-to-human of SARS-CoV might occur; nodal areas, comprising Hong Kong, Vietnam, Singapore, Canada, and Taiwan, with sustained local transmission in spring 2003 or entry of numerous persons from the potential zone of re-emergence; and low risk areas. SARS-related vigilance should be staged according to the zone in which a particular area is situated; for low risk areas, surveillance should be for clusters of “alert” cases among health care workers, other hospital staff, patients and visitors in the same health care unit. A SARS Alert is defined as two or more health care workers or hospital-acquired illness in at least three individuals (health care workers and/or other hospital staff and/or patients and/or visitors) in the same unit fulfilling the clinical case definition of SARS and with onset of illness in the same 10-day period. In the other zones, this should be supplemented by enhanced surveillance, plus special studies for SARS-CoV infections in animal and human populations in the potential zone of re-emergence.

Besides improving existing detection assays—for instance, PCR methods based on the amplification of the nucleoprotein gene may be intrinsically more sensitive, due to the coronaviral transcription strategy (Kuiken et al., 2003), and thus be valuable for early diagnosis—further laboratory research needs to include detailed physico-chemical analysis of SARS-CoV proteins to allow the development of novel compounds based on targeted drug design (Anand et al., 2003). Although an effective vaccine cannot be expected to be available soon, the relative ease with which SARS-CoV can be propagated in vitro is clearly helpful. A suitable animal model for SARS may be available in the form of cynomolgus macaques (*Macaca fascicularis*) (Kuiken et al., 2003). While the availability of vaccines against animal coronaviruses, such as avian infectious bronchitis virus, transmissible gastroenteritis coronavirus of pigs, and feline infectious peritonitis virus, is encouraging, the obvious lack of protective immunity in humans after infection with HCoV OC43 and 229E is not. There is also currently no commercial veterinary vaccine to prevent respiratory coronavirus infections, except for infectious bronchitis virus infections in chickens. Further research is also urgently needed to determine whether immune pathogenesis plays a rôle in SARS or whether immune enhancement may occur, the chances of developing an effective and safe vaccine therefore remain uncertain. It is to be hoped that after such an encouraging start in an atmosphere of open collaboration and mutual trust, progress in SARS-CoV research will not be impeded by patent matters (Gold, 2003).

References

- Anand K, Ziebuhr J, Wadhwani P, Mesters JR, Hilgenfeld R. Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. *Science* 2003;300:1763–7.

- Booth CM, Matukas LM, Tomlinson GA, Rachlis AR, Rose DB, Dwosh HA, et al. Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area. *JAMA* 2003;289:2801–9.
- Breiman RF, Evans MR, Preiser W, Maguire J, Schnur A, Bekedam H, MacKenzie JS. Role of China in the Quest to Define and Control Severe Acute Respiratory Syndrome. *Emerg Infect Dis* 2003;9(9):1037–41.
- Cavanagh D. Coronaviruses and Toroviruses. In Zuckerman, AJ, Bantvala, JE, Pattison, JR, editors. Principles and practice of clinical virology. 2000. Wiley, Chichester.
- Centers for Disease Control and Prevention. Update: Outbreak of severe acute respiratory syndrome—worldwide, 2003. *MMWR Morb Mortal Wkly Rep* 2003;52:241–6,248.
- Chan-Yeung M, Yu WC. Outbreak of severe acute respiratory syndrome in Hong Kong special administrative region: case report. *BMJ* 2003;326:850–2.
- Chan-Yeung M, Seto WH, Sung JJ. Severe acute respiratory syndrome: patients were epidemiologically linked. *BMJ* 2003;326:1393.
- Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. *Lancet* 2003a;361:2045–6.
- Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Treatment of SARS with human interferons. *Lancet* 2003b;362:293–4.
- Cyranoski D, Abbott A. Virus detectives seek source of SARS in China's wild animals. *Nature* 2003;423:467.
- Donnelly CA, Ghani AC, Leung GM, Hedley AJ, Fraser C, Riley S, et al. Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in Hong Kong. *Lancet* 2003;361:1761–6.
- Drosten C, Gunther S, Preiser W, van der WS, Brodt HR, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med* 2003a;348:1967–76.
- Drosten C, Preiser W, Günther S, Schmitz H, Doerr HW. Severe acute respiratory syndrome: identification of the etiological agent. *Trends Mol Med* 2003b;9:325–7.
- Dwosh HA, Hong HH, Austgarden D, Herman S, Schabas R. Identification and containment of an outbreak of SARS in a community hospital. *CMAJ* 2003;168:1415–20.
- Enserink M. SARS in China. China's missed chance. *Science* 2003a;301:294–6.
- Enserink M. SARS in China. The big question now: will it be back? *Science* 2003b;301:299.
- Fouchier RA, Kuiken T, Schutten M, van Amerongen G, van Doornum GJ, van den Hoogen BG, et al. Aetiology: Koch's postulates fulfilled for SARS virus. *Nature* 2003;423:240.
- Fowler RA, Lapinsky SE, Hallett D, Detsky AS, Sibbald WJ, Slutsky AS, et al. Critically ill patients with severe acute respiratory syndrome. *JAMA* 2003;290:367–73.
- Gold ER. SARS genome patent: symptom or disease? *Lancet* 2003;361:2002–3.
- Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, Cheung CL, Luo SW, Li PH, Zhang LJ, Guan YJ, Butt KM, Wong KL, Chan KW, Lim W, Shortridge KF, Yuen KY, Peiris JS, Poon LL. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science* 2003;302(5643):276–8. Epub 2003 Sep 04.
- Hawkey PM, Bhagani S, Gillespie SH. Severe acute respiratory syndrome (SARS): breath-taking progress. *J Med Microbiol* 2003;52:609–13.
- Hazleton PR, Gelderblom HR. Electron microscopy for rapid diagnosis of infectious agents in emergent situations. *Emerg Infect Dis* 2003;9:294–303.
- Holmes KV. SARS coronavirus: a new challenge for prevention and therapy. *J Clin Invest* 2003;111:1605–9.
- Hon KL, Leung CW, Cheng WT, Chan PK, Chu WC, Kwan YW, et al. Clinical presentations and outcome of severe acute respiratory syndrome in children. *Lancet* 2003;361:1701–3.
- Hong Kong Department of Health. Outbreak of severe acute respiratory syndrome (SARS) at Amoy Gardens, Kowloon Bay, Hong Kong. Main findings of the investigation. Published online 17 April 2003. http://www.info.gov.hk/info/ap/pdf/amoy_e.pdf.
- Huang P, Ni H, Shen G, Zhou H, Peng G, Liu S. Analysis of the 1991–2000 influenza epidemic in Guangdong Province, China. *Southeast Asian J Trop Med Public Health* 2001;32(4):787–90.
- Koren G, King S, Knowles S, Phillips E. Ribavirin in the treatment of SARS: a new trick for an old drug? *Can Med Assoc J* 2003;168:1289–92.
- Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* 2003;348:1953–66.
- Kuiken T, Fouchier RA, Schutten M, Rimmelzwaan GF, van Amerongen G, van Riel D, et al. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. *Lancet* 2003;362:263–70.
- Lai MM, Cavanagh D. The molecular biology of coronaviruses. *Adv Virus Res* 1997;48:1–100.
- Lee N, Hui D, Wu A, Chan P, Cameron P, Joynt GM, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. *N Engl J Med* 2003;348:1986–94.
- Li G, Chen X, Xu A. Profile of specific antibodies to the SARS-associated coronavirus. *N Engl J Med* 2003;349:508–9.
- Lin L, Han Y, Yang ZM. Clinical observation on 103 patients of severe acute respiratory syndrome treated by integrative traditional Chinese and Western Medicine. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 2003;23:409–13.
- Lipsitch M, Cohen T, Cooper B, Robins JM, Ma S, James L, et al. Transmission dynamics and control of severe acute respiratory syndrome. *Science* 2003;300:1966–70.
- Liu JP, Manheimer E, Tsutani K, Gluud C. Medicinal herbs for hepatitis C virus infection. *Cochrane Database Syst Rev* 2001;4:CD003183.
- Ludwig B, Kraus FB, Allwinn R, Doerr HW, Preiser W. Viral zoonoses—a threat under control? *Intervirology* 2003;46:71–8.
- Marra MA, Jones SJ, Astell CR, Holt RA, Brooks-Wilson A, Butterfield YS, et al. The Genome sequence of the SARS-associated coronavirus. *Science* 2003;300:1399–404.
- Ng SKC. Possible role of an animal vector in the SARS outbreak at Amoy Gardens. *Lancet* 2003;362:570–2.
- Normile D, Enserink M. SARS in China. Tracking the roots of a killer. *Science* 2003;301:297–9.
- Peiris JS, Chu CM, Cheng VC, Chan KS, Hung IF, Poon LL, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet* 2003a;361:1767–72.
- Peiris JS, Lai ST, Poon LL, Guan Y, Yam LY, Lim W, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* 2003b;361:1319–25.
- Pensaert M, Callebaut P, Vergote J. Isolation of a porcine respiratory, non-enteric coronavirus related to transmissible gastroenteritis. *Vet Q* 1986;8:257–61.
- Poon LL, Wong OK, Chan KH, Luk W, Yuen KY, Peiris JS, Guan Y. Rapid diagnosis of a coronavirus associated with severe acute respiratory syndrome (SARS). *Clin Chem* 2003;49:953–5. Erratum in: *Clin Chem* 2003 Jul; 49 (7): 1234.
- Poutanen SM, Low DE, Henry B, Finkelstein S, Rose D, Green K, et al. National microbiology laboratory, Canada; Canadian severe acute respiratory syndrome study team. Identification of severe acute respiratory syndrome in Canada. *N Engl J Med* 2003;348:1995–2005.
- Rainer TH, Cameron PA, Smit D, Ong KL, Hung AN, Nin DC, et al. Evaluation of WHO criteria for identifying patients with severe acute respiratory syndrome out of hospital: prospective observational study. *BMJ* 2003;326:1354–8.
- Reilley B, van Herp M, Sermand D, Dentico N. SARS and Carlo Urbani. *N Engl J Med* 2003;348:1951–2.
- Riley S, Fraser C, Donnelly CA, Ghani AC, Abu-Raddad LJ, Hedley AJ, et al. Transmission dynamics of the etiological agent of SARS in Hong Kong: impact of public health interventions. *Science* 2003;300:1961–6.

- Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, Icenogle JP, et al. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science* 2003;300:1394–9.
- Ruan YJ, Wei CL, Ee AL, Vega VB, Thoreau H, Su ST, et al. Comparative full-length genome sequence analysis of 14 SARS coronavirus isolates and common mutations associated with putative origins of infection. *Lancet* 2003;361:1779–85.
- Seto WH, Tsang D, Yung RW, Ching TY, Ng TK, Ho M, et al. Effectiveness of precautions against droplets and contact in prevention of nosocomial transmission of severe acute respiratory syndrome (SARS). *Lancet* 2003;361:1519–20.
- Sizun J, Yu MW, Talbot PJ. Survival of human coronaviruses 229E and OC43 in suspension and after drying on surfaces: a possible source of hospital-acquired infections. *J Hosp Infect* 2000;46:55–60.
- Snijder EJ, Bredenbeek PJ, Dobbe JC, Thiel V, Ziebuhr J, Poon LL, Guan Y, Rozanov M, Spaan WJ, Gorbalenya AE. Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. *J Mol Biol* 2003;331(5):991–1004.
- So LK, Lau AC, Yam LY, Cheung TM, Poon E, Yung RW, et al. Development of a standard treatment protocol for severe acute respiratory syndrome. *Lancet* 2003;361:1615–7.
- Thiel V, Ivanov KA, Putics A, Hertzog T, Schelle B, Bayer S, Weissbrich B, Snijder EJ, Rabenau H, Doerr HW, Gorbalenya AE, Ziebuhr J. Mechanisms and enzymes involved in SARS coronavirus genome expression. *J Gen Virol* 2003;84:1305–15.
- Tsang KW, Ho PL, Ooi GC, Yee WK, Wang T, Chan-Yeung M, et al. A cluster of cases of severe acute respiratory syndrome in Hong Kong. *N Engl J Med* 2000;348:1977–85.
- Tsui SK, Chim SS, Lo YM. Chinese University of Hong Kong Molecular SARS Research Group. Coronavirus genomic-sequence variations and the epidemiology of the severe acute respiratory syndrome. *N Engl J Med* 2003;349:187–8.
- WHO Department of Communicable Disease Surveillance and Response: Consensus document on the epidemiology of severe acute respiratory syndrome (SARS). World Health Organization: Geneva, 2003. WHO/CDS/CSR/GAR/2003. 11
- WHO Environmental Health Team: Report on Amoy Gardens. World Health Organization Regional Office for the Western Pacific. 16 May 2003. Available at <http://www.info.gov.hk/info/ap/who-amoye.pdf>.
- Wong SF, Chow KM, de Swiet M. Severe acute respiratory syndrome and pregnancy. *BJOG* 2003a;110:641–2.
- Wong VW, Dai D, Wu AK, Sung JJ. Treatment of severe acute respiratory syndrome with convalescent plasma. *Hong Kong Med J* 2003b;9:199–201.
- World Health Organization (WHO). WHO multicentre collaborative networks for severe acute respiratory syndrome (SARS) diagnosis. *Wkly Epidemiol Rec* 2003a;78:121–2.
- World Health Organization (WHO). Severe acute respiratory syndrome—Singapore, 2003. *Wkly Epidemiol Rec* 2003b;78:157–62.
- World Health Organization (WHO). Severe acute respiratory syndrome (SARS): over 100 days into the outbreak. *Wkly Epidemiol Rec* 2003c;78:217–20.
- World Health Organization (WHO). Chronology of travel recommendations, areas with local transmission. *Wkly Epidemiol Rec* 2003d;78:258–9.
- World Health Organization Multicentre Collaborative Network for Severe Acute Respiratory Syndrome Diagnosis. A multicentre collaboration to investigate the cause of severe acute respiratory syndrome. *Lancet* 2003;361:1730–3.
- Zhao Z, Zhang F, Xu M, Huang K, Zhong W, Cai W, et al. Description and clinical treatment of an early outbreak of severe acute respiratory syndrome (SARS) in Guangzhou, PR China. *J Med Microbiol* 2003;52:715–20.