

# Severe acute respiratory syndrome: five years on

It is perhaps hard to believe that it is almost five years ago since the world woke up from the nightmare that was severe acquired respiratory syndrome. However, much work has been undertaken since 2003, as Richard A Collins explains.

'The first PCR assay for detection of the SARS-CoV genetic material was useful in the early stages of infection, but produced many false-negative results'

On 23 June 2003 the World Health Organization (WHO) declared Hong Kong free of severe acute respiratory syndrome (SARS), formally ending a traumatic 121-day period in which the world encountered the first potentially pandemic disease of the 21st century.

At the conclusion of the epidemic in Hong Kong, there had been 1755 cases of SARS, with 300 deaths. Among those who died were eight healthcare workers, six of whom were from public hospitals and two were private doctors.

Now, after five years, it is time to look at what we have learned about this new zoonotic disease, to assess the possibility for a renewed outbreak, and examine the lessons learned about SARS and infection control so that we can apply them to similar diseases.

## IN THE BEGINNING

On 22 February 2003, a resident of mainland China who was visiting Hong Kong was admitted to hospital with a case of "severe community-acquired pneumonia". Health authorities in Hong Kong had been monitoring such cases since 13 February, following media reports of an outbreak of an unusually severe influenza-like illness in southern China that had occurred in late January.

In early March, outbreaks of atypical pneumonia occur in health workers at several hospitals across Hong Kong. On 12 March, WHO issued a global alert about cases of acute respiratory illness in Vietnam, Hong Kong, and Guangdong Province in China. It had an unknown aetiology that appeared to place health workers at high risk.<sup>1</sup>

On 15 March, WHO issued an emergency travel advisory notice, which named the illness as severe acute respiratory syndrome and listed the main syndromes and signs.<sup>2</sup> However, there was no recommendation to restrict travel to any destination. The disease continued to spread among the local population, with the most notable outbreak occurring at a large (19,000 residents) public housing estate that resulted in 329 infections and 42 deaths.

Retrospective data indicate that the patient admitted to hospital in Hong Kong on 22 February was the index case for subsequent SARS outbreaks in Canada, Singapore and Vietnam, as a result of infecting 16 fellow residents at his hotel.

## IDENTIFICATION OF THE CAUSATIVE AGENT

During the outbreak a variety of pathogens were suspected as causing SARS, including avian influenza A (H5N1), *Chlamydia pneumoniae*, *Legionella pneumophila* and *Mycoplasma pneumoniae*. As the outbreak escalated, efforts to characterise the pathogen responsible intensified. Examination of tissue samples from suspected or probable SARS patients revealed a previously unrecognised

'Initially, avian influenza A, *Chlamydia pneumoniae*, *Legionella pneumophila* and *Mycoplasma pneumoniae* were all potential suspects'

coronavirus and a human metapneumovirus (a paramyxovirus). Around the world, 13 laboratories in 10 countries raced to identify the causative agent of SARS. On 8 April, a new coronavirus was identified tentatively as the causative agent of SARS.<sup>3</sup> The virus was isolated from two out of 50 patients with SARS included in the analysis. Subsequently, 45 out of 50 patients tested positive by serological and polymerase chain reaction (PCR) tests based on the new virus.

Evidence for a new coronavirus was confirmed on 10 April when genetic characterisation of a virus isolated from SARS patients indicated that it was only distantly related to known coronaviruses (50–60% nucleotide identity).<sup>4</sup> High concentrations of viral RNA (up to 100 million molecules/mL) were found in sputum.

On 14 April, the US Centers for Disease Prevention and Control (CDC) confirmed the genome sequence of the new coronavirus, thus paving the way for a series of rapid and accurate PCR-based molecular assays.<sup>5</sup>

## DIAGNOSIS

By early April 2003, WHO reported that three diagnostic tests for SARS were available but that all had limitations. An enzyme-linked immunosorbent assay (ELISA) detected antibodies against the SARS coronavirus (SARS-CoV) reliably but only from about day 20 following the onset of clinical symptoms. Therefore, it could not be used to detect cases at an early stage before they had a chance to spread the infection to others. The second

test, an immunofluorescence assay, detected antibodies reliably from 10 days after infection, but it was a demanding and comparatively slow test that required the growth of virus in cell culture.

The available PCR assay for detection of the SARS-CoV genetic material was useful in the early stages of infection, but it produced many false-negatives. Thus, many people who carried the virus may not have been detected, creating a dangerous sense of false security for a virus that was known to spread easily in close person-to-person contact. However, armed with the complete SARS-CoV genome, more sensitive PCR tests were developed quickly.

The SARS-CoV could be recovered easily from nasopharyngeal aspirate and stool samples up to 21 days after the onset of symptoms. In contrast, viral positivity in urine samples was only 50% on day 10 and 21% on day 21.<sup>6</sup>

### TREATMENT

During the early stages of the epidemic, before the causative agent was identified, treatment was given as clinically indicated. No clinical improvement was observed with antibiotics using, for example, combined therapy with  $\beta$ -lactams and macrolides.<sup>7</sup> The observation that the antiviral agent ribavirin, given intravenously in combination with high-dose corticosteroids, may have been responsible for some clinical improvement observed in critically ill patients in Hong Kong<sup>8</sup> was confirmed in other studies.<sup>7</sup> Intensive and good supportive care, with or without antiviral agents, also improved prognosis.<sup>8</sup>

Despite the lack of antibiotic efficacy against the causative agent, prophylactic antibiotic therapy on admission to hospital continued to be recommended to prevent secondary bacterial infection. In the meantime, convalescent plasma was used successfully to treat a case of SARS.<sup>9</sup>

A prospective study of the clinical, haematological, radiological and microbiological findings of 75 patients managed using the Hong Kong Hospital Authority's standardised treatment protocol of ribavirin and corticosteroid indicated that although fever and pneumonia initially responded to treatment in the majority of cases (>85%), the fever recurred, often with watery diarrhoea and radiological deterioration of the lungs.<sup>6</sup> These and other data suggested that the deterioration was not related to uncontrolled viral replication but to immunopathological damage.<sup>6</sup>

Eventually, a standard treatment protocol for adult SARS patients was reported.<sup>10</sup> A series of 31 patients received antibacterial agents and a combination of ribavirin and methylprednisolone. A standard dose regime was finalised with the first 11 patients, and this included pulse methylprednisolone. One patient recovered on antibacterial treatment alone, 17 showed a rapid and sustained response, and 13 achieved improvement



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**Fig 1.** Coloured transmission electron micrograph of SARS virus particles (red) in host cells (original magnification x56,000).

with step-up or pulse methylprednisolone. Four patients required a short period of non-invasive ventilation. No patient required intubation or mechanical ventilation. No mortality or treatment morbidity was seen in this series of patients.<sup>10</sup>

To date, there remains no formally recommended treatment for SARS.

### PREVENTION

Severe acquired respiratory syndrome is a viral respiratory disease spread by contaminated droplets or aerosols. Virus particles (Fig 1) are found in large quantities in respiratory secretions and also in faeces and urine.<sup>6</sup> Persons coming in contact with suspected SARS patients should wear face masks and exercise good personal hygiene. Caution should be exercised by healthcare staff when intubating patients or in using nebulisers because of the risk of contacting infected secretions and generating infectious aerosols.

Much work has been done since the SARS outbreak to produce an effective vaccine. The

goal of any vaccine is to prime the immune system to respond quickly and effectively should the vaccinated person be exposed to the pathogen against which the vaccine has been produced. Experimental approaches have included inactivated whole virus,<sup>11</sup> live attenuated,<sup>12</sup> chimaeric,<sup>13</sup> subunit<sup>14</sup> and DNA vaccines.<sup>15-17</sup> The advantages and disadvantages of these approaches are shown in Table 1.

An ideal SARS vaccine should 1) elicit highly potent neutralising antibody responses against a broad spectrum of viral strains; 2)

**'Severe acquired respiratory syndrome originated in Southern China and was probably acquired by humans from exotic animals, especially civet cats'**

**Table 1.** Advantages and disadvantages of potential SARS vaccines.

Vaccine	Advantages	Disadvantages
Whole killed	SARS-CoV can be grown efficiently in cell culture. Requires less knowledge of pathogen biology than other vaccine types	Often requires multiple doses to elicit and sustain an effective immune response
	Whole virus particle includes many proteins (nucleocapsid, membrane, envelope and spike) that may serve as antigens to induce neutralising antibodies and protective responses	Production workers are at risk of infection when handling concentrated live SARS-CoV
	Virus can be inactivated easily (eg formaldehyde, UV light, $\beta$ -propiolactone)	Incomplete virus inactivation may cause SARS outbreaks among vaccinated populations
	Vaccines are relatively inexpensive to produce	Viral proteins may induce harmful immune or inflammatory responses
Live attenuated	Induces a more balanced immune response with longer-lasting immunity than do killed vaccines or immune globulins	Vaccine might contain adventitious agents (viruses and toxins derived from accidental contamination)
	Second in efficacy only to exposure to the unattenuated pathogen	The vaccine virus might cause illness in immunocompromised or otherwise healthy individuals, or may lose attenuation during manufacture or replication
	Vaccine can be administered in low doses (as it is self-replicating)	Not as stable as other types of vaccine
	Relatively inexpensive to manufacture	
Subunit	Very safe. Virtually impossible for live virus to reassemble, replicate or cause infection	Not as efficacious in terms of strength and duration of immune response as are live attenuated vaccines (usually only B-cell immunity is triggered)
	Less antigenic competition, as only a few viral components are included in the vaccine	Large amounts of antigen are needed to adequately trigger the immune system. Generally require strong adjuvants that may induce tissue reactions. Booster shots are usually required
	Can target the vaccine to the site where immunity is required	Purification of subunit material is relatively expensive
	Vaccine consists of defined molecular components, which in general elicit fewer adverse reactions and are easier to manufacture reproducibly than are whole cell vaccines	
	Antigens can be engineered to elicit unique neutralising antibodies that can be used to differentiate vaccinated subjects from those infected naturally	
DNA	Rapid and large-scale production is available at costs considerably lower than those for traditional vaccines	Long-term efficacy and safety of DNA vaccines have not yet been established in humans
	Responses include both cytotoxic T cells and antibodies, which persist for long periods of time	Some pathogens have outer capsids composed of polysaccharides. This limits the use of DNA vaccines because they cannot substitute for polysaccharide-based subunit vaccines
	Plasmid vectors can be constructed and produced quickly	
	DNA vaccines encoding several antigens or proteins can be delivered to the host in a single dose. A dose of 1 $\mu$ g plasmid can induce immune responses	
	Very temperature stable, making storage and transport much easier	
	No risk of infection for the recipient	
	Allows antigen presentation by both MHC class I and class II molecules	
	Are able to polarise T-cell help towards type 1 or type 2 responses.	
Allows the immune response to be focused on just one antigen of interest		

induce protection against infection and transmission; and 3) be safe by not inducing any infection-enhancing antibodies or harmful immune or inflammatory responses.<sup>18</sup>

The efficacy of live and attenuated vaccines to protect against coronavirus infection has been known for decades in the context of infectious bronchitis virus in a range of domestic animals (eg chickens, cattle, pigs, cats and dogs). Drawing on this prior experience, inactivated or live attenuated vaccines were obvious candidates for initial development. In general, prior to identifying the protein that contains the major neutralising epitopes, inactivated viruses may be used as first-generation vaccines because it is easy to generate whole killed virus particles. However, once the neutralising epitopes have been identified, the

inactivated virus vaccine should be replaced by vaccines based on fragments containing neutralising epitopes, as they are safer and more effective.<sup>18</sup>

After successive tests in mice,<sup>11</sup> rabbits<sup>19</sup> and rhesus monkeys<sup>20</sup> human trials on the safety and immunogenicity of an inactivated whole virus vaccine were reported.<sup>21</sup> Thirty-six subjects received two doses of 16 SARS-CoV units (SU) or 32 SU inactivated SARS-CoV vaccine, or a placebo control. Seroconversion reached 100% for both vaccine groups on day 42. The geometric mean titre of neutralising antibody peaked two weeks after the second vaccination, and then decreased four weeks later.

The third-generation DNA vaccines have many advantages over first-generation (whole killed, live attenuated) and second-generation

(chimaeric, subunit) vaccines (Table 1).<sup>22</sup> The first DNA vaccines to be investigated targeted the SARS-CoV spike protein (S) gene.<sup>15</sup> Soon, the nucleocapsid (N) gene,<sup>16</sup> membrane protein (M) gene<sup>17</sup> and envelope protein (E) genes<sup>17</sup> were targeted. Despite their obvious potential, DNA vaccines remain experimental and none have been licensed for use in humans to prevent any disease.

### POSSIBILITY OF RE-EMERGENCE

Severe acquired respiratory syndrome originated in Southern China and was probably acquired by humans from exotic animals, especially civet cats, captured in the wild and then kept at live-animal markets. The captured wildlife had probably been infected with a progenitor coronavirus derived from horseshoe bats.<sup>23</sup> This would then have

been amplified in the wildlife and transmitted to humans. These wild-animal markets have now been closed down in most parts of southern China, although a black market probably still exists for these animals. The re-emergence of SARS remains a possibility.

### LESSONS LEARNED

The SARS epidemic has profound implications for future epidemic preparedness. The spread of infection from Hong Kong to Canada, Singapore and Vietnam after a group of travellers were exposed briefly to the SARS-CoV in a hotel lift demonstrated clearly the risk posed by rapid international travel on the spread of disease. Health checks for travellers at points of departure and arrival may be useful in identifying infected persons and in isolating and treating them quickly. Restrictions on who may travel might be necessary in times of pandemic outbreaks.

The powers available to local and national governments in times of crisis should be reviewed, as public health legislation is often out of date with respect to emerging diseases. In Hong Kong, the residents occupying about 250 flats of an entire tower block in a public housing estate were quarantined for 10 days during the height of the SARS epidemic to prevent the disease spreading. During and after the SARS outbreak, relevant health ordinances in Hong Kong were revised to amend the powers available to the health authorities in times of epidemic disease, and to clarify the circumstances under which these powers could be exercised.

Stockpiling of appropriate medicines (eg broad-spectrum antibiotics and antivirals), personal protective clothing, portable isolation units and equipment for mobile field units to facilitate isolation should be considered in order to relieve the burden on hospitals. This would ensure that public health resources are devoted to maintain an adequate surge capacity at hospitals in times of urgent need. Public education, not only on legal rights and obligations but also to promote a cleaner, safer and more caring community, should be enhanced.

Environmental health practices contributing to disease spread (eg preventing the accumulation of refuse, and vermin and disease vector control) were enhanced after SARS. The maintenance of good health practices, especially in public buildings, was

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**Table 2.** Numbers and percentages of infected healthcare workers (from ref 24).

Area	Number of probable cases	Number of healthcare workers infected (%)
Mainland China	5327	1002 (19)
Hong Kong, China	1755	386 (22)
Taiwan, China	665	86 (13)
Canada	251	108 (43)
Singapore	238	97 (41)
Vietnam	63	36 (57)

enhanced. For example, control panels in many lifts in public areas (shopping malls, housing estates etc) are now covered with a protective plastic sheet to allow easier and more thorough disinfection. Furthermore, drains should be maintained properly, with, for example, U-bends cleaned with bleach and topped up regularly, and cracked and leaking pipes repaired, especially in public housing. One of the major causes of the spread of SARS in the quarantined public housing estate mentioned earlier was the release of plumes of infectious aerosols from poorly maintained sewerage and drainage pipes.

Healthcare workers around the world bore the brunt of the SARS epidemic (Table 2). Various deficiencies in hospital infection control practices were identified by the SARS Expert Committee,<sup>24</sup> and these included, for example, insufficient numbers of infection control personnel in hospitals, ineffective infection control in many hospitals, unclear guidelines to hospital staff, the needed more training in dealing with infectious diseases, and uncertainties and confusion among hospital staff regarding the standard and supply of personal protection equipment.

These deficiencies were not unique to Hong Kong at the time of the SARS outbreak. However, in the era of pandemic preparedness in which we now live, they should be taken as reminders of the simple steps that can and should be taken in all hospitals to prepare for epidemic infectious disease.

### CONCLUSIONS

Severe acquired respiratory syndrome was the first epidemic of a new zoonotic disease in the 21st century, but it will not be the last. Of the 1415 species of infectious agent known to cause disease in humans, 868 (61%) are zoonotic (ie a disease that can be transferred between humans and animals). Over the past 25 years, 38 new pathogens have moved from animals to humans, and human interference can be considered a primary cause of exposure to new pathogens.

The hunting and eating of bushmeat in Africa has been postulated as the means by which human immunodeficiency virus (HIV) was introduced into the human population, just as the capture and consumption of wild animals resulted in the amplification and spread of SARS-CoV. The distribution of disease vectors is changing as a result of

global warming, which in turn affects the viability of many potential pathogens. The potential for the occurrence of a new or re-emerging pathogen is obvious.

The 2003 SARS epidemic may be regarded as a dry run for a potentially larger global pandemic. While the heroism and selfless devotion to duty of many healthcare workers during the SARS outbreaks doubtless saved many lives, many other aspects of basic infection control and pandemic preparedness were found wanting. The time between pandemics should be spent in preparation and training, as next time there will be no second chance to get it right. ■

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## 'Over the past 25 years, 38 new pathogens have moved from animals to humans, and human interference can be considered a primary cause'

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Dr Richard A Collins FIBMS is a research grant administrator in Hong Kong specialising in infectious disease research.

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