Severe Acute Respiratory Syndrome

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Foreword

The participation of infectious disease experts from across the globe in this book addressing a topic unknown just two years ago is witness to the tempo of the 21st Century. This book will be enormously helpful to those who must help to design national policies, and to those contemplating joining the research enterprise on SARS, and the multitude of present and prospective infectious disease threats.

If I could think of snappy Greek terms, I'd be talking about some synonyms of travelosis and global-osis as the trajectory we can look forward to in the century opening up before us. In the history of disease, many of its ingredients were anticipated by the AIDS pandemic 20 years earlier. But the long chronicity of HIV infection makes it far less dependent on modern air transport for its dissemination. SARS, for now, takes pride of place with its exploitation of the jetliner as its mode of global dissemination. Prompted by HIV-AIDS, SARS, and a score of other major outbreaks, we have only begun to contemplate the implications of the 21st Century human condition: population crowding often in huge conurbations, typically in close proximity to jet aircraft airports, counting almost 2 million passengers bound for international destinations every day. All this is compounded by intense stratification of material income and health services. What a cauldron![1]

If SARS uses high-tech vectors, it has primitive origins in dietary zoonosis, but arguably augmented by urban appetites nourished by 2003-style levels of affluence. Then the quality of public health infrastructure over the surrounding terrain will be all-important in determining the level of spread of the new infection. New therapeutic measures are hard to come by, and take years to develop and validate; so it is no surprise that all that was available for SARS was the classic repertoire of case-identification, isolation, and contact tracing and management (e.g. quarantine). Wherever and whenever the political will was consolidated, these were the measures that successfully contained the outbreak of 2002–2003.

The response to SARS is beautifully exemplified in the papers in this volume: the extraordinarily rapid and competent identification of the agent of SARS as a novel coronavirus, and the ensuing development of biomolecular technologies for diagnosis and the detective work of tracking the origins and spread of SARS.

In this pause we might contemplate the prospects of new emerging infections in general, where they might come from, what if anything we can do to raise our guard. We know virtually nothing of the ultimate evolutionary origins of viruses. Dependent on a host's metabolism, they are unlikely to be primitive; and likely most viruses are offshoots of host genomes, including their organelles—which in turn may be of symbiotic origin. We then ask, what are the logical possibilities for sources of DNA (read also 'or RNA' in all that follows). Viral innovations are more likely to occur as

mutations or recombinants or fragmentary additions to existing viruses which have already trodden the tortuous path to adaptation to human, animal, plant or microbial hosts. Where can such DNA be found? Arguably from the hosts themselves, and then by extension also hypothetically from any fragment, plasmid, virus inhabiting those hosts. The cardinal lesson, learned from the practice of biotechnology, is the nearly universal promiscuity of DNA; homologous sequences will pair and recombine no matter what their origin; and more rarely non-homologous segments can also be patched together. What happens in the lab must surely be feasible somewhere in our vast biosphere.

'Animal–vegetable–mineral'

1 Anthroponoses
2 Zoonoses (vertebrate or invertebrate)
3 Phytonoses
4 Bacteria and their viruses, and other unicellulars
5 Synthetic DNA

1 Anthroponoses

Confining ourselves for the moment to viruses, the majority of anthroponoses are all too familiar in our history, like smallpox and paediatric viruses. Vaccines are available for almost all of the ancient ones. HIV-AIDS has rapidly transformed itself from a primate zoonosis to the most devastating viral disease now on the planet. The hepatitides are just being sorted out. The very recent discovery of metapneumovirus, and most recently, yet another novel human coronavirus (NL-63) is a caution that still further discoveries may be in the offing: we have not reached the end of the catalog. So many pneumonias remain undiagnosed even post mortem, we can hardly be reassured. Blood transfusion and organ transplants must now be added to sexual transmission, close personal contact, and arthropods as vectors, all augmented by 21st Century transport.

We are also beset with a handful of nearly benign infections, rhinoviruses and other 'common colds'. Any of these, or all of the above, have the potential, in principle, of mutating to far more aggressive strains, or of recombining with already virulent strains and augmenting their transmissibility. It is some reassurance that this has not come about (at least to our knowledge) during the past hundred years, more or less, of relevant observation.

One of the urgent priorities for the genomic revolution will be the meticulous cataloguing of 'every' human virus, and more enlightened insight of the prognosis, based on the dissection of pathogenetic pathways. How clumsy are our purported explanations of the seasonality of respiratory disease. These clumsy explanations show how much we still have to learn.[2]

2 Zoonoses

We should not be surprised that the majority of new outbreaks are of zoonotic origin.[3] A myriad of host species harbor a matching number of endogenous zooviruses, that have hardly begun to be indexed. These zooviral infections are often nearly benign in their primary host, with coevolution of host and parasite toward a mutually favorable symbiosis. For most zooviruses, human encounter will have no consequence: either the virus is equally well adapted to the human host; else it may be poorly adapted to the cellular environment of the new human host. These then escape our notice, though some may break through the level of detectability by serosurveys. In a few examples, like monkeypox or H5N1 influenza or hantavirus, we may see sporadic human disease, sometimes quite virulent, but little or no human–human transmission that would ignite a pandemic. The barriers to such transmission are ill-understood: do they reflect disease disproportionate to viral shedding? Host idiosyncrasies: genotype or intercurrent infection or cross-reacting immunity? There is no guarantee that such hypothetical barriers will remain unbreached, with intense selection for mutants that can transcend them, as perhaps

occurred in the transition of animal coronaviruses to human SARS.

It is anachronistic that one must enter a plea, on behalf of global health, to regulate the consumption of 'bush-meat' (whether in its African or Asiatic manifestations) and of the international transport of exotic pets. Our zoos must learn to use the best of veterinary science and art. This is not merely to protect the consumers, but to avert the fallout when exotic diseases spread.

3 Phytonosis

No animal viruses of plant origin come to mind, though I am unaware of any concerted effort e.g. to elicit expression of TMV RNA in human cell culture. Pseudomonad bacteria might be expected to elicit opportunistic infection on any substrate.

4 Bacteria and their viruses, and other unicellulars

Numerous hints prevail of lateral transfer of bacterial and animal host genes, from genomic studies. There are recurrent claims of expression of bacteriophage and plasmid genes in mammalian cell culture. Recently, the Courvalins[4] have demonstrated the delivery of genes by Shigella internalized in epithelial cells. This was intended to be a vector for DNA vaccines, but it is easy to imagine recombination between the bacterial DNA with other viral DNA already entrained in the target cells.

Similar prospects might apply to other unicellular parasites (*Plasmodium*, *Leishmania*).

5 Synthetic DNA

Here we enter the 'mineral' world, mainly artefacts of the laboratory. Free DNA has been found to be remarkably potent in entering muscle and other cells, and thereby opened the door to DNA vaccines. This mode of entry bypasses the need for specific receptors, applicable to virus DNA as well: witness the infectivity of synthetic poliovirus.[5,6] Free DNA may also occur in abundance with lysis of pus, heavily laden with bacterial flora, witness the market for deoxyribonuclease in symptomatic relief of viscous clogging of airways in cystic fibrosis.

This discussion inevitably leads to the odious topic of malicious construction of hyperpathogens and their application to biowarfare, in the hands of terrorists or of organized states. The use of contagious agents is an attack on the whole world's population, insane for any imaginable political agenda.

Were it not for our actual experiences of the last 20 years, from AIDS to SARS, my remarks might be put down as overenthusiastic ravings. There has been one alarm after another, and the beginnings of a concerted international response. The technical cooperation elicited by the SARS episodes, and the monumental contributions of researchers from the PRC, Europe, Canada, the US are encouraging for the prospect of more effective responses, but they still pale before the magnitude of the threats from unforeseeable new emergents. The unique position of the Hong Kong SAR, as the pivotal point of entry and exit, of pathogens and of knowledge about them, has been highlighted.

A new flu reassortment, matching the 1918–1919 episode, is on everyone's mind; but we know our current capability to generate stocks of a new vaccine is totally 'out of synch' with the speed of its spread in the modern world. We must renew our cooperative planning with a recognition of the magnitude of the threat, and humility about the paucity of our knowledge of the globe's viral agents and how they might break out. If we can achieve that, we may retrospectively view the SARS of 2002–2003 as a blessing, that those who perished may not have died in vain.

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We dedicate this book to health care workers the world over who were affected and afflicted by SARS, and in a most special way, to those gave their lives in the pursuit of their profession.

Chapter 1

SARS: A Historical Perspective from Hong Kong

Kwok-yung Yuen and Nam-shan Zhong

History is apt to repeat itself. In 1894, an outbreak of plague started in Canton, China. The epidemic soon spread to Hong Kong and was first described by Dr James Lowson, a government medical superintendent.[1] It was later carried by ships to California and then on to port cities in South America, Africa and Asia. This epidemic led to the discovery of the plague bacillus,[2] *Yersinia pestis*, after a race between two groups of researchers to identify the cause. The rat flea was subsequently identified as the vector and the main animal reservoir recognized to be *Rattus rattus* and *Rattus norvegicus*.[3–5]

Similarly, this is what happened with an outbreak of atypical pneumonia in late 2002. It is notable that the first intelligence of both epidemics came from journalists, whose pursuit of the truth keeps the public well-informed. Their reporting in the Hong Kong media on 10 February 2003 of an outbreak of a rapidly spreading atypical pneumonia in Guangdong province came earlier than the official announcement. However, not all media reports served to keep the public well-informed of developments. For instance, rumours about the death of many young patients, including doctors and nurses, helped incite panic buying of vinegar and traditional Chinese medicinal herbs in Guangdong province.The public believed that vapour released by boiling vinegar could kill germs circulating in the atmosphere, while herbal extracts could detoxify and dissipate the heat generated by infection caused by this atypical pneumonia.

In the same week, the Hong Kong government formed an expert panel to study the risk of a similar outbreak in the Hong Kong Special Administrative Region (HKSAR) and started the surveillance of severe pneumonia cases in the region. At that time, hospital surveillance did not reveal any increase in the number of cases of severe community-acquired pneumonia. Unfortunately, official exchange of information between the HKSAR and Guangdong province in China was not readily established at that juncture because of an inadvertent 'firewall' created by the 'one country–two systems' situation. In fact, for 2 months cases of severe atypical pneumonia had been recorded in five cities around Guangzhou.[6] But obtaining medical information along official channels from Guangdong province proved bureaucratic and problematic for HKSAR authorities. The first such case of atypical pneumonia was reported in Foshan, a city 24 kilometres away from Guangzhou, on 16 November 2002. A month later, a chef from Heyuan who worked in a Shenzhen restaurant was also infected. He had an occupational history of regular contact with wild game-food animals. His wife, two sisters and seven hospital staff who had contact with him were also affected. All patients had high fever, respiratory symptoms and infiltrate on chest radiograph. In China from 16 November 2002 to 9 February 2003, 305 cases of atypical

pneumonia were reported, 105 of which were in health-care workers. The epidemiology of the disease was described with initial consensus among medical experts in China.[7] It was an atypical pneumonia of unknown aetiology but probably viral in origin. The incubation period ranged from 1 to 11 days. There was clustering of cases in families and hospitals, which suggested the necessity of close contact for transmission, probably by respiratory droplet. Thus single-room isolation, environmental decontamination and hospital-staff protection with the use of masks and hand washing were recommended as control measures, in addition to the notification of the disease and contact tracing.

With the HKSAR epidemic, the index patient and the key connection to the global epidemic was CCL, a professor of nephrology from a teaching hospital in Guangzhou, China.[11] Between 11 and 13 February 2003, he had a history of contact with patients suspected to have this unusual atypical pneumonia. CCL then developed flu-like symptoms, including a fever and cough, which he self-treated with penicillin and ofloxacin before coming to Hong Kong. He checked into the Metropole Hotel on 21 February and within a period of 24 hours had infected 16 people. This was one of those unexplained superspreading events that eventually took on global significance. The professor was admitted to the Kwong Wah Hospital the next day where his condition rapidly deteriorated. No pathogenic bacteria, viruses, fungi, or parasites could be found in his respiratory secretions, blood or other body fluids. The turning point of the whole event was that his brother-in-law, YPC, was soon admitted with a similar condition. After upper respiratory tract specimens and a bronchoalveolar lavage failed to yield an aetiological agent, the decision was made to carry out an open lung biopsy. The sample was sent to the Queen Mary Hospital, the teaching hospital of the University of Hong Kong, for microbiological analysis. This turned out to be the key specimen in identifying the aetiological agent of SARS.

As a result of this superspreading event, visitors to the Metropole Hotel unwittingly carried the disease to other hospitals in the HKSAR and by air travel to Vietnam, Canada, Singapore, the Philippines, the United Kingdom, the United States and back again to China[12] (Fig. 1.1). Dr Carlo Urbani, a physician stationed at the World Health Organization (WHO) office in Hanoi, answered the call for assistance in investigating a patient who had stayed in the Metropole Hotel while in the HKSAR. Within the next 2 weeks, nosocomial transmission occurred affecting health-care workers, patients and visitors to the hospital in Hanoi. Dr Urbani recognized that he was probably dealing with a hitherto unknown disease and he initiated measures to control this hospital outbreak. He described this unusual disease and informed WHO.[13] The WHO subsequently labelled this atypical pneumonia as severe acute respiratory syndrome (SARS). Dr Urbani's description of the disease, to which he later succumbed, alerted the authorities and resulted in an unprecedented collaboration of 11 research laboratories in 9 countries. This international collaboration involved daily teleconferencing and exchange of specimens and ultimately led to the rapid discovery of the aetiological agent of SARS.

Discovery was by educated guess and trial and error. The differentiation between typical and atypical pneumonia is a historical one, and is often difficult.[8] Diagnosis rests on the clinical syndromes, the microbiological analysis and the therapeutic response to beta-lactam antibiotics. In general, typical pneumonia has a very acute onset with fever, chills, pleuritic chest pain, tachypnoea and cough with rusty-coloured sputum. It is mainly caused by pyogenic bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Staphylococcus aureus*. Peripheral blood counts usually show an increase in neutrophils. The disease responds very well to beta-lactams. These bacteria are therefore

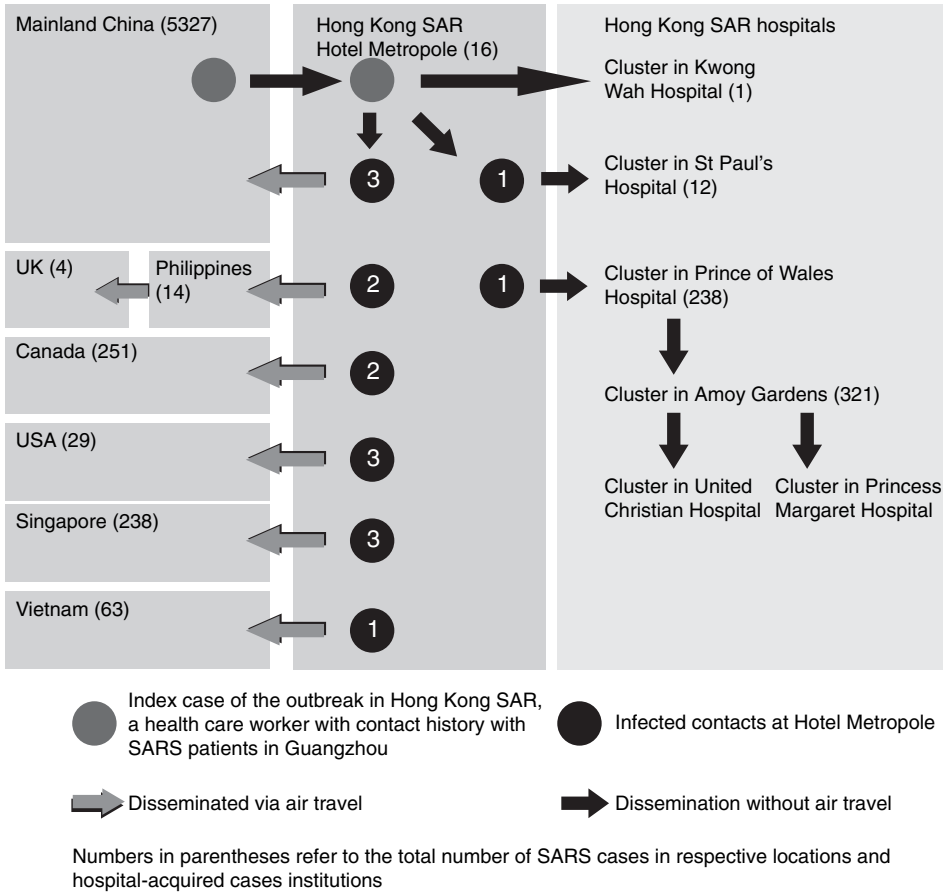

Figure 1.1 Superspreading of SARS.

unlikely to be the cause of a major outbreak in the hospital setting where antibiotics are commonly prescribed.

Atypical pneumonia usually has a less acute onset with preceding upper respiratory tract symptoms.[9] The cough is often nonproductive. The findings on clinical examination are disproportionate to the chest radiographic changes. Historically, cases have been caused mainly by *Mycoplasma pneumoniae*, followed by *Chlamydia pneumoniae*, *Chlamydia psittaci*, *Coxiella burnetti*, *Legionella pneumophila* and less commonly various respiratory viruses. Peripheral blood examination often shows either normal or increased white blood cells. Patients often do not respond to treatment with beta-lactams. The most common cause of atypical pneumonia is *Mycoplasma pneumoniae*, a bacterium that does not have a cell wall. Moreover, the beta-lactams do not penetrate into infected cells very well and would not be very effective against the other causes of atypical pneumonia, which are predominantly intracellular pathogens. All four bacterial agents are sensitive to treatment with the macrolides, tetracyclines and quinolones. They are often

associated with a specific antibody response in the convalescent serum of patients. The only remaining differential diagnosis in atypical pneumonia is a viral cause.

The influenza virus was therefore high on the list of differential diagnoses because the season was late winter/early spring. However, investigations can easily be misled by reasonable anticipations that turn out to be red herrings. On 11 February 2003, a 33-year old man was admitted to the Princess Margaret Hospital in the HKSAR for a severe community-acquired pneumonia and was soon followed by his 9-year-old son. A week earlier, his youngest daughter had died of pneumonia when the family were visiting Fujian in China. The man died on 17 February, while his son eventually recovered. Influenza A subtype H5N1 virus was isolated from the man and his son. This led to the World Health Organization (WHO) issuing a global alert on avian flu on February 19. Previously in 1997, the HKSAR had been struck by an epidemic of influenza A subtype H5N1 involving 18 patients and 6 deaths.[10] The outbreak was finally brought under control by the territory-wide slaughter of 1.5 million chickens. Isolated outbreaks of avian flu continued to occur at farms and markets in 2001 and 2002. On 10 December 2002, dead waterfowl found in a recreational park in the HKSAR and subsequently some farm chickens were shown to have influenza A subtype H5N1 virus. These recent events placed a strong bias on the suspected cause of the growing number of atypical pneumonia cases reported in China. In February 2003, the anticipation of an outbreak of influenza A subtype H5N1 turned out to be a red herring.

The other possibility was an antigenically drifted human influenza A or B, which happens every few years. This theory was followed by the very ominous possibility of an antigenically shifted influenza A. But these were considered unlikely because cell cultures and chick-embryo inoculation at the University of Hong Kong were optimized for influenza. Moreover, reverse transcription-polymerase chain reaction (RT-PCR) assays for the matrix gene and antigen tests for nucleoprotein were uniformly negative for influenza. Clinically, patients did not respond to antiviral drugs specific for influenza virus, such as amantadine and oseltamivir.

Other possibilities had to be considered in view of the absence of evidence favouring the diagnosis of different types of influenza viruses. Other types of viruses that have an outbreak potential in hospitals include the adenovirus, the parainfluenza viruses, the respiratory syncytial virus, the metapneumovirus, the rhinovirus and the coronaviruses. Only influenza and adenovirus are known to cause severe pneumonia in immunocompetent young adults. The rest are much less likely to cause such a serious illness. Further laboratory investigations with RT-PCR, PCR using consensus primers and antigen testing excluded the first five types of virus. However, these initial investigative findings could not exclude a coronavirus or another novel virus.

As was the case in 1894 with the bacillus plague, the initial claims of discovery were conflicting. Though experts in China found electron microscopic evidence of chlamydial infection in the postmortem lung tissue of early cases of SARS,[14] this was not found in patients from other countries and most SARS patients did not respond to antichlamydial antibiotics. There was a subsequent report that scientists from the Chinese Academy of Military Medical Science in China might have observed coronavirus-like particles under the electron microscope on 26 February.[15] On 19 March, two research groups in Germany and the HKSAR announced the finding of paramyxovirus-like particles in samples from SARS patients. Between 21 and 27 March, groups from the HKSAR, the USA and Germany independently found a novel coronavirus from SARS patients after mounting specific antibody response tests on whole virus immunoassays using im-

munofluorescent or ELISA format.[16–18] The initial fragments of genes obtained by differential display PCR were used in the development of RT-PCR for rapid diagnosis. On 16 April, Koch's postulates for the viral causation of SARS by this novel coronavirus were satisfied in a primate model.[19] On 12 April, the full genome of the virus was sequenced and available on the internet to all researchers.[20,21] This rapid progress in investigating the aetiological agent of SARS is one of the most amazing achievements in medical history. Within 2 months of its discovery, the complete deciphering of the full genome of a pandemic microbe has markedly accelerated the research on the pathogenesis, diagnostics, antivirals and vaccines for SARS.

Although no vector like the rat flea that transmitted the plague was involved in SARS, there were other horrifying mechanisms that amplified the outbreak in the HKSAR. On 4 March, a visitor to the Metropole Hotel was admitted to the Prince of Wales Hospital in the HKSAR with acute community-acquired pneumonia. The administration of albuterol by a nebulizer on this patient led to a hospital outbreak that involved 238 persons, mostly health-care workers, patients and visitors.[22] One of these affected was a patient on haemodialysis. He was admitted to the Prince of Wales Hospital on 15 March with suspected atypical pneumonia, which was subsequently attributed virologically to influenza A. The patient improved and was discharged on 19 March. He stayed at an apartment in Block E at Amoy Gardens, a private housing estate. What followed was a devastating outbreak involving 321 people in that estate. The amplification mechanism was traced to a faulty sewage system.[23] The U-trap of the bathroom floor-drains in most apartments were dry and therefore had lost their function as a seal to the soil stack (Fig. 1.2). The common use of an exhaust fan in a closed bathroom generated a strong negative pressure that drew infected droplets from the soil stack into the bathrooms. On 21 March, a leak occurred in the water pipe to Block E and led to a shutdown of the flushing system. It has since been shown that the SARS coronavirus can survive for 4 days in faecal matter. Therefore, the diarrhoeal stool of

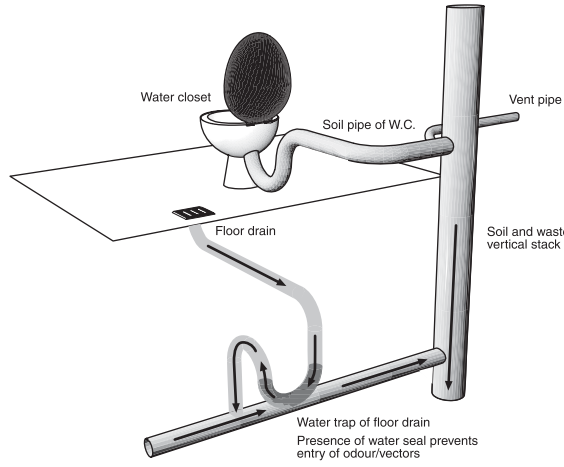

Figure 1.2 Properly maintained U-trap. (Reproduced with permission from Report of the SARS Expert Committee.)

the haemodialysis patient was passed into the sewage system[24] and infected droplets were drawn into the bathroom and followed the current of the exhaust fan through the light well to various floors in Block E. Other mechanisms of amplification of this epidemic at this housing estate by infected cats, rats or passive carriage by cockroaches were suggested but remain unproven despite the positive isolation of the SARS coronavirus from domestic cats with seropositivity towards this virus.

This large outbreak at the housing estate was an excellent opportunity for the study of the natural progression of SARS since the patients were exposed at almost the same time. They were home-quarantined and admitted to hospital on the first day of the onset of any symptoms. Their clinical symptoms and signs, blood parameters, radiological changes, microbiological findings and outcome could be prospectively monitored and analysed. A unique pattern of changes of viral load in their respiratory secretions was documented and had implications in the design of treatment strategies for future epidemics.[25] The observation that 70% of these patients developed diarrhoea led to the discovery of a large amount of virus being present in the stool. This has strengthened the argument that the major amplification mechanism of the SARS outbreak was due to the faulty architectural design of the bathrooms.

Finding the responsible virus and defining the syndrome of SARS are just the beginning of a long and difficult journey. The ultimate goal is to treat and prevent SARS effectively. The necessary tools are rapid and sensitive diagnostic tests, effective and safe antiviral drugs, pragmatic and compliable infection-control measures, and finally a safe and effective vaccine. None of these will come about without painstaking research. The antibody test using the infected cell line as antigen only starts to show a positive response at around day 10 of infection. It takes 28 days before most SARS patients can mount an antibody response. The use of recombinant viral proteins as the antigen might lead to the production of a more sensitive and specific antibody test.[26] Again, the rapid RT-PCR assay is only positive in 25% of SARS patients at the time of admission. The sensitivity of the assay is being improved to over 80% after a careful optimization of the RNA extraction,[27] the choice of the primer sequence, the testing conditions, an additional amplification step called a nested reaction, and a final hybridization step.

The histopathological findings from the tissues of deceased patients suggest that part of the damage to the lung is mediated by macrophages, which are highly activated as a result of the viral infection.[28] Moreover, viral-load study shows that the viruses increase in number during the first 10 days and then decrease irrespective of whether the patient is improving or deteriorating. Thus at least part of the damage might be related to the immune dysregulation of the host, which may be alleviated by steroids. This may decrease the need for intubation and the workload of the intensive care unit. However, such immunosuppressive treatment is also associated with other potentially fatal side-effects that include bacterial or fungal superinfections. The answer to the treatment of SARS must lie in the development of effective antiviral agents that decrease the peak viral load and the associated immune-mediated damage. This is supported by the viral load study at days 10–15 which showed that the manifestations of SARS such as respiratory failure, hepatitis, diarrhoea, abnormal urinalysis and death are closely related to viral genome copies in the relevant clinical specimens.[29] At the time of writing, there is still no randomized placebo control trial to show whether any antiviral is effective. Ribavirin by itself did not appear to have sufficient antiviral effect.[30] This was used in the early stage of the outbreak as a broad-spectrum antiviral agent when the aetiological agent was still unknown. Later, a relatively low dose of ribavirin combined with a protease inhibitor or an interferon called alfacon-1 were used in clinical trials with only historical

control.[31,32] Ribavirin may also function as an immunomodulator because the drug was reported to be highly effective in the treatment of fulminant hepatitis in mice caused by a mouse coronavirus.[33]

Because the complete genome sequence of four different SARS coronavirus strains was available within a month of the discovery of the virus by four different research centres in Canada, the United States and the HKSAR, we know that there are a number of enzymatic targets for antiviral therapy. These include the RNA replicase, the helicase, the proteases and mRNA methyltransferase.[34] Researchers are now rushing to screen combinatorial chemical libraries to find effective antiviral treatment for SARS. One of the most important surface targets for antiviral treatment is the spike protein, which makes up the surface projections that give the coronavirus the morphology of a crown. It functions like a claw, which allows the virus to attach to human cells and enables the virus envelope to fuse with the host cell membrane. This fusion process allows the virus to enter the cell to start a new cycle of infection. Short chains of amino acids are designed to glue-up the mechanical apparatus of this claw so that the virion can no longer enter the human cell. Short interfering RNA inhibiting viral transcription is another treatment modality that is also undergoing investigation.[35] However, all these potentially exciting new drugs will only move from the laboratory and on to the clinical trial stage after at least another year, and they will not be commercially available for a further 2–3 years. A great many *in vitro* and animal experiments are needed before trials in humans are justified.

The same difficulties will apply to the development of a vaccine. The relative importance of systemic or mucosal immunity against surface targets such as spike, membrane, and envelope proteins by neutralizing antibody or by cytotoxic T-lymphocyte response against nucleoprotein and other targets is still unclear. But recent studies suggested that neutralizing antibody against Spike (the spike protein) appeared to be very important. The use of live attenuated coronavirus is out of the question for fear of reversion to virulence or recombination with wild strains to form new wild types. The most readily available vaccine to undergo clinical trials will be an inactivated SARS coronavirus. However, laboratory safety is a major issue when culturing a huge stock of coronavirus. This is particularly worrying in light of a recent reports of a laboratory-acquired cases of SARS in Singapore, Tawain and China. Recombinant proteins expressed in mammalian cells or yeast are alternatives for inducing good neutralizing antibody response. DNA vaccines delivered by non-replicating viral vectors such as modified vaccinia virus Ankara and adenovirus are likely to induce both good antibody and cytotoxic T-cell response. Non-replicating coronavirus particles will be safe and will closely mimic the live coronavirus in terms of immunogenicity. Irrespective of which approach is used for immunization, it is important to note that immune enhancement disease has occurred in feline peritonitis coronavirus,[36] inactivated measles and respiratory syncytial virus vaccination.[37,38] The economic viability of a SARS vaccine depends on whether and when SARS comes back and whether antigenic variation will affect the effectiveness of this vaccine.

Whether SARS will come back depends on the existence of ongoing mildly symptomatic infection in humans, persistent animal reservoirs or unsafe laboratories working with SARS. On 24 May 2003, a collaboration between the University of Hong Kong and the Shenzhen Centre for Disease Control (CDC) identified a precursor of the SARS coronavirus in civet cats and a raccoon dog.[39] Sera from people in close contact with these animals had a high prevalence of antibody against the SARS coronavirus. In essence, SARS is probably a zoonosis. The virus may have jumped the human barrier repeatedly without being noticed, but it has not yet mutated sufficiently to cause human-to-human spread. Comparing the animal precursor with the

human isolates of SARS coronavirus, there is a 29-base-pair deletion between ORF 10 and 11 in the human isolates. The deletion and perhaps some other mutations may have sufficiently changed the property of the virus as to cause a pandemic. The situation is in some way similar to what has happened with influenza viruses. In 1997, genetic reassortment between avian influenza strains of H5N1 and H9N2 or H6N1 produced an epidemic of 18 patients with 6 fatalities in HKSAR.[40] Fortunately the genetic reassortment did not enable the new reassortant to spread efficiently among humans. But we are aware that more disastrous events have happened in the past hundred years. In 1918 the Spanish Influenza A pandemic, known as Spanish flu, swept across the world leaving between 20 and 40 million people dead.[41] However, the causative agent was not discovered until 1933 by Smith.[42] In February 1957, the Asian influenza H2N2 originated in Southern China, overwhelmed the region within a month, spread to Hong Kong and Singapore 2 months later, and became an obvious pandemic by November.[43] About a decade later history repeated itself in July 1968 with the emergence of the Hong Kong H3N2 influenza A pandemic, which is often remembered as the Hong Kong flu.[44] These experiences strongly suggest that SARS and influenza appeared to have brewed in southern China, where the density and proximity of human and animal populations are unmatched. The genetic change empowering the virus to spread between humans is the key event leading to all of these disasters.

The world, and the HKSAR in particular, has a lot to learn from this major epidemic that affected over 1700 people and resulted in almost 300 deaths. The 1894 outbreak of bacillus plague in Hong Kong was largely the result of negligence in personal and environmental hygiene, pest infestation and overcrowded living conditions. The HKSAR is now a modern cosmopolitan region with outspoken mass media, an elected legislature and groups of physicians and microbiologists working at the cutting edge of research in infectious diseases. Hong Kong has also grown from a small fishing village to become a vital doorway into China and an international centre for trade, finance, business and communications. The HKSAR has the highest throughput container port and the busiest airport in terms of passengers and international cargo in the world. Since China adopted an open economic policy in 1978, increasing numbers of visitors and cargo flow between China and the HKSAR, providing an annual average of 23% in trade value. Since the political transition in 1997, the integration of the HKSAR with the Pearl River Delta of Southern China has accelerated. The open door policy and economic boom in Southern China is naturally associated with an increasing population density and subsequent demand for food animals as a source of dietary protein. This has obviously facilitated the increase in the number of farm and market animals and consequently the flow of emerging pathogenic microbes from animals to humans. Infectious diseases know no international boundaries. Increasing regional and international travel can rapidly import emerging or re-emerging infections into Hong Kong and export them to the rest of the world. The huge population density of China and the HKSAR provide an ideal incubator for brewing and spreading new infectious agents and antimicrobial resistance. Although new agents are often discovered in Hong Kong, their source could well be on the mainland in China. Such detection is made possible because of the HKSAR's relatively better surveillance and laboratory infrastructure.

Known infectious diseases are less likely to pose a major threat in the HKSAR because the behaviour of the diseases is already well established and the government is usually prepared. What led to the recent disaster with SARS was that we faced a previously unknown emerging infectious disease. The first lesson to learn is how to face the un-