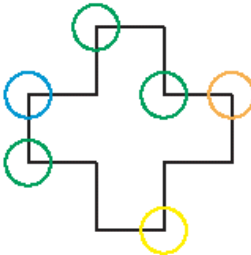


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Chapter 3: Virology

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The search for the causative agent of SARS

The epidemic of severe atypical pneumonia which was observed in the Chinese province of Guangdong and [reported internationally on February 11, 2003](#), was initially suspected to be linked to a newly emerging influenza virus: on February 19, 2003, researchers isolated an avian influenza A(H5N1) virus from a child in Hong Kong. This virus was similar to the influenza virus originating from birds that caused an outbreak in humans in Hong Kong in 1997, and new outbreaks of similar strains were expected. However, bird 'flu', possibly of poultry origin, was soon ruled out as the cause of the newly termed Severe Acute Respiratory Syndrome, or SARS.

Investigations then focussed on members of the *Paramyxoviridae* family, after paramyxovirus-like particles were found by electron microscopy of respiratory samples from patients in Hong Kong and Frankfurt am Main. Further investigations showed that human metapneumovirus (hMPV; [van den Hoogen](#)) was present in a substantial number of, but not in all, SARS patients reported at the time. Further tests did not confirm these findings.

At about the same time, China reported the detection, by electron microscopy, of Chlamydia-like organisms in patients who had died from atypical pneumonia during the Guangdong outbreak. Again, this finding could not be confirmed by other laboratories in SARS patients from outside China, although a concurrent *Chlamydia pneumoniae* infection was also found in the index patient from the outbreak in Frankfurt am Main ([Drosten](#)).

On March 17, 2003, the WHO called upon eleven laboratories in nine countries to join a network for multicenter research into the etiology of SARS and to simultaneously develop a diagnostic test (<http://www.who.int/csr/sars/project/en/>). The member institutions communicated through regular telephone conferences (initially held on a daily basis) and via a secure website and exchanged data, samples and reagents to facilitate and speed up research into the etiology of SARS.

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Discovery of SARS Co-V

The etiologic agent of severe acute respiratory syndrome (SARS) is a coronavirus which was identified during the third week of March 2003, when laboratories in Hong Kong, Germany, and the United States found evidence of a novel coronavirus in patients with SARS. This evidence included isolation on cell culture, demonstration by electron microscopy, demonstration of specific genomic sequences by polymerase chain reaction (PCR) and by microarray technology, as well as indirect immunofluorescent antibody tests ([Ksiazek](#), [Drosten](#), [Peiris](#)).

The discovery of the novel agent was made less than two weeks after the WHO-led laboratory network came into existence. Three weeks later, on April 16, 2003, following a meeting of collaborating laboratories in Geneva, the WHO officially announced that this new coronavirus, never before seen in humans or animals, was the cause of SARS. This announcement came after research done by the then 13 participating laboratories from ten countries had demonstrated that the novel coronavirus met all four of Koch's postulates, necessary to prove the causation of disease: the pathogen must be found in all cases of the disease, it must be isolated from the host and grown in pure culture, it must reproduce the original disease when introduced into a susceptible host, and it must be found in the experimental host so infected.

The new virus was provisionally termed SARS-associated coronavirus (SARS-CoV). Shortly thereafter, complete genome sequences of the new coronavirus ([Marra](#), [Rota](#)) were published by a Canadian laboratory and the [CDC](#). The genome sequence data available so far from several SARS-CoV strains reveal that the novel agent does not belong to any of the known groups of coronaviruses, including two human coronaviruses, HCoV-OC43 and HCoV-229E. ([Drosten](#), [Peiris](#), [Marra](#), [Rota](#)), to which it is only moderately related. It has been proposed that the new virus defines a fourth lineage of coronavirus (Group 4). ([Marra](#)) The sequence analysis of SARS-CoV seems to be consistent with the hypothesis that it is an animal virus for which the normal host is still unknown and that has recently either developed the ability to productively infect humans or has been able to cross the species barrier ([Ludwig](#)).

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Morphology

Negative-stain transmission electron microscopy of patient samples and of cell culture supernatants reveals pleomorphic, enveloped coronavirus-like particles with diameters of between 60 and 130 nm. Most but not all viral particles display the characteristic appearance of surface projections, giving rise to the virus' name (corona, Latin = crown) ([Ksiazek](#), [Peiris](#)).

Examination of infected cells by thin-section electron microscopy shows coronavirus-like particles within cytoplasmic membrane-bound vacuoles and the cisternae of the rough endoplasmic reticulum. Extracellular particles accumulate in large clusters, and are frequently seen lining the surface of the plasma membrane ([MMWR 2003; 52: 241-248](#)).

Detection

SARS Co-V has been detected in multiple specimens including extracts of lung and kidney tissue by virus isolation or PCR; bronchoalveolar-lavage specimens by electron microscopy and PCR; and sputum or upper respiratory tract swab, aspirate, or wash specimens by PCR or isolation. ([Ksiazek](#), [Drosten](#))

High concentrations of viral RNA of up to 100 million molecules per milliliter were found in sputum. Viral RNA was also detected at extremely low concentrations in plasma during the acute phase and in feces during the late convalescent phase, suggesting that virus may be shed in feces for prolonged periods of time. ([Drosten](#))

Coronaviridae

The coronaviruses (order Nidovirales, family *Coronaviridae*, genus Coronavirus) are members of a family of large, enveloped, positive-stranded RNA viruses that replicate in the cytoplasm of animal host cells (Siddell).

There are three groups of coronaviruses; groups 1 and 2 contain mammalian viruses, while group 3 contains only avian viruses. The viruses are associated with a variety of diseases in humans and domestic animals, including gastroenteritis and disease of the upper and lower respiratory tract. Whereas animal coronaviruses may cause

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severe disease in animals (i.e., porcine transmissible gastroenteritis virus, murine hepatitis virus, feline infectious peritonitis virus, and avian infectious bronchitis virus), human strains were previously associated only with mild diseases.

Human coronaviruses (HCoVs) are found in both group 1 (HCoV-229E) and group 2 (HCoV-OC43) and are a major cause of mild respiratory illnesses ([Makela](#)). They can occasionally cause serious infections of the lower respiratory tract in children and adults and necrotizing enterocolitis in newborns (McIntosh, [El-Sahly](#), [Folz](#), [Sizun](#)). The known human coronaviruses are able to survive on environmental surfaces for up to 3 hours ([Sizun](#)). Coronaviruses may be transmitted from person-to-person by droplets, hand contamination, fomites, and small particle aerosols ([Ijaz](#)).

SARS-related CoV seems to be the first coronavirus that regularly causes severe disease in humans.

Stability and resistance

Work is ongoing to evaluate the stability of SARS-CoV and its resistance against various environmental factors and disinfectants.

The preliminary results, obtained by members of the WHO multi-center collaborative network on SARS diagnosis (see: http://www.who.int/csr/sars/survival_2003_05_04/en/index.html), show that the virus is stable in faeces and urine at room temperature for at least 1-2 days. The stability seems to be higher in stools from patients with diarrhoea (the pH of which is higher than that of normal stool).

In supernatants of infected cell cultures, there is only a minimal reduction in the concentration of the virus after 21 days at 4°C and –80°C. After 48 hours at room temperature, the concentration of the virus is reduced by one log only, indicating that the virus is more stable than the other known human coronaviruses under these conditions. However, heating to 56°C inactivates SARS-CoV relatively quickly. Furthermore, the agent loses its infectivity after exposure to different commonly used disinfectants and fixatives.

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Antiviral agents and vaccines

Efforts are underway at various institutions to assess potential anti-SARS-CoV agents in vitro. Ribavirin, a "broad spectrum" agent, which is active against various RNA viruses ([Tam](#)) has also been used clinically in SARS patients, but seems to lack the in vitro efficacy.

Likewise, efforts are underway to develop a vaccine that offers protection against SARS-CoV infection and/or disease. Although an effective vaccine cannot be expected to be available within less than at least one to two years, both the relative ease with which SARS-CoV can be propagated in vitro and the availability of vaccines against animal coronaviruses such as avian infectious bronchitis virus, transmissible gastroenteritis coronavirus of pigs and feline infectious peritonitis virus, are encouraging.

Outlook

The discovery of the SARS-associated coronavirus was the result of an unprecedented global collaborative exercise co-ordinated by the WHO. The rapid success of this approach 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](#)) to molecular techniques, in order to identify a novel agent. It demonstrates how an extraordinarily well orchestrated effort may be able to address the threat of emerging infectious diseases in the 21st century.

Control of the SARS epidemic will require the development of reliable diagnostic tests to diagnose patients and to monitor its spread, as well as of vaccines and antiviral compounds to prevent or treat this disease. Vaccines are successful in preventing coronavirus infections in animals, and the development of an effective vaccine against this new coronavirus is a realistic possibility. However, vaccination against coronaviruses in animal diseases is not uniformly successful. As is the case for the development of any vaccine, time is needed. Suitable animal models must demonstrate efficacy, and time is necessary in order to be able to demonstrate the safety of the new vaccine in humans.

With the availability of different laboratory methods, a number of highly important questions regarding the natural history of the SARS-

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associated coronavirus are now being addressed as a matter of urgency:

- When during the course of infection is virus shedding highest? What is the concentration of the virus in various body compartments? In what way does the "viral load" relate to the severity of the illness or the likelihood of transmission?
- Do healthy virus carriers exist? If so, do they excrete the virus in amounts and concentrations sufficient to cause infection?
- Does virus shedding occur following the clinical recovery? If so, for how long? Is this epidemiologically relevant?
- Why are children less likely to develop SARS: Do they have a lower clinical manifestation index, or do they possess a (relative) (cross-?) immunity against SARS-CoV?
- What is the role of potential co-factors such as *Chlamydia spp.* and hMPV? Are they related to a clinically more severe illness or to a higher degree of infectiousness ("super-spreaders")?
- What is the origin of SARS-CoV? What is the animal reservoir, if any? Has this cross-species transmission to humans been a singular event or is there constant re-introduction?
- Are there environmental sources of SARS-CoV infection, such as food items, water, sewage?
- How stable is SARS-CoV under different conditions? How can efficient disinfection be achieved? How long can the virus "survive" in the environment on both dry surfaces and in suspension, including in fecal matter?
- How important is genetic diversity among SARS-CoV strains?

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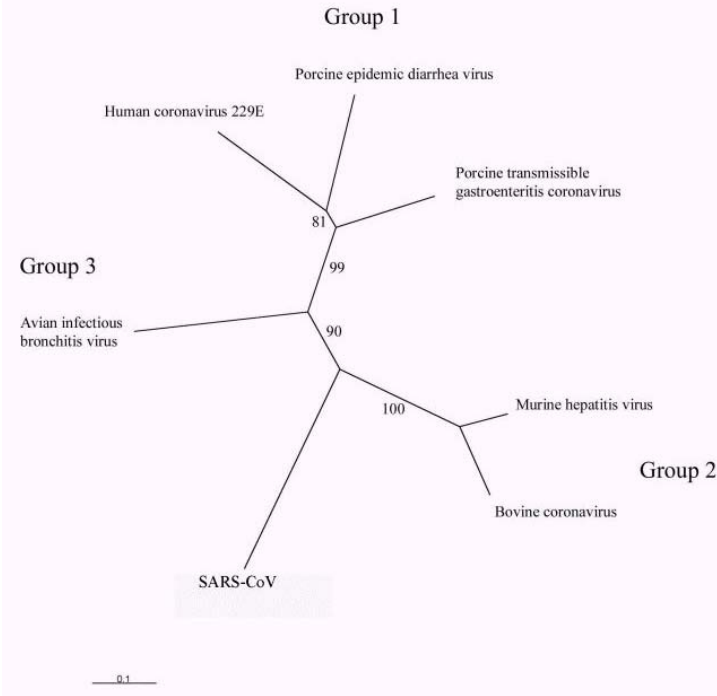
Figure 1. Electron micrograph of coronavirus-like particles in cell culture, supernatant after ultracentrifugation and negative staining with uranyl acetate. (Source: Department of Virology, Bernhard Nocht Institute for Tropical Medicine; Director: H. Schmitz; full-size picture: http://SARSReference.com/archive/coronavirus_em.jpg)



Figure 2. Cytopathic effect in Vero cell culture caused by SARS-associated coronavirus 24 hours post inoculation; for comparison: uninfected cell culture. (Source: Institute for Medical Virology, Director: H. W. Doerr; full-size picture: <http://SARSReference.com/archive/cytopathiceffect.jpg>, <http://SARSReference.com/archive/uninfectedcells.jpg>)



Figure 3. Phylogenetic tree of the SARS-associated coronavirus (Source: S. Günther, Department of Virology, Bernhard Nocht Institute for Tropical Medicine; Director: H. Schmitz; full-size picture: <http://SARSReference.com/archive/phylogenetictree.jpg>)



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