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Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) (*Coronaviridae*)

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Glossary

Lymphopenia Reduction in the lymphocytes in the circulating blood below the normal range for age.
Pathognomonic Characteristic and diagnostic of a particular disease.
PEGylated A molecule conjugated with polyethylene glycol (PEG).
Radiological abnormalities Pathological findings observed by medical imaging procedures (e.g., chest X-ray).
Rhinorrhea Runny nose.

History

Coronaviruses OC43 and 229E were first discovered in the 1960 as causes of mild upper respiratory disease. The discovery that a novel coronavirus was the cause of severe acute respiratory syndrome (SARS) in 2003 led to increased activity of research on human coronaviruses, resulting in the discovery of NL63, HKU1 and, in 2012 the identification of Middle East respiratory syndrome (MERS)-coronavirus as a novel zoonotic disease with epidemic potential. Both SARS and MERS coronaviruses are identified by WHO as pathogens of greatest concern for global public health for which countermeasures are urgently needed.

Emergence of SARS

From November 2002 to January 2003, clusters of cases of an unusually severe atypical pneumonia were observed in Guangdong Province, China. The disease was characterized by the lack of response to conventional antibiotic therapy and the occurrence of clusters of cases within a family or health care setting. In retrospect, these were the first known cases of the disease that was later called severe acute respiratory syndrome (SARS). In January, the numbers of cases "atypical pneumonia" continued to increase with examples of "super-spreading incidents" that were to punctuate the course of the subsequent SARS epidemic.

On February 21, 2003, a health care worker in a hospital in the city of Guangzhou, the provincial capital of Guangdong, arrived in Hong Kong and checked in at Hotel M. He had treated patients with "atypical pneumonia" in Guangzhou and had been ill himself since 15 February. His one-day stay on the ninth floor at this hotel led to the infection of at least 17 other guests or visitors, some of whom traveled later to Hanoi, Toronto, Vancouver, Singapore, USA, Philippines, Guangzhou, and Australia. Five of these secondary cases initiated clusters of infection in Hanoi, Singapore, Toronto and two clusters of infection in Hong Kong. This was the most significant single event in the global spread of SARS, and arguably the most dramatic single event in the global spread of any infectious disease.

In response to the outbreaks in Hanoi and Hong Kong, on 12 March WHO issued a Global Health Alert warning of a pneumonia that was a particular risk to health care workers. Subsequently, Singapore and Toronto also reported clusters of cases. On 15 March, WHO issued a Travel Advisory. The new disease was named Severe Acute Respiratory Syndrome (SARS) and a preliminary case definition was provided. Within weeks, SARS had spread to affect 8096 patients in 29 countries across five continents with 744 fatalities, an overall case-fatality rate of 9.6%. Health care facilities served as a major amplifier of the infection, constituting 21% of all reported cases. By 21–24 March, the etiological agent of SARS was identified to be a novel coronavirus, subsequently named as SARS coronavirus (SARS-CoV). Early case detection and isolation of infected individuals reduced and interrupted SARS-CoV transmission throughout the world. By July 5, 2003, WHO announced that all chains of human transmission of SARS were broken and the outbreak was at an end. Although SARS was subsequently to re-emerge to cause limited human disease (and in one instance, limited human-to-human transmission) as a result of laboratory escapes and zoonotic transmission from live game animal markets of Guangdong in December 2003–January 2004, the human outbreak of SARS had been efficiently controlled.

Emergence of MERS: Almost a decade later, a virus was identified from a patient with a severe pneumonia in Saudi Arabia and later identified to be a novel betacoronavirus, initially named EMC, but later renamed as Middle East Respiratory Syndrome coronavirus (MERS-CoV). The report of this discovery via Promed on September 20, 2012 led to recognition of other cases, and to a retrospective recognition that a previously undiagnosed cluster of patients with pneumonia in Jordan in 2012 was linked to the same virus. As of October 2020, ca. 2600 confirmed cases of MERS with 880 deaths (34%) have been reported to WHO from 27 countries.

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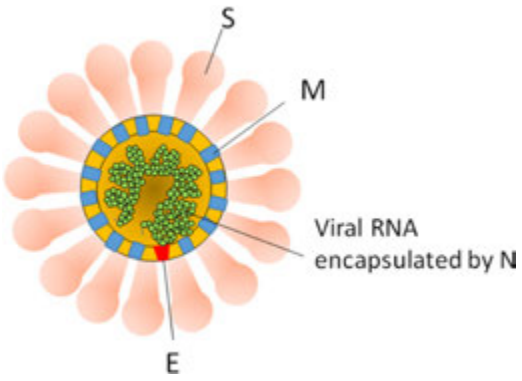

Fig. 1 Schematic drawing of a SARS or MERS viral particle.

Virology

SARS- and MERS-CoVs

Coronaviruses are currently genetically classified into 4 genera (α, β, γ, and δ) and a putative one (ε). SARS- and MERS-CoVs are now classified within the sub-genus Sarbecovirus and Merbecovirus, respectively, in the genus *Betacoronavirus* within the family *Coronaviridae*, under the order *Nidovirales*. Coronaviruses are enveloped positive-sense, single-stranded RNA viruses with a genome size of approximately 30 kb. Their virus particles are about 100–140 nm in diameter with a distinctive corona of petal-shaped spikes on the surface which is comprised of the spike glycoprotein (S) (Fig. 1). The S protein is in a trimeric form on the viral surface and it has an N-terminal subdomain (S1) which contains the motifs responsible for receptor binding. A more conserved C-terminal subdomain (S2), which contains heptad repeats and a coil-coil structure, is important in the membrane fusion process. The S1–S2 subdomains remain in a non-cleaved form in newly synthesized progeny viral particles and cleavage of S into S1 and S2 subunits is essential for the viral entry process. The envelope also contains a transmembrane glycoprotein M and in much smaller amounts, an envelope (E) protein. The M protein is a triple-spanning membrane protein and it has a key role in coronavirus assembly. The hemagglutinin-esterase (HE) glycoprotein, found in some betacoronaviruses, are absent in both SARS- and MERS-CoVs. The nucleocapsid protein (N) interacts with the viral genomic RNA to form the viral nucleocapsid. Within the cell, viral replication complexes are located at double-membraned vesicles or autophagosomes within the cell for viral RNA synthesis.

SARS- and MERS-CoV Genomes

The genome organization of SARS- or MARS-CoV is that of typical coronaviruses. The viral genomes of SARS- and MERS-CoVs have, respectively, at least 14 and 11 open reading frames (ORFs) (Fig. 2(A)). Both genomes code for 16 nonstructural proteins (nsp1–16) in their replicase genes (Fig. 2(B)). The capped genomic RNA encoding the replicase gene functions as mRNA to generate polyproteins 1a and 1ab. The translation of ORF1b is directed by a –1 ribosomal frameshift (RFS) signal that contains a nucleotide slippery sequence and an RNA pseudoknot. By contrast, the structural and accessory proteins are products derived from capped subgenomic RNA (sgRNA) which are synthesized by discontinuous RNA transcription.

The polyproteins 1a and 1ab generated from the replicase gene of SARS- or MERS-CoV are cleaved by a papain-like proteinase (part of nsp3) and a 3C-like proteinase (nsp5 or 3CLpro) to generate 16 nsps (Fig. 1(B)). As SARS-CoV is relatively well characterized, the biological functions of these nsps described below are primarily based on the findings of studies on SARS-CoV. Nsp12 is a primer-dependent RNA-dependent RNA polymerase (RdRp), whereas nsp8 is a noncanonical RdRp (nsp8) synthesizing primers utilized by nsp12. The N-terminus of Nsp12 has a domain (nidovirus RdRp-associated nucleotidyltransferase, niRNA) which is unique to nidoviruses. In addition, eight nsp7 and eight nsp8 subunits are able to form a hexadecamer with a hollow, cylinder-like structure. RNA-binding studies and the overall architecture of this nsp7–nsp8 complex suggest that it may encircle RNA and confer processivity of nsp12. The nsp9 is a single-stranded RNA-binding protein and is able to interact with nsp8. The dimerization of nsp9 is essential for virus replication. The nsp10 contains two zinc finger motifs and is suggested to be a regulator of vRNA synthesis. The nsp13 is a helicase and unwinds duplex RNA (and DNA) in a 5′-to-3′ direction. The nsp14, nsp15, and nsp16 have been shown to have 5′-to-3′ exonuclease, endoribonuclease, and 2′-*O*-ribose methyltransferase activities, respectively. These three proteins, together with nsp3 (see below) are distantly related to cellular enzymes involved in RNA metabolism. These observations may be relevant to viral RNA processing. The exonuclease activity of nsp14 is known to be essential for replication fidelity. The nsp10, 13, 14 and 16 are the subunits essential for forming the cap structure of viral RNA. The nsp3 contains multiple domains and it has 8 domains which are conserved in all coronaviruses (ubiquitin-like domain 1, hypervariable region, X domain, ubiquitin-like domain 2, papain-like protease 2 domain, zinc-finger domain and Y1 and CoV-Y domain). Apart from facilitating viral RNA synthesis and releasing nsp1-nsp3 from the polyprotein 1a and 1ab, nsp3 is also involved in various post-translation modifications (de-ADP-ribosylation, deubiquitination, and de-ISGylation) and evasion of host innate immunity. The biological functions of nsp1, nsp2, nsp4, nsp6, and nsp11 are not entirely clear. The nsp1 is reported to induce chemokine dysregulation and

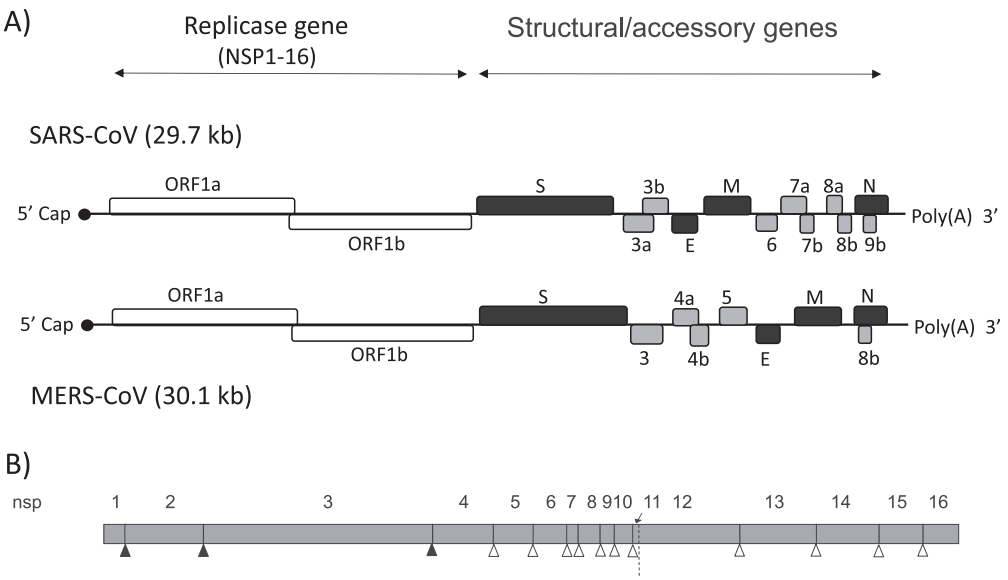

Fig. 2 SARS and MERS CoV genomes. (A) Genomic organization of SARS and MERS CoVs. The ORFs are expressed from the genomic RNA and a nested set of subgenomic mRNA (not shown) that all have a common leader sequence derived from the 5′ end of the genome will be expressed. The genomic RNA and all sgRNA contain a 5′ cap and a polyadenylated tail at the 3′ end. (B) Domain organization of the proteins for ORF1ab. Black and white arrow heads represent the sites cleaved by papain-like and 3C-like proteinases, respectively. The ribosomal frameshift (RFS) site is highlighted by a broken line.

host mRNA degradation, and regulate host gene expression. The nsp2 is reported to be involved in mitochondrial biogenesis and intracellular signaling, but it is dispensable for virus replication. Both nsp4 and nsp6 contain a putative transmembrane domain and they are suggested to be essential for membrane rearrangement. The majority of nsps of MERS-CoV (nsp3, 5, 7–10, 12–16) are assumed to have similar biological functions, whereas the functions of other MERS-CoV nsps are not clear.

Both SARS- and MERS-CoVs have 5 structural proteins (S, E, M, ORF3a and N) encoded by the corresponding ORFs as indicated (**Fig. 2(A)**). The proteins are the basic protein components of the viral particles. Some studies suggest that ORF3a of SARS-CoV is also a structural protein. This protein can induce membrane rearrangement and activate NF-κB and the NLRP3 inflammasome in infected cells. However, the precise function of this protein in these virions is not entirely clear.

Apart from the ORFs encoding the replicase and structural proteins, SARS-CoV and MERS-CoV genomes contain additional ORFs that code for accessory proteins (SARS-CoV: 3b, 6, 7a, 7b, 8a, 8b, and 9b and MERS-CoV: 3, 4a, 4b 5 and 8b). The accessory proteins of SARS- and MERS-CoV have very different sequences, suggesting that the proteins may have very different biological functions. Genetically modified recombinant SARS- and MERS-CoVs without these accessory ORFs have been shown to be replication competent in cell cultures, indicating that the accessory ORFs may not be essential for virus replication *in vitro*. However, recombinant viruses with deletions in these regions are attenuated *in vivo*, suggesting that these proteins may have functions that are important for viral replication, immune evasion and pathogenesis *in vivo*.

The accessory proteins of ORF3b and ORF7a of SARS-CoV induce apoptosis in transfected cells. ORF3b is reported to act as an interferon antagonist during the infection. There is also evidence suggesting that the 7a protein is incorporated into virions. The protein encoded by ORF6 has been shown to inhibit the nuclear import of STAT1 and function as an interferon antagonist in SARS-CoV-infected cells. In addition, this protein is also reported to interact with the viral RNA transcription complex. These properties may be related to virus virulence. Interestingly, comparative sequence analysis of SARS-CoV isolated from palm civets (see below) and humans showed that all animal strains contained a 29-nucleotide (nt) sequence which is absent from most human strains obtained in the later phase of the SARS outbreak. The ORF8 in human SARS-CoVs encodes 8a and 8b proteins, whereas the corresponding ORF in the animal isolates encodes a single protein, known as the 8ab protein. These proteins from the animal and human ORF8 have differential binding affinities to various SARS-CoV structural proteins. Furthermore, the expression of E can be downregulated by 8b but not 8a or 8ab in infected cells. These observations may suggest that the 29-nt deletion modulates the replication or pathogenesis of the human SARS-CoV. The crystal structure of the 9b protein suggests that it may be a lipid binding protein and that it may be a virion-associated accessory protein. But its function is yet to be identified. Overall, these accessory proteins may have a role in viral replication and pathogenesis.

The biological functions of MERS-CoV accessory proteins, except ORF4a and ORF4b, are not clear. All the accessory proteins are dispensable for *in vitro* virus cultures. ORF4a and ORF4b of MERS-CoV are known to suppress innate sensing triggered by dsRNA. Thus, these proteins may have essential roles in virus pathogenesis. Interestingly, deletions in ORF3 and ORF4b sequence can be found in MERS-CoV circulating in West and North African dromedary camels, but not in those animals found in East Africa and the Arabian Peninsula (see below).

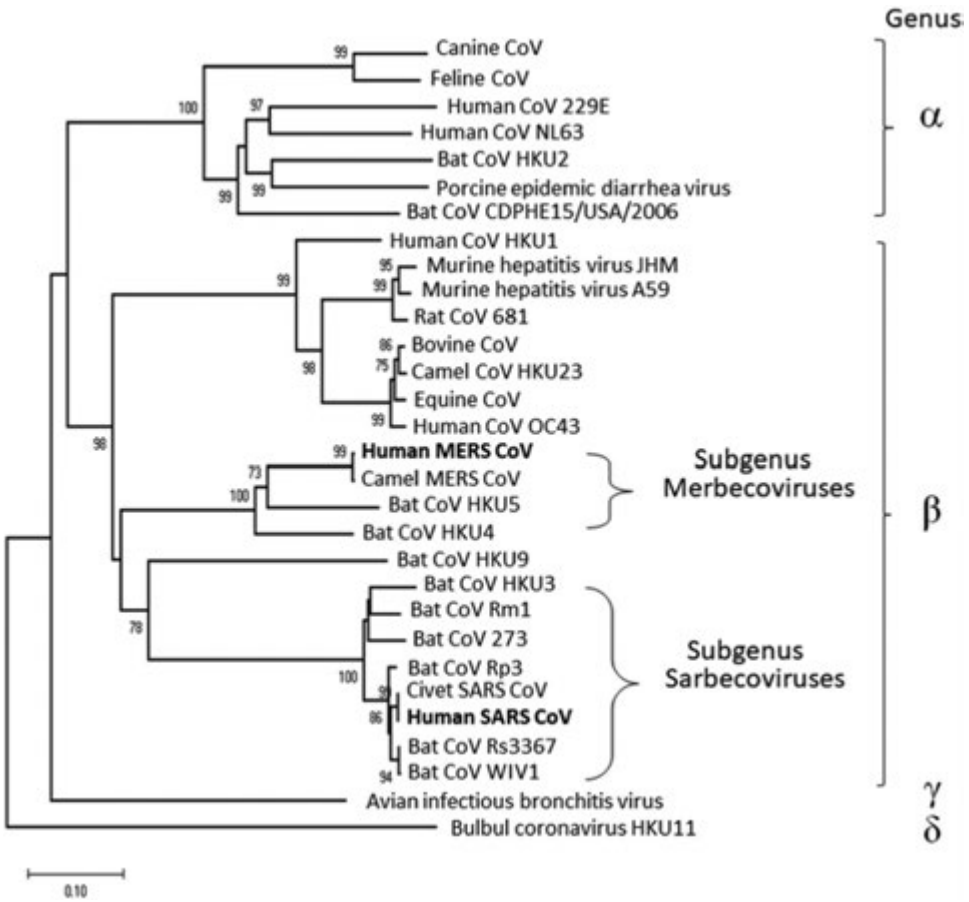

Fig. 3 Phylogenetic analysis of representative RNA-dependent RNA polymerase sequences from α, β, γ and δ coronaviruses. The phylogenetic tree was constructed by the neighbor-joining method and bootstrap values were determined with 500 replicates. Bootstrap values are shown as indicated. Scale bar indicates an estimated genetic distance.

Phylogeny

The phylogeny of nidoviruses is based on the concatenated 5 highly conserved domains in 3 nsps (nsp5: 3CLpro; nsp12: NiRAN and RdRp; nsp13: ZBD and HEL1). Currently, viruses under the same subgenus have patristic pairwise distances less than 0.22. As formal virus taxonomy analyses primarily focuses on (almost) full genome sequences, partial virus sequence fragments derived from potentially novel virus species are not considered in these analyses. Thus, virus diversity within each subgenus is expected to be underestimated. SARS-CoV, MERS-CoV and their related animal viruses are grouped into subgenera *Sasbecovirus* and *Merbecovirus*, respectively (**Fig. 3**).

The search for the precursor of SARS-CoV led to the discovery of a number of novel coronaviruses in both wild and domesticated animals. In particular, a vast number of alphacoronaviruses and betacoronaviruses were identified in bats. Interestingly, these recently discovered bat coronaviruses appear to be in evolutionary stable while many other mammalian coronaviruses still appear to be under evolutionary selection pressure, raising an intriguing possibility that bats may in fact be the precursors, not only of SARS-CoV, but also for many other mammalian coronaviruses. It is suggested that some other human coronaviruses, such as NL63 and 229E primarily originated from bat coronaviruses.

Genetic and phylogenetic analysis indicates that the viruses associated with the early phase of the human SARS outbreak are more closely related to the viruses found in palm civets and other small mammals in the live game animal markets in Guangdong. The genomes of viruses in the early phase of the human outbreak in 2003 were observed to be under strong positive selective pressure, suggesting that the virus was rapidly adapting to the new host, humans. Furthermore, SARS-CoV in civets was also found to be under strong positive selection pressure, supporting the view that civets were not the natural host of the precursor SARS-like coronavirus.

MERS-CoV found in humans and camels can be divided into 3 clades (A–C). Both clades A and B viruses were found in humans and camels from the Arabian Peninsula, whereas clade C viruses found in African camels are very diverse. The genomes of these viruses are genetically relatively stable, but some studies suggest that the S genes of these camel and human viruses are under positive selection pressure. This may be due to adaptive evolution of these viruses to achieve better attachment to the cells of their new hosts. Deletions in accessory genes of MERS-CoV were found in camel and human viruses. In addition, recombination

between MERS-CoV in camels or humans has been reported, for example, giving rise to the lineage 5 clade B viruses currently causing outbreaks of human disease in the Arabian Peninsula.

Virus Receptors

The S1 subunit of coronavirus spike proteins contains two distinctive structural domains, the N-terminal domain (NTD) and the receptor binding domain (RBD). The RBD binds to the major functional receptor to achieve viral attachment and entry. However, work on other coronaviruses indicate that the NTD may also have strain-specific binding affinity to other host proteins or sugar residues. Both SARS- and MERS-CoVs can bind to multiple non-functional receptors and it is generally assumed that the NTDs may also facilitate virus-host interactions.

The functional receptor for SARS-CoV in human cells is the angiotensin-converting enzyme 2 (ACE-2) which binds the RBD (amino acid residues 424–494) of the SARS-CoV S protein. While the human SARS-CoV S protein binds efficiently to both human and civet ACE-2, the civet-like SARS-CoV S protein binds efficiently to ACE-2 from civets but poorly to human ACE-2. These findings explain the increased human transmissibility of SARS-CoV in the later stages of the SARS outbreak. An observation that human SARS-CoV efficiently infects civets under experimental conditions, the poor virulence and transmissibility of re-emergent SARS in December 2003–January 2004 when humans are believed to have been infected with a civet-like SARS-CoV and the observation that there was approximately 20% sero-prevalence to SARS-CoV in those working in Guangdong live game animal markets although none of them had SARS-like disease. The spike protein of some bat SARS-like coronavirus binds to human ACE-2. Other cell-surface molecules such as L-SIGN, DC-SIGNR, DC-SIGN (CD209), and LSECtin may serve as binding receptors but they do not appear to be functional viral receptors in the absence of ACE-2. They may, however, promote cell-mediated transfer of the virus to other susceptible target cells.

The RBD of MERS-CoV S protein (AA residues 484–567) can bind to its human functional receptor, dipeptidyl peptidase-4 (DPP4). The RDB of human MERS-CoV is identical to that of dromedary camel virus. MERS-CoV can experimentally infect animals having DPP4 with conserved critical binding residues to those of human DPP4 (e.g., camelids, primates, and rabbits). By contrast, animals with critical DPP4 residues that are different to those of human DPP4 are not susceptible to MERS-CoV infection (e.g., mice, rats and ferrets). MERS-CoV is also known to bind to sialic acid, CEACAM5 and GRP78.

Epidemiology

Ecology, Animal Reservoir and Zoonotic Transmission

Both SARS- and MERS-CoVs are of animal origin, but their host origins and modes of zoonotic transmission differ (see below).

SARS

In the early phase of SARS epidemic (before the end of January 2003), 39% of patients with SARS in Guangdong had handled, killed, or sold wild animals or prepared and served them as food. However, such risk factors were found in only 2%–10% of cases from February to April 2003 when the virus had adapted to efficient human-to-human transmission. Thus, the early epidemiological evidence pointed to the live game animal trade as an interface for the emergence of the SARS-CoV. SARS-like coronaviruses were identified in a number of small mammalian species sold in the live game animal markets in Guangdong, including the palm civet (*Paguma larvata*), raccoon dog (*Nyctereutes procyonides*), and the Chinese ferret badger (*Melogale moschata*). A high proportion of individuals working in these markets had developed antibodies to SARS-CoV, although none of them had a history of the disease. Viruses isolated from the re-emergent SARS cases in Guangdong in December 2003–January 2004 were more similar to those found in civets in these markets, rather than to viruses causing the global outbreak in early 2003. These observations strongly implicated that the live game animal trade is the interface for interspecies transmission of a precursor animal SARS-like coronavirus to humans.

SARS-CoV is shed for weeks in experimentally infected palm civets but many of the other species appear to clear the virus more rapidly. While civets in live-animal markets tested positive for SARS-like coronavirus RNA and antibody, civets tested in the farms that supply these markets and those caught in the wild rarely had evidence of infection. Thus, although palm civets and other small mammals sold in these markets were likely the intermediate host that amplified and maintained the virus in these markets and provided the source of virus for repeated human exposure to zoonotic infection, they were not the natural reservoir of the precursor SARS-CoV. Several sarcoviruses related to SARS-CoV have now been identified in *Rhinolophus* bats in China. Such bats were also sold live in these game animal markets. So far, none of the reported bat CoV is genetically identical to SARS-CoV. It is now believed that SARS-CoV is a recombinant virus of bat sarbecoviruses. Studies in Yunnan, China, has revealed several bat coronaviruses containing all genetic elements needed for generating SARS-CoV in a single geographical location.

MERS-CoV

Is enzootic in dromedary camels which is the main source of zoonotic infection. Bactrian camels have no evidence of infection. The majority of dromedary camels in the Middle East, Africa and Central Asia have serological evidence of MERS-CoV infection. Infection in dromedary camels is characterized as a mild upper respiratory infection. Virus shedding in camel herds is highly seasonal (winter months and the calving season) and is most commonly seen in calves although adults can get re-infected. Settings

where camels from different sources intermingle (camel markets, abattoirs, racing meets) are associated with higher rates of virus detection and may show less pronounced seasonality. Zoonotic transmission is stochastic and uncommon and the routes by which such transmission occurs are unclear. Direct contact with infected camel upper respiratory secretions is one presumed route of infection to humans. A gastro-intestinal route of infection has also been proposed, based on studies of virus tropism in the human gastrointestinal tract and experimental animal models. While virus RNA has been detected in camel milk, presumably derived by the suckling calf contaminating the udder and thence the milk. Viable virus has not been isolated from milk and it is likely that passive maternal antibody found in camel milk would neutralize infectious viruses.

Most zoonotic transmission appears to be asymptomatic or mild, while those individuals with underlying co-morbidities are more likely to manifest severe disease. Over 50% of patients with laboratory confirmed "primary" MERS infection (not explainable by potential contact with other MERS patients) had recent direct or indirect contact with camels. Case-control studies have confirmed direct exposure to dromedaries within two weeks of disease onset as a significant risk factor.

MERS-CoV transmission between humans can lead to outbreaks, especially within health care facilities. Transmission may also occur within households or other community settings but this is less frequent. MERS patients have been diagnosed in 27 countries to date, some associated with index cases acquiring infection in the Arabian Peninsula. Zoonotic MERS has only been reported in the Arabian Peninsula, not in Africa where the majority ($>70\%$) of infected camels are found. The reason for this is at present unclear. MERS-CoV found in African camels is genetically highly similar but phylogenetically distinct from those found in camels in the Arabian Peninsula.

MERS-CoV neutralizing antibody was detected in archived serum samples collected in 1983, indicating that the virus has been circulating in camels for over 30 years. It is unclear whether dromedary camels are the primary reservoir of MERS-CoV. There are several merbecoviruses found in *Tylonycteris* and *Pipistrellus* bats, suggesting that the precursor(s) of MERS-CoV derives from bats but a closely related ancestor is yet to be found.

Human-to-Human Transmission

Both SARS and MERS-CoV appear to be more stable and remain viable for longer on surfaces and in aerosols than other enveloped respiratory viruses. Both viruses are more stable at low humidity and low temperature environment such as those found in air-conditioned spaces. This may explain why the major outbreaks of both have occurred in air conditioned environments such as modern hospitals. The findings are also compatible with epidemiological observations that transmission may occur via fomites, large droplet and aerosol routes. Aerosol generating procedures such as tracheal intubation, manual ventilation before intubation, non-invasive ventilation and tracheostomy have contributed to the transmission in health care settings. SARS-CoV is present in high titers in feces and this may well be a source of infection. The largest single outbreak of SARS in an apartment block in Hong Kong where over 300 individuals were infected from a single index case, is believed to have been caused by aerosols generated from infected feces via a faulty sewage system.

The incubation period for both SARS and MERS ranges from 2 to 14 days. While a asymptomatic infection is rare in SARS it appears to be more common in MERS. The majority of cases did not transmit disease at all and only a few patients accounted for a disproportionately large number of secondary cases, referred as "super-spreading events". While some host factors may play a role in these super-spreading events, in many cases there was a unique combination of host factors and environmental circumstances that facilitated transmission. In contrast to the high transmission rates in these super-spreading events and within hospitals, there was less evidence of secondary transmission within the family or households.

SARS-CoV viral load in the upper respiratory tract was lower in first few days of illness and it peaked in the beginning of the second week of the disease. This correlated with minimal transmission occurring in the first five days of illness and explains why health care-associated outbreaks was more common because hospitalization and use of aerosol generating procedures are more common towards the end of the first week of illness. Taken together, with the lack of asymptomatic infections the public health measures of aggressive case detection and isolation lead to efficient interruption of transmission of the SARS-CoV outbreak in 2003. The characteristics of the epidemiology of MERS is generally comparable to that of SARS, except that asymptomatic infections are more common in MERS.

The basic reproduction number (Ro) of SARS is estimated to range from 2 to 4 while that for MERS over the course of an outbreak is <1. However, there is considerable heterogeneity of effective reproduction number (Rt) during the course of an outbreak, being 1.3–5.4 in the early stages of a hospital outbreak followed by a decrease to <1 within 2–6 weeks.

Clinical Features

The clinical features of SARS and MERS are not pathognomonic. Fever, cough, headache, myalgia, nausea, diarrhea and malaise are commonly reported at early stages of the disease followed by progression to shortness of breath, acute respiratory distress syndrome (ARDS) and multiple organ failure in more severe cases. Rhinorrhea was uncommon in both diseases (**Table 1**). Lymphopenia was common in both diseases. Differences seen in MERS compared to SARS include a higher proportion of patients who are older and/or with co-morbidities and the presence of hypotension that lead to the need for vasopressors. In both diseases, symptoms may be atypical in immune-compromised patients. A contact history with SARS-CoV positive individuals was an important epidemiological risk factor for SARS. A travel history to the Arabian Peninsula, direct contact with dromedary camels or visiting health care facilities in these affected areas are important factors relevant to initiating specific diagnostic tests for MERS.

Table 1 Comparison of virological, clinical and epidemiological features of SARS and MERS

	SARS	*MERS*
Virus taxonomy	Genus betacoronavirus; subgenus Sarbecovirus	Genus betacoronavirus; subgenus Merbecovirus
Receptor	ACE2	DPP4
Animal reservoir and zoonotic source	Natural reservoir Rhinolophus bats, Zoonotic interface – live game animal markets	Natural reservoir: unknown. Natural host and source of zoonotic infection: Dromedary camels
Clinical and epidemiological aspects (see notes below)		
Health care workers	21%	Approx. 17%
Males	47%	Approx 63%
Fever	++++	++++
Headache	++	+
Myalgia	+++	++
Cough	+++	+++
Shortness of breath	++	++
Sore throat	+	+
Nausea/vomiting	+	+
Diarrhoea	+	+
Rhinorrhoea	(+)	(+)
Co-morbidities		
Diabetes	+	++
Hypertension	Not noted	++
Chronic lung disease	+	+
Chronic renal failure	<1%	+
Chronic heart disease	+	+
Malignancy	+	+

Approximate frequency of occurrence: + 1%–25%; ++ 26%–50%; +++ 51%–75%; ++++ 76%–100%.

Radiological abnormalities were observed in >60% of cases at initial stages of the disease and preceded lower respiratory tract symptoms in approximately 41% of patients with SARS. In MERS, chest radiographs or computed tomography scans showed multilobar airspace disease, ground glass opacities, and sometimes, pleural effusions, but some patients with mild or asymptomatic infection may show minimal changes in lungs. Liver dysfunction and lymphopenia are seen in both infections. High serum levels of chemokines (IL-8, CCL2, and CCl10) and pro-inflammatory cytokines have been seen in patients with SARS. In MERS, higher serum levels of IL-6 was associated with a severe disease.

The overall case fatality ratio for confirmed SARS and MERS cases has been 9.6% and 35%, respectively. Since MERS-CoV infections, unlike SARS, can often be mild and unrecognized by the health care system, this apparently higher fatality rate may be misleading. In both diseases, infections of children and young adults have been mild. Case-fatality rate increased progressively with increasing age in SARS and with the presence of co-morbidities such as diabetes, cardiac disease, obesity, chronic respiratory disease, end stage renal disease in the case of MERS.

Autopsy findings of those who died in the first 10 days of illness with SARS were diffuse alveolar damage, desquamation of pneumocytes, and hyaline membrane formation. Viral RNA was detected by quantitative polymerase chain reaction (PCR) at high copy numbers in the lungs, intestine and lymph nodes, and at lower levels in the spleen, liver and the kidneys. In the lungs of the patients who died within the first 10 days after disease onset, viral antigen and viral nucleic acid were detectable by immunohistochemistry and *in situ* hybridization in alveolar epithelial cells and to lesser extent in macrophages. Some studies also reported virus particles or viral RNA in other organs but these findings require independent confirmation.

There are limited autopsy studies on MERS. A diffuse alveolar damage was seen in a man who died approximately 12 days after onset of illness. MERS-CoV antigen was detected in pneumocytes and syncytial epithelial cells. No evidence of infection in the kidneys was observed. In an immunocompromised patient with a T cell lymphoma who died of MERS around 3 weeks after onset, histopathological examination showed necrotizing pneumonia, pulmonary diffuse alveolar damage, acute kidney injury, portal and lobular hepatitis and myositis with atrophic changes in the muscles. The brains and the heart were histologically normal. Virus infection was demonstrated in pneumocytes, pulmonary macrophages and renal proximal tubular epithelial cells.

Laboratory Diagnosis

Specimens from the lower respiratory tract such as sputum or endotracheal aspirates have higher viral load and give a higher diagnostic sensitivity than those from the upper respiratory tract for both SARS or MERS. Highly sensitive and specific real-time PCR assays for the detection of viral RNA remain the best choice for early diagnosis. For diagnosis of MERS, initial screening by

RT-PCR targeting the E gene followed by confirmatory tests with RT-PCR for the ORF1 gene region is recommended and only patients positive in both assays are diagnosed as confirmed cases of MERS.

While SARS-CoV RNA remains detectable in the respiratory secretions and feces for many weeks after the onset of illness, specimens rarely yield a virus isolate after the third week of illness, likely because of the emerging antibody responses. Viral RNA can be detected in feces, serum, and urine in patients with SARS, later in the illness. MERS-CoV RNA may also be detected in serum approximately in one-third of patients, especially those with a more severe disease; and in the stool of around 15% of patients, less often that was seen in SARS. In both SARS and MERS, a negative PCR result in an individual specimen does not exclude the disease. Testing multiple specimens improves the diagnostic accuracy. Both viruses can be cultured from clinical specimens in Vero or Vero-E6 cells but the sensitivity of virus culture is much lower as compared to RT-PCR.

Immunofluorescence or neutralization tests have been used for serological diagnosis or sero-epidemiology of SARS-CoV. A MERS-CoV S1 ELISA is available for the detection of MERS-CoV antibody. Positive results are best confirmed by neutralization tests with live MERS-CoV or spike-pseudotyped virus. Seroconversion occurs during the second week of illness in both diseases and can provide reliable retrospective diagnosis. While a seroconversion is invariable in patients with a severe MERS disease, antibody responses may be marginal or absent in those with a mild MERS infection. Waning antibody responses need to be considered in sero-epidemiologial studies. MERS-CoV-specific T cell response has also been used as an indicator of past infection that may complement conventional serology.

Animal Infection Models

Experimental animal models are important in the studies related to pathogenesis, transmission and the effectiveness of therapeutics and vaccines. Experimental animal models for both SARS and MERS are sub-optimal, especially in relation to pathogenesis and there are only few that serve to investigate transmission.

SARS

Experimental infection with SARS-CoV leads to virus replication in Cynomolgous and Rhesus macaques, African green monkeys and marmoset monkeys, with variable manifestations of the disease. Part of the variation in the development of the disease may relate to age (older animals develop more severe disease) or virus strains used.

Mice (BALB/c, C57/BL6, 129SvEV-lineage), Syrian golden and Chinese hamsters, ferrets, and cats are also susceptible to SARS-CoV. Only some of these animals develop pathological lesions in the lungs (ferrets, hamsters, marmosets, aged BALB/C mice). Interestingly, while SARS-CoV replicates in the lung of both young and aged (12–14 months) BALB/c mice, only aged mice manifest clinical symptoms and histological evidence of lung pathology. This is reminiscent of the disease in humans where children suffer only a mild form of the disease. Serial passages in mice has led to the development of virus strains (MA15) with increased pathogenicity in mice. Ferrets are susceptible to SARS-CoV infection and can transmit the virus to other ferrets. They develop fever and show variable histological changes in the lungs but there is no mortality. In most experimental models, viral titers peak early (within first 4 days) and the virus is cleared by the end of the first week, which is not comparable to the human disease. Furthermore, few animal models reproduce the gastrointestinal manifestations of the illness. While an ideal animal model for SARS pathogenesis or transmission is lacking, those that support viral replication (with or without clinical disease) are adequate ones for evaluating the efficacy of vaccines.

MERS

Mice, ferrets, guinea pigs, ferrets and hamsters are not suitable experimental models to study MERS-CoV because the DPP4 receptors of these species do not support virus attachment. Human DPP4 has been transduced into mouse lungs by adenovirus vectors and these mice are susceptible to infection and they develop lung pathology. Human DPP4 (hDPP4) transgenic mice are susceptible to MERS-CoV infection but because the expression of hDPP4 was not physiological in its tissue distribution, infection lead to unusual manifestations such as central nervous system disease, which is not comparable to the one seen in humans. On the other hand, hDPP4 knock-in mice have key regions of the mouse DPP4 replaced with amino acid residues of the hDPP4 and these mice are susceptible to infection. Adapting virus strains by passage in such mice led to virus strains that caused a disease.

Rabbits have DPP4 similar to that of humans and they are, therefore, susceptible to MERS-CoV infection. In contrast to humans, the distribution of DPP4 is predominantly in the upper respiratory tract and consequently, infected rabbits do not develop a lung disease. Marmosets are also susceptible to an infection and a disease although these findings have not been reproduced by different researchers. Rhesus macaques are susceptible to an infection and they manifest a mild form of disease. Dromedary camels, alpacas and llamas are all susceptible to experimental MERS-CoV infection and they can be used as experimental models for infection and transmission. Camels have been used to study the effectiveness of vaccines.

Pathogenesis

SARS

The primary mechanism of lung damage appears to be due to infection of type 1 and type 2 pneumocytes which are the key target cells of the virus. Type 2 pneumocytes are important in the repair of lung injury and infection of these cells can potentially impair the regenerative processes of the lung and aggravate the respiratory impairment. While mice deficient in NK, T or B lymphocytes display similar kinetics of viral replication to that seen in normal mice, infection of mice with defects in the STAT1 signaling pathway results in a more prolonged viral replication and a more severe disease. These findings indicate the importance of innate immune responses in the control of the infection, at least in the mouse. Infection of epithelial cells, macrophages, and myeloid dendritic cells fails to induce a type 1 interferon response although IFN-inducible genes are activated. Multiple accessory proteins mediate innate immune evasion leading to innate immune dysregulation. Patients with a severe SARS had higher plasma levels of IFN-γ, IL-1, IL-6. IL2, TGBβ, CCL2, CXCL10, CXCL9, and IL8 compared to those with an uncomplicated disease. There is evidence of viral replication within intestinal epithelial cells but there is minimal cellular infiltrate or disruption of intestinal architecture and the pathogenesis of diarrhea in SARS remains unclear.

MERS-CoV

DPP4, the receptor for MERS-CoV is less abundant in upper respiratory tract epithelium but it is expressed in the epithelial cells of the distal airways and alveoli as well as in the kidneys, intestine, liver, thymus and the bone marrow. Patients with a chronic lung disease have an increased DPP4 expression. Limited autopsy studies and in ex vivo cultures of human lung specimens infected with MERS-CoV have identified evidence of virus infection in ciliated epithelial cells of the bronchioles, type 1 and 2 alveolar epithelial cells in the lung, alveolar macrophages, lung microvascular endothelial cells, renal proximal tubular epithelial cells and human primary intestinal epithelial cells or small intestine explants. The DPP4 distribution and tropism of the virus explains why MERS-CoV causes severe lower respiratory tract infections with minimal upper respiratory symptoms and also explains the mild renal dysfunction observed in patients with MERS and virus detection in the stool and urine in some patients. Unlike SARS-CoV, MERS-CoV can infect human dendritic cells and can infect activated T cells leading to apoptosis. Thus, MERS-CoV may impair adaptive immune responses. As with SARS, MERS-CoV has potent mechanisms for innate immune evasion which contribute to innate immune dysregulation and pathogenesis. Patients with a severe MERS had higher plasma levels of IL-6, IL8, CXCL10 and CCL5 compared those with a mild disease.

Treatment

In the absence of specific antiviral therapies of proven efficacy, good supportive care remains the major form of therapy for both SARS and MERS.

SARS

SARS emerged as a disease of unknown etiology and empirical therapeutic options were initially tested including broad spectrum antivirals and immunomodulators such as ribavirin, intravenous immune globulin, type 1 interferon, SARS convalescent plasma and corticosteroids. However, in the absence of controlled clinical trials, no conclusions can be drawn on the efficacy or adverse effects of these interventions.

A clinical trial of 400 mg lopinavir with 100 mg ritonavir orally every 12 h (added to an existing regimen of ribavirin and corticosteroid therapy) appeared to provide clinical benefit compared to historical controls. However, the lack of concurrent controls makes it difficult to draw conclusions. Similarly, a limited clinical trial of 13 patients using interferon alfacon-1 treatment showed a trend toward improved radiological and clinical outcomes, without, however, reaching a statistical significance. A meta-analysis of observational studies of passive immunotherapy for SARS suggested a decrease in mortality with therapy with convalescent plasma from patients with SARS, particularly if there was evidence of the presence of neutralizing antibodies to SARS-CoV.

Studies in primate models demonstrated prophylactic and therapeutic benefit for PEGylated recombinant interferon α-2b and from small interfering RNA therapies.

Anti-SARS-CoV activity has been demonstrated in vitro, and sometimes in experimental animal models, for several therapeutic substances already in clinical use for other diseases, including lopinavir–nelfinavir, glycyrrhizin, baicalin, reserpine and niclosamide. Screening of combinatorial chemical libraries *in vitro* has lead to identification of potential inhibitors of the viral protease, helicase and spike protein-mediated entry.

MERS

Given the promising observational data with the use of convalescent antibody therapy with SARS, a similar approach was attempted for MERS. However, the poor convalescent antibody levels and waning immunity in patients with MERS and because

many patients are elderly or have co-morbid conditions, it turned out very difficult to collect adequate amounts of convalescent plasma for good clinical trials.

A human polyclonal antibody to MERS-CoV produced in transchromosomic cattle has been shown to be safe in phase 2 clinical trials (AIMS). Similarly, several anti-S monoclonal antibodies with neutralizing activity against MERS-CoV are under development and they have shown *in vivo* efficacy in transgenic mice and *Rhesus macaque* models. Some of these antibodies are proceeding to phase 1 clinical trials.

Corticosteroid treatment of MERS-CoV infected patients was not significantly associated with a difference in mortality but it was associated with a delay in MERS-CoV RNA clearance suggesting a lack of efficacy of corticosteroids against SARS-CoV infection.

Based on the data from *in-vitro* and *Rhesus macaque* challenge studies where high doses of ribavirin and IFN-α2b administered within 8 h post challenge showed partial efficacy in reducing clinical symptoms and viral loads, varying combinations of ribavirin with several interferons (IFN-α2b, IFN-α2a, IFN-β1a) have been used to treat patients with MERS. A retrospective cohort study showed no effect on mortality or viral load, after adjusting for confounding factors. It is now recognized that ribavirin concentrations required to inhibit MERS-CoV need to be far higher than what is clinically acceptable for humans.

A double-blind randomized trial comparing a combination of lopinavir/ritonavir and recombinant IFN-β1b to placebo is presently ongoing in Saudi Arabia.

Vaccines and Immunity

SARS

A wide range of strategies have been explored for the development of SARS vaccines, including inactivated whole virus vaccines, subunit vaccines (baculovirus expressed S1 subdomain or the complete trimeric spike protein of the virus expressed in mammalian cells), DNA vaccines expressing S (full-length and fragments), N, M, or E proteins; and vectored vaccines based on modified vaccinia Ankara (MVA) virus, vesicular stomatitis virus, adenoviral vectors carrying S, M, or N proteins and attenuated parainfluenza virus type 3 vectored vaccines carrying S, E, M, and N proteins. Neutralizing antibody responses and where appropriate, cell-mediated immune responses have been measured as correlates of immunity. Some of these vaccines have been evaluated in experimental models by challenging with infectious SARS-CoV.

It has been shown that the S protein is the principal immunogen that induces antibody response with neutralization-mediated protection. Passive immunization with human monoclonal antibodies against the S protein has been successful at protecting mice and ferrets from an experimental challenge by reducing viral load in the lungs but not in the nasopharynx. However, S protein-based vaccines have also been shown to elicit Th2 immunopathology following experimental live virus challenge.

A newly emerged SARS outbreak will probably arise from an animal reservoir and it is, therefore, important to investigate cross-protection against animal SARS-like CoV. While human SARS-CoV S protein was neutralized by antibodies to the civet SARS-like virus, civet-like S protein was not neutralized by antibodies against the human SARS-CoV. On the other hand, antibodies against human SARS-CoV appeared to enhance the infectivity of the GD03 and SZ3 pseudo-typed viruses. The development of vaccines that can prevent re-emergence of SARV-CoV from its zoonotic reservoir remains a challenge.

MERS

There are no licensed vaccines against MERS so far, although there are some in clinical trials. Pre-clinical studies have shown that both antibody responses to the receptor binding domain of the spike protein and cellular immune responses are required for full protection against MERS-CoV infection. In spite of genetic heterogeneity of MERS-CoV in Africa, virus neutralization epitopes remain conserved and a protective neutralizing antibody-based vaccine to any MERS-CoV strain is likely to cross-protect against most MERS-CoV strains currently circulating in camels. MERS vaccines based on the virus spike protein, live-attenuated, viral vectored vaccines, DNA vaccines and virus-like particle-based vaccines are in the development and they have undergone initial dose-response tolerability and immunogenicity studies. T cell responses have been consistently observed in MERS survivors and they are more long-lasting than antibody responses. In pre-clinical models, intranasal vaccination with the N protein was shown to generate airway memory CD4 T cells responses that protected against a challenge against multiple CoV.

As with SARS, the possibility of disease enhancement by vaccination needs to be considered. Pulmonary Th2-immunopathology, associated with eosinophilic infiltration and increased pro-inflammatory cytokines in the lungs has been observed after immunization with an inactivated MERS vaccine followed by a wild-type MERS-CoV challenge in a transgenic mice model.

Vaccines have also been developed for vaccinating dromedary camels with an idea of reducing zoonotic infections. A vaccinia (MVA) vectored vaccine administered by an intranasal route was shown to protect camels from an experimental challenge. However, an experimental challenge was carried out within a few weeks of vaccination and it remains unclear whether a vaccine will provide protection for several years in field-settings. It is known that a natural infection does not protect seropositive camels against mucosal re-infection of the nasopharynx. It may be that mucosal immunity is more important than systemic serum antibody-mediated. On the other hand, it is possible that even if the vaccine is not providing sterilizing immunity, vaccination may lead to lower peak viral titers, shorter duration of virus shedding, reduced transmission within camel herds and reduce zoonotic transmission. An important aspect of acceptance of such a vaccine by camel owners is the fact that it also protects against camel pox, which is considered as a more important disease of camels than MERS-CoV infection which is a very mild illness in camels.

Conclusion

Given that the precursor viruses of SARS remain in bats, it is conceivable that SARS may return as a public health threat. However, the wild game animal markets which allowed the virus to transmit to humans are now prohibited in mainland China, thus reducing the possibility of potentially new SARS-like viruses to infect humans. However, the situation is very complex, which is evidenced by the emergence of COVID-19 pandemic caused by a SARS-CoV-2 which likely originated from wild game.

MERS-CoV continues to cause zoonotic infections, followed by outbreaks in health care facilities associated with transmission between humans. Such repeated outbreaks may provide the virus an opportunity to further adapt to humans in order to become a virus with a higher potential to infect humans. In addition, we do not know how extensive MERS-CoV infection is in camel-exposed humans in Africa where >70% of infected dromedary camels are found. It is important to understand the extent of zoonotic transmission and disease, if any, in Africa.

Further Reading

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