MICROBIOLOGY AUSTRALIA, 2021, 42, 4–9
https://doi.org/10.1071/MA21003

The threat of zoonotic coronaviruses

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Abstract. Since 2002, three zoonotic coronaviruses (CoV), SARS-CoV, MERS-CoV and SARS-CoV-2 have emerged in humans, establishing that emergence of coronaviruses from animal reservoirs represents a significant pandemic threat. SARS-CoV and MERS-CoV led to smaller epidemics with very high case fatality rates while SARS-CoV-2 resulted in a global pandemic. These zoonotic coronaviruses have their likely origins in bat species and they transmit to humans through intermediate hosts. Coronaviruses can occasionally jump between host species due to their high rate of recombination. Pandemic preparedness requires surveillance in animals and occupationally exposed humans and prevention and treatment strategies that have broad activity against coronaviruses.

Received 13 February 2021, accepted 1 March 2021, published online 12 April 2021

Introduction

Zoonotic transmission of viruses represents a significant threat to human health and viruses that spread via the respiratory route increase their pandemic potential. To date, seven CoVs have been identified in humans, four are endemic in the human (h) population (hCoV-NL63, hCoV-229E, hCoV-OC43 and hCoV-HKU1) and generally cause mild respiratory illness[1]. Since 2002, zoonotic CoVs severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) emerged and led to epidemics associated with severe disease[2,3]. The potential pandemic risk of zoonotic CoVs was cemented when SARS-CoV-2 emerged in 2019, leading to the coronavirus disease 2019 (COVID-19) pandemic.

Coronavirus epidemics and pandemics

The SARS epidemic, caused by SARS-CoV, occurred in 2002-2003 and led to 8098 infections and 774 deaths (9.6% case fatality rate (CFR))[3–5]. The virus emerged in a live-animal market in Guangdong, China[6] and, SARS-CoV spread to 29 countries over eight months[5]. Fortunately, public health measures halted the epidemic in July 2003[7]. With the exception of a small cluster of cases the following year, SARS-CoV has not been detected in humans since[8].

MERS-CoV was first isolated from a patient who died of multiorgan failure following severe pneumonia in Saudi Arabia in April 2012[9]. Since then, 2519 cases and 866 deaths have been reported (34.3% CFR)[10] from 27 countries[2] due to sporadic zoonotic transmission from dromedary camels to humans, with person-to-person transmission limited to healthcare or household settings[2,10].

In December 2019, seven individuals in Wuhan, China were hospitalised with fever, cough, chest discomfort and bilateral lung infiltration[11]. The etiological agent of this new respiratory illness was a novel coronavirus, initially called 2019-nCoV and subsequently formally named SARS-CoV-2 by the International Committee on Taxonomy of Viruses[12]. On 11 March 2020, the World Health Organization (WHO) declared COVID-19 a public health emergency of international concern. Since January 2020, SARS-CoV-2 has spread to every continent with 106 008 375 cases and 2 313 677 deaths (2.2% CFR, Johns Hopkins University Coronavirus Resource Centre) as of 7 February 2021.

Virology

The *Coronaviridae* family are classified into four genera: alpha-, beta-, gamma- and deltacoronaviruses. Betacoronaviruses are further split into four lineages (A, B, C and D). HCoVs NL63 and 229E are alphacoronaviruses and HKU-1 and OC43 are lineage A betacoronaviruses. SARS-CoV, MERS-CoV and SARS-CoV-2 are lineage B betacoronaviruses (Figure 1). CoVs encode a ~30 kb strand of single-stranded positive-sense RNA coated in nucleocapsid (N) protein and enclosed within a lipid bilayer with spike (S), membrane (M) and envelope (E) proteins.

The S protein is comprised of two subunits; S1 is the surface-exposed subunit that includes the receptor binding domain (RBD) that

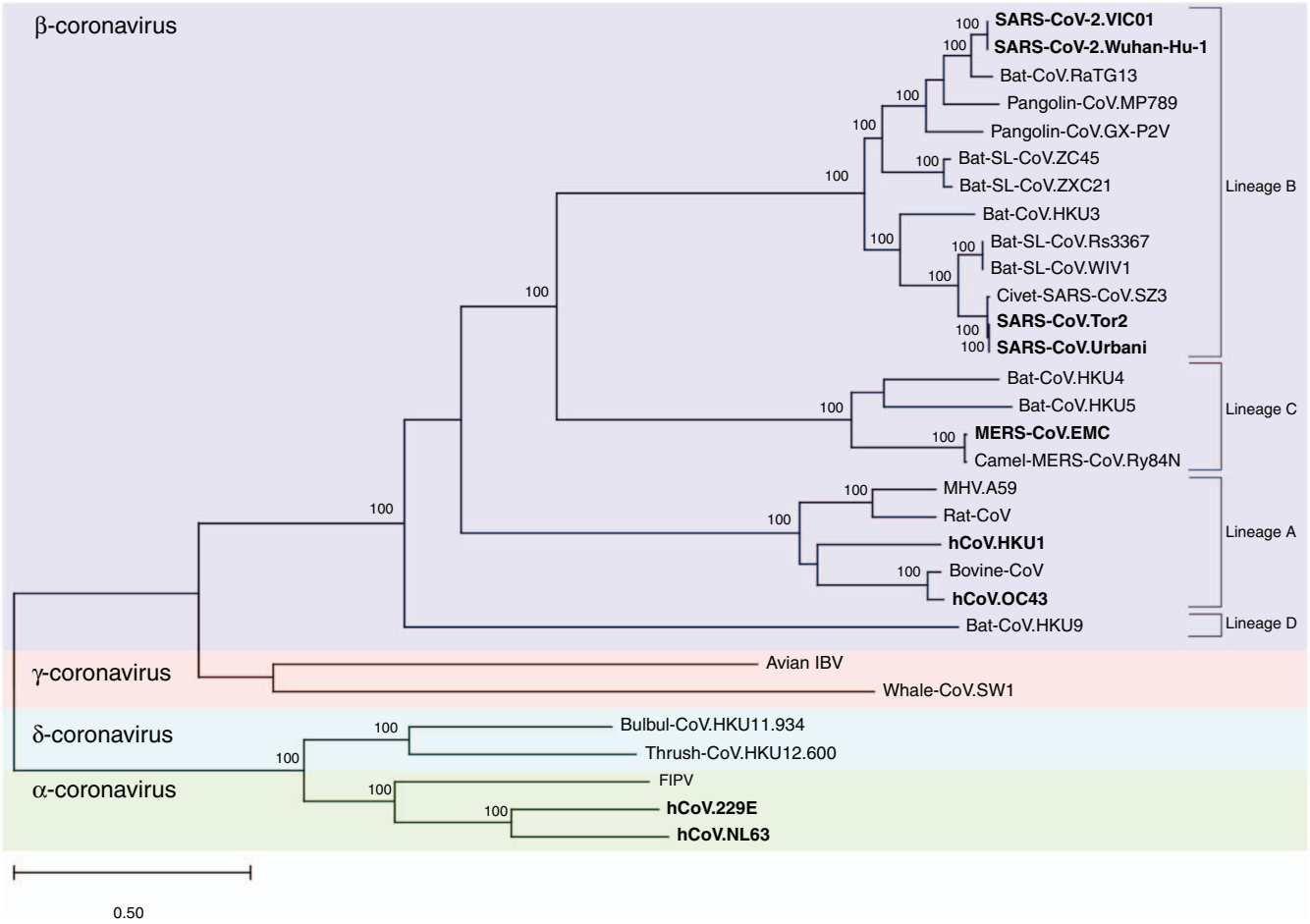

Figure 1. Phylogenetic relationships between coronaviruses. A maximum likelihood tree was generated using the general time reversible method (GTR) incorporating gamma-distributed rate variation among invariant sites. Variance estimation was achieved through 500 bootstrap replicates. Values shown on the tree represent bootstrap values. The scale bar represents substitutions per nucleotide. Coronaviruses are colour-coded by genus: green, alpha-coronaviruses; dark blue, beta-coronaviruses; red, gamma-coronaviruses; light blue, delta-coronaviruses. B-coronaviruses are further split into Lineage A, B, C and D. The sequence labels of coronaviruses that infect humans are in bold.

attaches to the host receptor, and S2 domain that mediates fusion of viral and host membranes[13]. The receptor for SARS-CoV-2, SARS-CoV and hCoV-NL63 is angiotensin converting enzyme 2 (ACE2) and for MERS-CoV is dipeptidyl peptidase 4 (DPP4). Following receptor attachment, the S protein is proteolytically cleaved at two sites to mediate fusion (Figure 2)[13,14]. SARS-CoV, SARS-CoV-2 and MERS-CoV have all been shown to mediate entry through direct membrane fusion or through endosomal entry, depending on the cell type[14].

Replication of CoVs occurs within double membrane vesicles, where positive-sense RNA acts as a template for synthesis of full-length negative-sense RNA, which in turn serve as templates for production of full-length positive-sense RNA to be packaged into virions[14]. Unlike other RNA viruses, coronavirus Nsp14 encodes an exoribonuclease with proof-reading function limiting their error-rate during RNA synthesis[14] in substitutions/nucleotide/replication cycle from 9×10^{-7} for SARS-CoV versus 10^{-3} to 10^{-6} for other RNA viruses[15].

Transcription of CoVs follows a unique discontinuous process that leads to high rates of recombination among CoV genomes during co-infection (Figure 3). CoV genomes contain a transcription-regulatory 'leader' sequence (TRS-L) at the 5′ end[16] and each open reading frame (ORF) contains a 'body' transcription-regulatory sequence (TRS-B) immediately 5′ of the ORF[14]. During negative-strand synthesis, the RNA-dependent RNA polymerase (RdRp) pauses when it reaches the TRS-B and switches template to the TRS-L, fusing the 'leader' sequence to the 5′ end of the gene being copied. Positive strand subgenomic messenger RNAs (mRNAs) are transcribed and translated from these fused negative-strand intermediates[14]. Assembly and release of CoVs requires the nucleocapsid-bound RNA and structural proteins M, S and E to combine near the cell membrane and bud from the cell surface[14].

Reservoirs of coronaviruses

CoVs infect a wide range of host species. Interspecies transfer occurs sporadically (reviewed in[1]). Human CoVs are believed to have originated from bats, bovine and murine hosts[1]. Bats are the

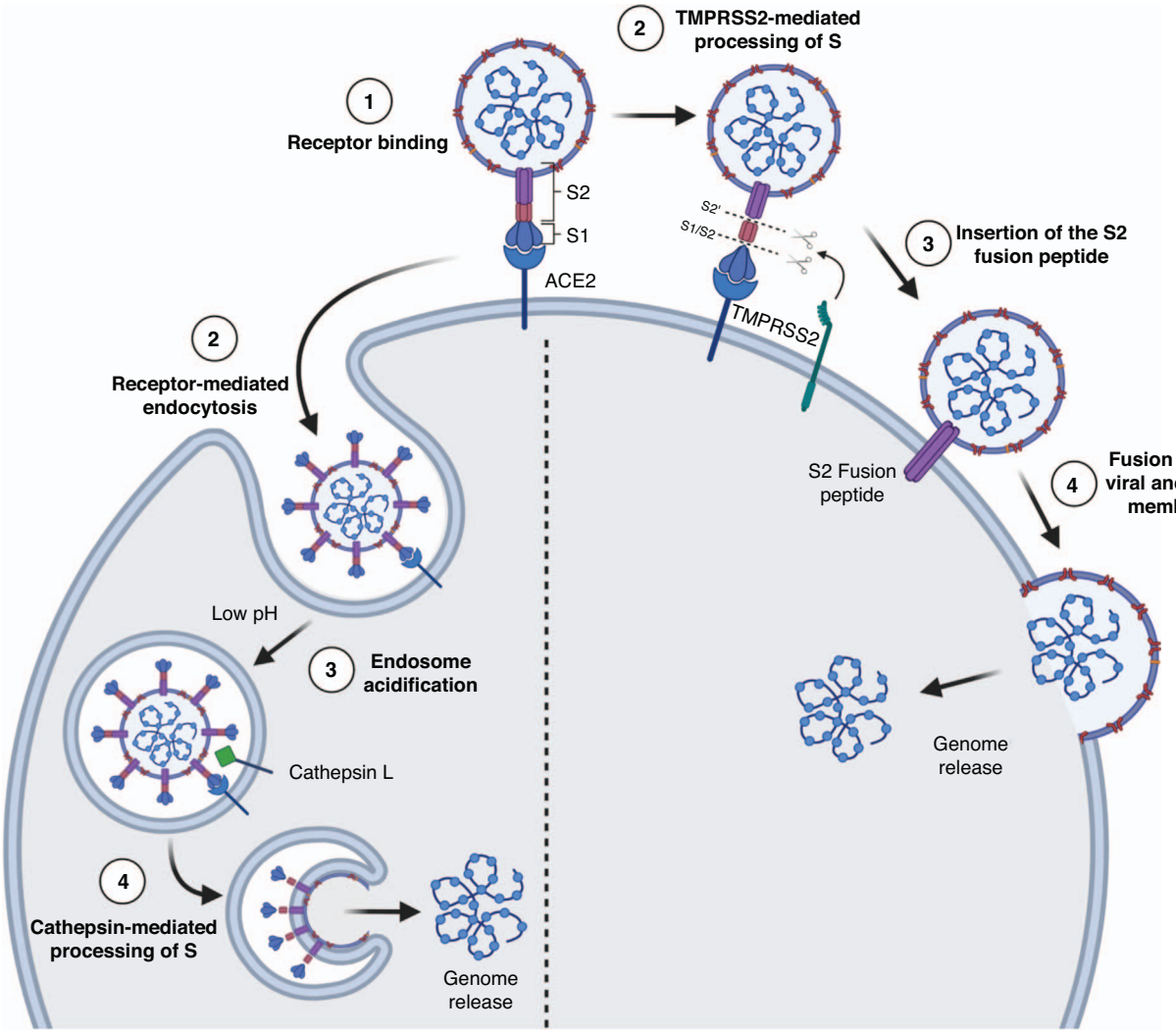

Figure 2. Mechanisms of coronavirus entry. Coronaviruses enter host cells through either endosomal entry (shown on left.) or TMPRSS2-mediated fusion at the cell membrane (shown on right). Left: (1) Following engagement of the S1 subunit of spike to the host receptor (ACE2 for SARS-CoV, SARS-CoV-2 and hCoV-NL63, DPP4 for MERS-CoV), (2) the receptor-viral particle complex is endocytosed, usually through clathrin-mediated endocytosis. (3) Endosome acidification leads to the cleavage of S by endolysosome-associated proteases cathepsin L and cathepsin B (4), leading to release of the genome into the cytosol. Right: (1) Following engagement of the S protein with its cognate host receptor, host membrane-associated protease (2) TMPRSS2 cleaves the S at the S1/S2 junction and the S2' region, leading to activation of the S2 domain for viral fusion. (3) The fusion peptide within S2 inserts into the host cell membrane to facilitate fusion between the viral and host cell membranes (4), leading to release of the viral genome into the host cytosol. Figure created using Biorender.com.

most divergent, widely distributed non-human mammalian species on earth[17]. They harbor a wide range of viruses that cause disease in humans and animals, including coronaviruses, henipaviruses, filoviruses and lyssaviruses[17].

Horseshoe bats were identified as the likely natural reservoir of SARS-CoV based on detection of antibodies to SARS-CoV N protein in bat populations in China[18]. Furthermore, diverse SARS-like-CoVs have been detected in bat populations in Asia, Africa and Europe[1]. While no direct progenitor of SARS-CoV has been isolated, related genomes were identified in Chinese horseshoe bats[19], that could infect cells expressing ACE2 from humans, civets and Chinese horseshoe bats. Zoonotic transmission of SARS-CoV likely occurred either through direct bat-human contact[19], or through an intermediate host like palm civets or racoon dogs in live-animal markets[6].

Case reports of MERS patients identified contact with dromedary camels as a risk factor for MERS-CoV infection[20]. Studies in dromedary camels from the Middle East found high seroprevalence (80–100%) to MERS-CoV[21] that may have been circulating in camels since 1983[1]. Furthermore, MERS-like-CoV sequences have been detected in nasal and rectal samples from dromedary camels in the Arabian Peninsula[2]. Additional studies revealed diverse MERS-like CoVs in bat populations in Africa, Europe, Asia and the Middle East[1]. Taken together, these findings suggest that bats were the origin of MERS-CoV, while dromedary camels are the intermediate host.

Since the first SARS-CoV-2 sequence was publicly available in January 2020, researchers have sought to understand the origins of the virus. The epidemiological link to a seafood market in Wuhan suggested that zoonotic transmission may have occurred at the market[11] and SARS-like CoVs with 91.02% identity to SARS-

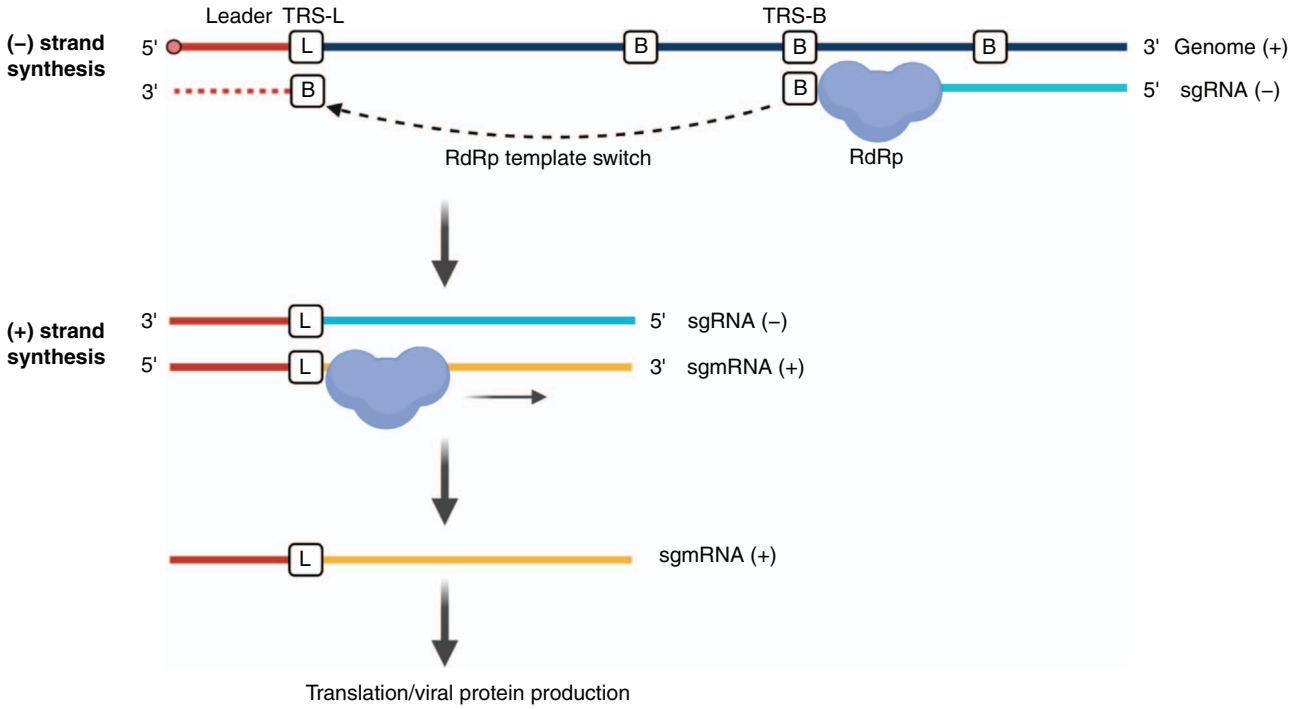

Figure 3. Discontinuous transcription of coronavirus RNA. The RNA-dependent RNA polymerase (RdRp) complex is involved in producing negative and positive sense RNAs. During negative-strand synthesis, when the RdRp approaches the body transcription regulatory sequence (TRS-B) encoded at the start of each open reading frame, the RdRp can switch templates and 'jump' to the leader transcription regulatory sequence (TRS-L), leading to fusion of the TRS-B region to the TRS-L. This process leads to the synthesis of negative sense subgenomic RNAs, which are then copied by the RdRp into positive sense subgenomic mRNAs for protein synthesis. Figure created using Biorender.com.

CoV-2 were reported in lung samples from two dead Malayan pangolins two months earlier[22,23]. However, all of the earliest reported cases of SARS-CoV-2 infection in humans were not linked to the wet market[24]. Phylogenetic analysis suggests RaTG13 and RmYN02 from horseshoe bats in Yunnan, China are most closely related to SARS-CoV-2, with 96.2% and 93.3% similarity, respectively[11,25]. However, these isolates are more than 30 years evolutionarily divergent from SARS-CoV-2[26]. Therefore, additional sampling of CoVs in animals is required to identify the reservoir and possible intermediate hosts for SARS-CoV-2.

Pandemic potential of emerging coronaviruses

Coronaviruses exist in bats in heterogenous, quasispecies pools[17]. CoVs are highly recombinogenic[26]; small regions of the genome are derived from independent CoVs by homologous recombination. Studies have shown that the SARS-CoV genome has a mosaic ancestry, with subgenomic regions from multiple origins[27,28] including HCoV-229E, mouse hepatitis virus, avian infectious bronchitis virus, bovine coronavirus, transmissible gastroenteritis virus and porcine epidemic diarrhea virus[27]. Furthermore, substitutions of entire S1 and S2 regions may play an essential role in mediating expansion of CoV host range. Swapping the RBD of a SARS-like bat CoV, with that of SARS-CoV allowed the new hybrid virus to replicate efficiently in human airway epithelial cells[29], suggesting that recombination insertion of a different CoV RBD may be a key step in driving cross-species transmission of CoVs. Ultimately, the high frequency of CoV recombination and the propensity to jump host-species mean that emerging CoVs will remain a future pandemic risk in humans.

Several conditions must be met for a novel CoV to emerge and pose a pandemic threat. It must attach, infect and replicate in human cells often using a host receptor with a human homologue expressed in an accessible anatomical location e.g. the gastrointestinal or respiratory tract. Second, it must be able to transmit efficiently from person-to-person. Third, the human population should lack pre-existing immunity to the virus. Fourth, there must be sufficient exposure to the reservoir species through hunting and consumption of wild-caught animals, trading in live animal markets or occupational exposure in meat and poultry industries. Finally, the virus must cause overt disease in humans.

Pandemic preparedness

The COVID-19 pandemic confirmed the warnings that SARS and MERS epidemics provided of the pandemic potential of betacoronaviruses. Remarkable progress has been made to understand SARS-CoV-2 including global transmission dynamics and shifts in viral variants, development of monoclonal antibodies, antivirals and

vaccines, and public health strategies. These lessons must be implemented into preparing for future emerging CoVs. This relies on sampling and evaluation of CoVs circulating in the wild for their potential for human emergence of CoVs. Reverse genetics systems[30–32] can be applied to identify which novel CoVs are able to infect human cells. Surveillance in occupationally exposed people may be an effective approach to identifying animal CoVs that can cross the species barrier. The development of pan-CoV therapeutics will be valuable for responding to the emergence of novel CoVs.

Conclusions

The COVID-19 pandemic and recurrent zoonotic transmission of CoVs from animal reservoirs into humans, along with the large diversity of SARS-like CoVs circulating in animals suggests that CoVs will continue to pose a global public health threat and that we must be prepared for the next emerging CoV.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This study did not receive specific funding.

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Biographies

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