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Review

Human coronaviruses: The emergence of SARS-CoV-2 and management of COVID-19

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ABSTRACT

To date, a total of seven human coronaviruses (HCoVs) have been identified, all of which are important respiratory pathogens. Recently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has led to a global pandemic causing millions of infections and deaths. Here, we summarize the discovery and fundamental virology of HCoVs, discuss their zoonotic transmission and highlight the weak species barrier of SARS-CoV-2. We also discuss the possible origins of SARS-CoV-2 variants of concern identified to date and discuss the experimental challenges in characterizing mutations of interest and propose methods to circumvent them. As the COVID-19 treatment and prevention landscape rapidly evolves, we summarize current therapeutics and vaccines, and their implications on SARS-CoV-2 variants. Finally, we explore how interspecies transmission of SARS-CoV-2 may drive the emergence of novel strains, how disease severity may evolve and how COVID-19 will likely continue to burden healthcare systems globally.

1. History of human coronaviruses

In the late 1960s, virologists Tyrrell and Bynoe spearheaded studies on various strains of human and animal viruses. During this time, they isolated an unknown virus from the respiratory tract of an adult patient who presented with the common cold (Tyrrell and Bynoe, 1966). Around the same time, Hamre and Procknow isolated another infectious agent from medical students who also presented with the common cold (Hamre and Procknow, 1966). These viruses were eventually named OC43 and 229E respectively. Both newly discovered viruses were found to replicate in human embryo nasal or tracheal epithelium organ cultures. Upon performing electron microscopy to visualize the virus particles in the media from infected organ cultures, OC43 and 229E virus particles were found to have similar morphology, both of which contained membrane coatings with surface projections, and their sizes ranging between 80 and 150 nm. Further studies found that these viruses were morphologically similar to mouse hepatitis virus, infectious bronchitis virus and transmissible gastroenteritis virus of swine. These discoveries led to the identification of a new viral genus named coronavirus, denoting their crown-like appearance under electron microscopy due to the surface projections (Tyrrell et al., 1975). To further investigate the pathogenicity of these viruses in humans, researchers performed volunteer inoculation studies and found that human coronaviruses (HCoVs) predominantly caused upper respiratory infection with low pathogenicity in otherwise healthy individuals (Bradburne et al., 1967). In certain cases, however, some HCoV-infected infants and young adults developed pneumonia, and elderly individuals developed chronic bronchitis upon infection. Up until the early 2000s, HCoV-229E and HCoV-OC43 were the only two HCoVs discovered.

In November 2002, cases of life-threatening respiratory disease emerged in Guangdong Province, China, of unknown etiology. Researchers eventually identified a novel highly pathogenic HCoV to be the causative agent, eventually named Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) (Ksiazek et al., 2003). By July 2003, SARS-CoV had spread to 25 countries, resulting in an epidemic with over 8000 infections worldwide and 700 deaths, making its mortality nearly 10% (World Health Organization 2022; Perlman and Netland, 2009). In 2004 and 2005, respectively, two more novel HCoVs were identified, HCoV-NL3 and HCoV-HKU1, and were found to be the causative agents of the common cold. Similar to HCoV-OC43 and HCoV-229E, these two HCoVs also generally cause mild upper-respiratory tract illness in healthy individuals, but more severe disease in infants, elderly individuals and those who are immunocompromised. Together, the four endemic HCoVs are responsible for 10–30% of common cold cases

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(Paules et al., 2020). In June 2012, about a decade after the identification of SARS-CoV, a novel HCoV was isolated from a 60-year-old man in Saudi Arabia who died of acute respiratory illness and renal failure. This virus was later named Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (Zaki et al., 2012). To date, a total of 2574 MERS-CoV infections have been reported in 27 countries, mainly localized to the Middle East, Africa and South Asia, causing over 850 deaths making its mortality rate about 35% (World Health Organization 2021). In November 2019, less than a decade after the emergence of MERS-CoV, a cluster of cases of severe pneumonia of unknown etiology were reported in Wuhan, Hubei Province, China (Zhu et al., 2020). A novel HCoV, originally named 2019 novel coronavirus and now known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was subsequently identified as the causative agent of the emerging acute respiratory disease, now known as coronavirus disease 2019 (COVID-19) (Zhu et al., 2020). By early March 2020, 118,000 SARS-CoV-2 infections and over 4000 deaths were reported worldwide. On March 11, 2020, the World Health Organization (WHO) declared COVID-19 a worldwide pandemic due to the concerning increase in the spread and severity of SARS-CoV-2 infections (World Health Organization, 2021). Overall, a total of seven HCoVs have been identified to date (Fig. 1), all of which are considered important human respiratory pathogens.

As of July 27, 2022, over 570 million confirmed cases and over 6.3 million deaths globally have been recorded according to the WHO (World Health Organization, 2021). SARS-CoV-2 is highly transmissible, which has been highlighted by studies that have aimed to identify its basic reproductive number (R_0), defined as "the number of secondary infections when an index case is introduced into a fully susceptible population" (Ke et al., 2021). A review by Liu et al. (2020) reported that the average R_0 of the original strain of SARS-CoV-2 during the early epidemic period in early 2020 was found to be 3.28. Others have estimated that the ancestral strain of SARS-CoV-2 has a R_0 ranging between 3.6 and 6.1 (Ke et al., 2021). Despite the differences in these values, the R_0 of SARS-CoV-2 is higher than those of SARS-CoV and MERS-CoV, which are estimated to be around 2.0–3.0 and 0.9, respectively (Petersen et al., 2020). It is important to note that as SARS-CoV-2 continues to evolve and gives rise to novel variants, the R_0 is subject to change. For instance, studies have reported that several SARS-CoV-2 variants identified to date with increased transmissibility have a higher R_0 compared to the ancestral SARS-CoV-2 strain (Liu and Rocklöv, 2021; Liu and Rocklöv, 2022).

Due to its high transmissibility, cases exponentially increased worldwide, and health care systems rapidly became overwhelmed. In hopes of mitigating the spread of SARS-CoV-2 within the population, many countries around the world implemented non-pharmaceutical interventions including school and non-essential business closures, bans of gatherings, work-from-home mandates and in some cases curfews. As hospitalizations of individuals with severe COVID-19 increased, health care systems around the world resorted to cancelling all non-emergency procedures and appointments to redirect personnel and resources for COVID-19 patient care and prevent further spread of SARS-CoV-2. Overall, COVID-19 has caused detrimental biopsychosocial effects on individuals and devastating socioeconomic effects on society.

2. HCoV fundamental virology

Coronaviruses (CoVs) are enveloped, single-stranded positive-sense RNA (+ssRNA) viruses. Their genome is about 30 kb in length, and are among the largest known RNA viruses (V'kovski et al., 2021). CoVs belong to the order *Nidovirales,* suborder *Cornidovirineae,* and family *Coronaviridae* (V'kovski et al., 2021). This viral family is further classified into four different genera based on genetic and antigenic studies of both human and animal coronaviruses. While *Alphacoronaviruses* and *Betacoronaviruses* primarily infect mammalian species including humans, *Gammacoronaviruses* and *Deltacoronaviruses* primarily infect avian species (V'kovski et al., 2021). *Alphacoronaviruses* include HCoV-229E and HCoV-NL63 while *Betacoronaviruses* include HCoV-OC43, HCoV-HKU1, SARS-CoV, MERS-CoV and SARS-CoV-2 (Heinz and Stiasny, 2020).

HCoV viral particles are spherical with a diameter of 80–120 nm (Fung and Liu, 2019). Both membrane (M) glycoproteins and the envelope (E) protein are embedded into the host-derived lipid bilayer that surrounds the surface of the virion. The virion surface contains protruding trimeric Spike (S) glycoproteins, consisting of the S1 and S2 subunits, which gives the virus its crown-like appearance that is seen under electron microscopy. In the case of SARS-CoV-2, the S1 subunit containing the receptor-binding domain (RBD) directly binds the cell surface receptor such as angiotensin converting enzyme 2 (ACE2) for SARS-CoV-2 (Huang et al., 2020), which is expressed on the surface of cells within the human respiratory and gastrointestinal tract and mediates viral entry. Meanwhile, the S2 subunit mediates membrane fusion. The S protein is also the primary target of neutralizing antibodies against SARS-CoV-2 (Huang et al., 2020). Within the virion, the nucleocapsid (N) protein binds the viral RNA genome, which together form a helical structure (Kumar et al., 2020) (Fig. 2a).

The HCoV genome, one of the largest genomes of all RNA viruses, contains a 5′-cap structure and a 3′-polyadenylated tail that flank its 27 to 32 kb genome (Perlman and Netland, 2009). The genome contains 6 to 11 open reading frames (ORFs) depending on the HCoV, which encode a total of 22 to 29 viral proteins (Hartenian et al., 2020). Two large overlapping ORFs, ORF1a and ORF1b, make up about two-thirds of the viral genome and encode non-structural proteins (NSPs) that are involved in viral replication (Fig. 2b) (Perlman and Netland, 2009). The remaining ORFs encode accessory proteins that have diverse roles in virus-host interactions and structural proteins that assemble together to form the viral particle (V'kovski et al., 2021). Even though the viral genome contains a single ribosome entry site, HCoVs have evolved two important mechanisms that allow many proteins to be synthesized from a single viral RNA. First, a slippery sequence that is followed by an RNA pseudoknot enables for a −1 ribosomal frameshift, which allows host ribosomes to bypass the stop codon between ORF1a and ORF1b. As a result, either ORF1a alone can be translated into a shorter polyprotein, pp1a that encodes nsp1 to nsp11, or both ORF1a and ORF1b can be translated into a longer polyprotein, pp1ab, encoding nsp1 to nsp16. The

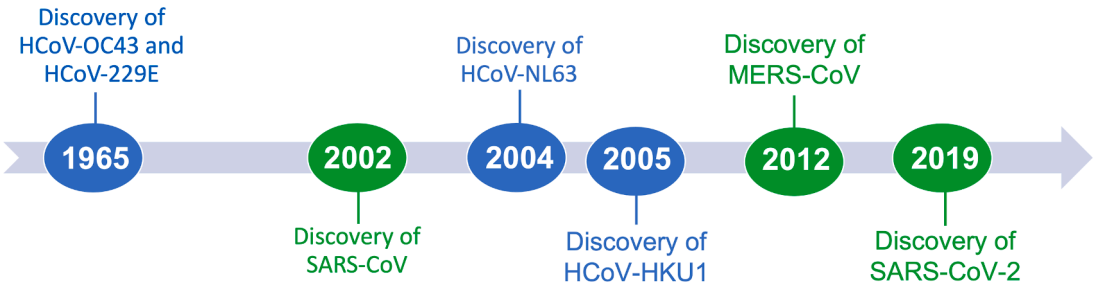

Fig. 1. Timeline of HCoV discoveries. Blue represents the discovery of endemic HCoVs that generally cause the common cold. Green represents the discovery of highly pathogenic HCoVs.

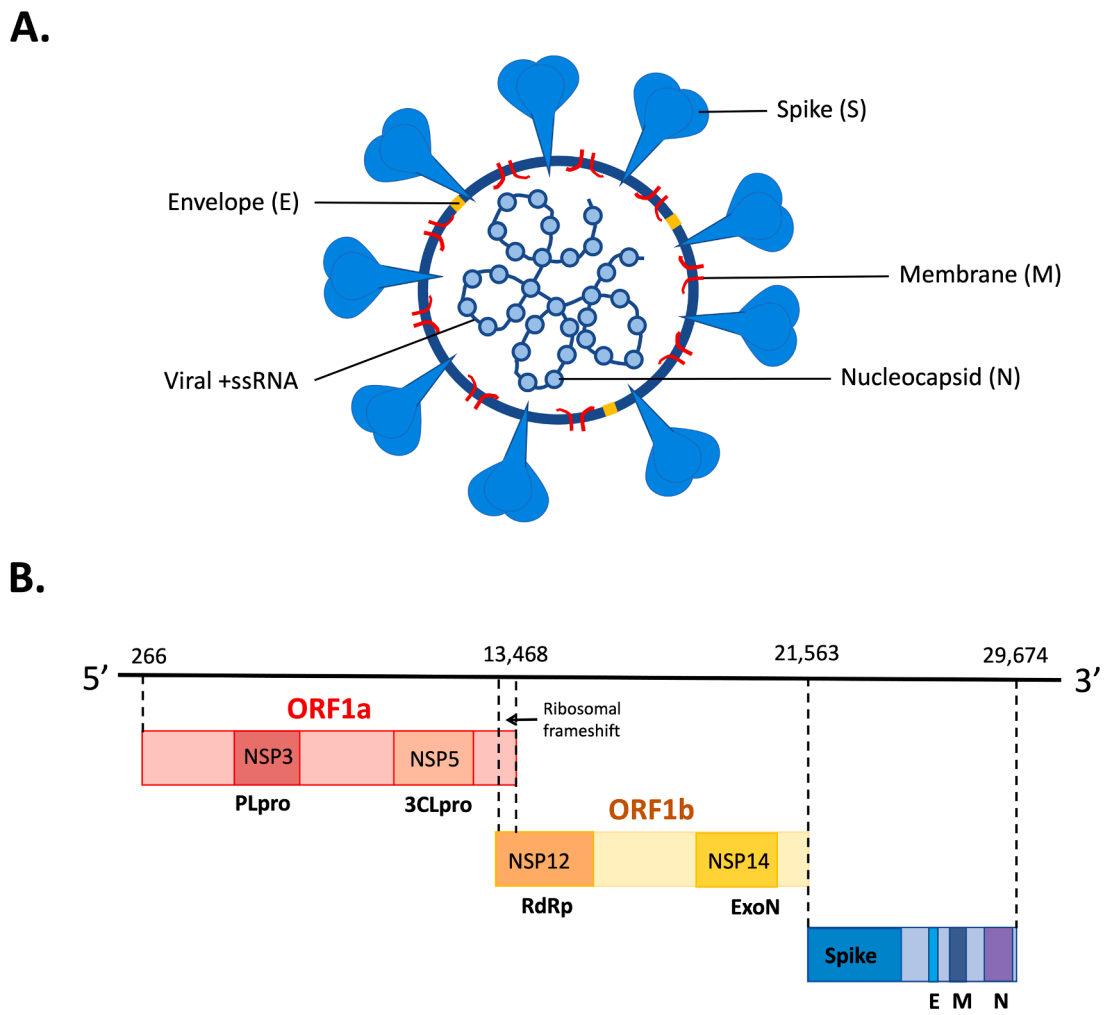

Fig. 2. Schematic of HCoV viral particle and genome. (A) HCoV virions contain membrane and envelope proteins embedded into the lipid bilayer surrounding the viral particle. S proteins protrude from the surface of the viral particle. The +ssRNA genome is encapsulated by nucleocapsid proteins. (B) Schematic of the SARS-CoV-2 genome. Viral NSPs are predominantly involved in viral replication and are encoded by ORF1a and ORF1b. The remaining ORFs encode accessory and structural proteins, including the spike, envelope, membrane and nucleocapsid proteins.

polyproteins are subsequently processed into individual replicase proteins by viral proteases including main protease (Mpro), also known as chymotrypsin-like protease (3CLpro), and papain-like proteases (Irigoyen et al., 2016; Cascella et al., 2021).

Additionally, sub-genomic RNAs (sgRNAs), which encode both structural and accessory proteins, are synthesized through discontinuous transcription, a process unique to the order *Nidovirales,* using the +ssRNA genome as a template to synthesize negative sense sgRNAs (Hartenian et al., 2020). This process is controlled by two different types of transcription-regulating sequences (TRS) that contain identical conserved sequences and serve to slow down the replication-transcription complex: body-TRS (TRS-B), which are located upstream of each 3′-proximal gene, and a single leader TRS (TRS-L), which is located at the 5′ end of the viral genome. At these TRS-Bs, the synthesis of the negative-strand RNA stops and the TRS-B on the nascent negative sense sgRNA hybridizes to the TRS-L on the positive sense genomic RNA through the conserved sequences allowing for leader-body joining, also known as template switching. Negative sense sgRNA synthesis is reinitiated at the TRS-L resulting in the addition of the negative sense leader sequence to the negative sense sgRNA (Sola et al., 2015). The negative sgRNAs are subsequently used as a template to synthesize positive sense sgRNAs that encode both structural and accessory proteins.

3. Zoonosis and interspecies transmission of HCoVs

All seven HCoVs identified to date are of zoonotic origin (Fig. 3a) (Forni et al., 2017). Field studies have enabled researchers to identify and sequence viruses related to HCoVs in wildlife animal reservoirs, which can be used for phylogenetic reconstruction to better understand how these viruses have been introduced into the human population (Forni et al., 2017). All HCoVs are thought to have originated in bats except for HCoV-HKU1 and HCoV-OC43, which are thought to have originated in rodents (Forni et al., 2017). While bats or rodents act as the natural hosts for HCoVs, intermediate reservoir hosts have also been identified for several HCoVs. For example, bovines, palm civets and dromedary camels have been shown to be the intermediate host for HCoV-OC43, SARS-CoV and MERS-CoV, respectively. In the case of HCoV-229E, camelids are suspected to be the intermediate host reservoirs, though this has yet to be confirmed (Ye et al., 2020). Alternatively, it is possible that HCoV-229E was transmitted to humans directly from bats. To date, the intermediate host reservoirs of HCoV-NL63 and HCoV-HKU1 remain unknown (Ye et al., 2020). Similarly, the zoonosis of SARS-CoV-2 remains unknown. While viruses that are closely related to SARS-CoV-2 have been identified in bats and pangolins in South-East Asia, including in China, significant phylogenetic distance exists (Lytras et al., 2022; Zhou et al., 2021), which reflects decades of evolutionary divergence (Holmes et al., 2021). With the animal host of SARS-CoV-2

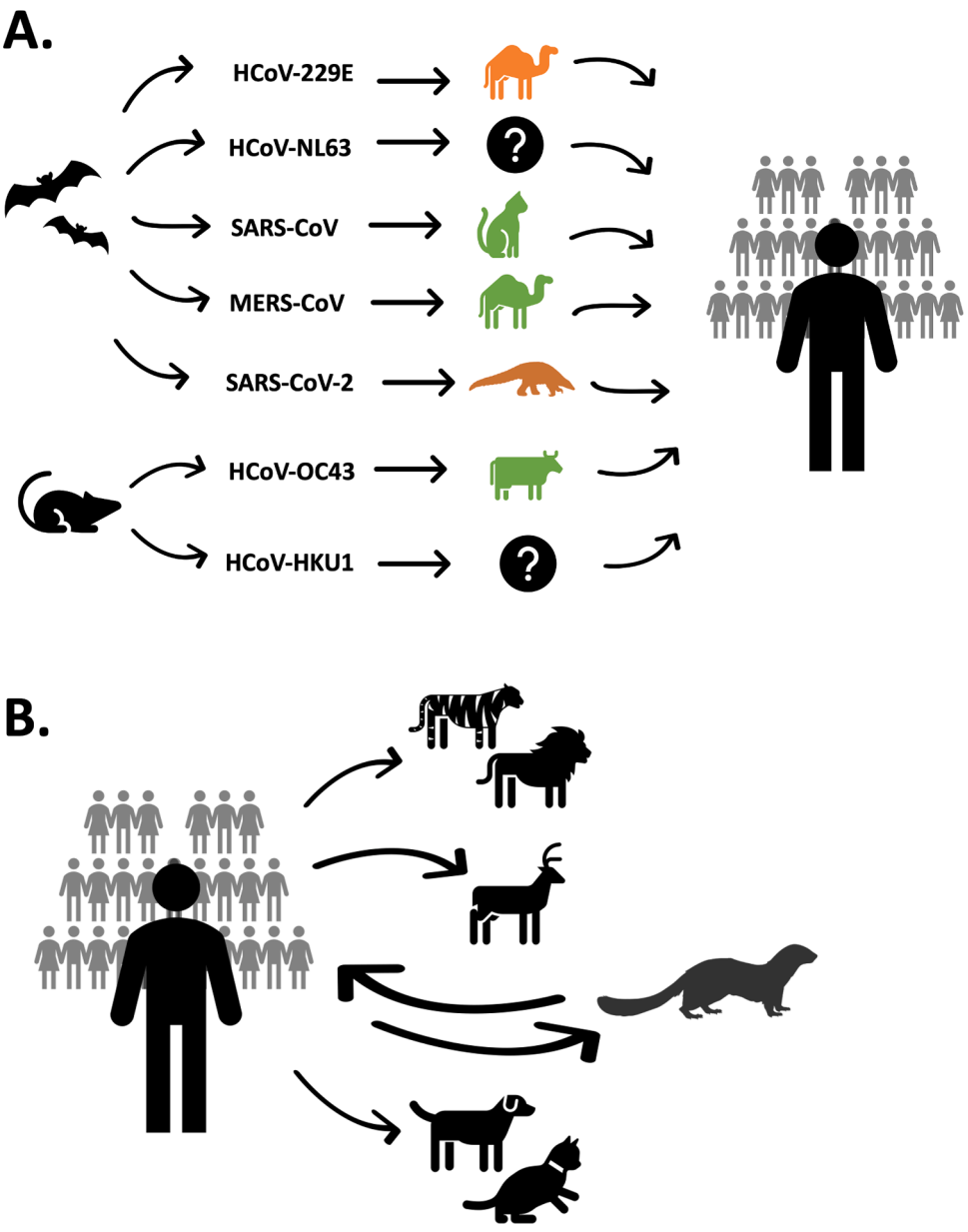

Fig. 3. Zoonosis of HCoVs and interspecies transmission of SARS-CoV-2. (A) Schematic of zoonotic transmission of HCoVs from their respective reservoirs to their immediate host and subsequently to the human population. Green represents confirmed intermediate hosts and orange represents suspected intermediate hosts. Question marks represent unknown intermediate hosts. (B) Schematic of interspecies transmission of SARS-CoV-2 reported to date. Larger arrows represent large outbreaks and smaller arrows represent few documented cases.

yet to be determined, evidence suggests that the Huanan wet market in Wuhan, China, where live animals among other goods are sold, might be an important spreading location where zoonosis may have occurred (Gao et al., 2022; Worobey et al., 2022).

One unique aspect of SARS-CoV-2 that has not been reported for other HCoVs is its ability for zoonotic transmission from infected humans back to a variety of mammalian species including farmed minks, lions and tigers in zoos, wild white-tailed deer as well as dogs and cats (Garigliany et al., 2020; McAloose et al., 2020; Sit et al., 2020; McAleer, 2021; Chandler et al., 2021) (Fig. 3b). As the wide host range of SARS-CoV-2 facilitates cross-species transmission events, the virus will likely evolve to adapt to its new host. Establishing reservoirs in new hosts may provide SARS-CoV-2 the opportunity to acquire adaptive mutations that confer enhanced virus transmissibility, pathogenicity as well as evasion of host immune response and ultimately give rise to novel SARS-CoV-2 strains. Given that the SARS-CoV-2 species barrier appears to be weak, a major concern is the possibility that novel SARS-CoV-2 strains that evolve in animal species may spill back into humans and potentially give rise to future SARS-CoV-2 variants. For example, just a few weeks after COVID-19 was declared a global pandemic, SARS-CoV-2 was detected in farmed minks in the Netherlands in April and May 2020 and later in Denmark (Oreshkova et al., 2020; Larsen et al., 2021). Sequence analysis of viruses collected from the minks suggested that the virus was most likely transmitted from humans (Oreshkova et al., 2020). Toward the end of June 2020, more than half of the workers employed at the mink farms with SARS-CoV-2-infected animals tested positive for the virus or had SARS-CoV-2 antibodies (Munnink et al., 2021). Sequence analysis of virus collected from employees at infected mink farms showed that reverse zoonosis had occurred, resulting in the virus spilling back into the human population (Munnink et al., 2021) with reported human-to-human transmission (Wang et al., 2021). While the virus was transmitted and evolved within the mink population, SARS-CoV-2 acquired many mutations. Of particular concern, the Y453F mutation within the RBD of the S protein was reported to enhance the affinity of the S protein for human ACE2 (Bayarri-Olmos et al., 2021), potentially contributing to enhanced transmission and pathogenicity in humans. Moreover, the highly mutated SARS-CoV-2 that was transmitted from minks back to humans

in Denmark was found to evade neutralization by antibodies from convalescent sera (Larsen et al., 2021). Although the culling of millions of minks prevented further transmission of mink-derived SARS-CoV-2 in the human population, cross-species transmission of this virus may serve an important role in viral evolution and contribute to future emerging viral strains and variants.

4. SARS-CoV-2 variants: overview, experimental challenges and possible origin

Generally, RNA viruses are prone to mutation due to the intrinsic error-prone properties of RNA-dependent RNA polymerases (Holmes, 2009). Unlike other RNA viruses, however, SARS-CoV-2 and other HCoVs encode a conserved protein, NSP14 which bears exonuclease activity and hence the proofreading function (Smith et al., 2013; Gribble et al., 2021). Despite its proofreading activity that increases replication fidelity to maintain the sequence stability of the large viral genome, SARS-CoV-2 continues to acquire mutation throughout the replication process. One possible mechanism for the high mutation rate observed in SARS-CoV-2 infection is the extraordinarily high rates of viral transmission and replication in the human populations, which naturally results in increased opportunities to accumulate mutations. Moreover, HCoVs frequently undergo recombination events at the template-switching step in discontinuous transcription (Su et al., 2016), which closely resembles a high-frequency homology-assisted recombination process and accelerates association of mutations. The mutations that arise within the viral genome, which can either be beneficial, inconsequential, or deleterious to the virus, drive genetic variation and viral evolution. Those that enhance viral fitness and thus confer a competitive advantage increase in frequency through natural selection while those that reduce viral fitness are eventually outnumbered.

Since October 2020, a series of SARS-CoV-2 variants with novel mutations have been identified (Tao et al., 2021), some of which contain mutations that influence the epidemiological and clinical aspects of COVID-19. Variants containing mutations that confer enhanced rates of virus transmission, increased risk of reinfection, cause more severe disease or evade the host immune response are known as variants of concern (VOC) (Tao et al., 2021). To date, five VOCs have been identified, which have spread globally (Fig. 4). The first VOC, B.1.1.7, was reported in November 2020 in the United Kingdom. Retrospective analyses found that this variant, now known as the Alpha-variant, was detected in clinical samples as early as September 2020 (Tao et al., 2021) and appeared to have enhanced infection and transmission efficiency (Starr et al., 2020). About one month later, two additional VOCs, B.1.351 (Tegally et al., 2021) and P1 derived from the B.1.1.28 lineage (Faria et al., 2021), were reported in South Africa and Brazil, respectively. These variants are now known as the Beta- and Gamma-variants, respectively, and retrospective analyses found that these variants were detected in clinical samples as early as May 2020 and October 2020 (World Health Organization, 2022).

To date, studies investigating the various SARS-CoV-2 variants have been heavily focused on the mutations within the S protein due to its role in viral entry and transmission, and its role as a target for antibody-mediated neutralization. The D614G mutation in the S protein emerged in May 2020 prior to the detection of VOCs. D614G is located outside of the RBD (Plante et al., 2021), has been reported to prevent S1 shedding, leading to increased spike density on the virion surface (Zhang et al., 2020) and enhancing SARS-CoV-2 infectivity. The N501Y mutation in the S protein is common to the first three identified VOCs (Otto et al., 2021). N501Y is located within the six amino acids that are critical in establishing a direct interaction between the RBD and the ACE2 receptor (Yang et al., 2021; Teruel et al., 2021). By enabling new interactions between the viral protein and the host cell receptor through hydrogen bonds, this mutation has been reported to enhance the binding affinity between these two proteins, which likely enhances viral entry (Liu et al., 2022). E484K is another S protein mutation common to the first three VOCs. To date, the functional properties of E484K mutation have yet to be elucidated. In February 2021, another VOC, B.1.617.2 and now known as the Delta-variant, was reported in India following a dramatic surge in SARS-CoV-2 infections throughout the country (Banu et al., 2020). Compared to the previously identified VOCs, the Delta-variant had more than twice the number of mutations of interest within the S protein, many of which are thought to confer enhanced binding affinity to human ACE2. The Delta-variant was also associated with a higher viral load, longer duration of infectiousness and ability to evade the host immune response resulting in higher rates of reinfection (Choi and Smith, 2021; van Kampen et al., 2021; Townsend et al., 2021). By the middle of 2021, the Delta-variant became the globally dominant variant.

Most recently, at the end of November 2021, a novel SARS-CoV-2 VOC, the Omicron-variant, was identified in South Africa and contains deletions and around 50 mutations that are distributed across both structural and non-structural proteins (Karim and Karim, 2021), making it the most mutated VOC identified to date. Over half of the mutations in the SARS-CoV-2 Omicron-variant are located within the S protein, many of which are mutations that are also common to the other VOCs (Tian et al., 2022). By January 6, 2022, the WHO announced that the Omicron-variant was reported in at least 149 countries. Based on the rapid global spread of the Omicron-variant within just a few weeks, this VOC was expected to have mutations that conferred significantly enhanced transmissibility compared to the other VOCs identified to date. Early assessments found that individuals infected with the Omicron-variant have a significantly reduced risk of hospitalization and death compared to individuals infected with the Delta-variant (Wolter et al., 2022; Ulloa et al., 2022). Although accumulating evidence suggests that the Omicron-variant evades both natural infection- and vaccine-induced neutralizing antibodies (Cao et al., 2022; Planas et al., 2022), it is likely that innate and cellular immunity in previously infected and vaccinated individuals protect from severe diseases, which may be a confounding factor in evaluating the intrinsic virulence of this SARS-CoV-2 variant. Preliminary reports have compared the mutations of interest within the Omicron-variant to those of other VOCs and have suggested that many confer increased transmissibility, higher viral binding affinity and enhanced antibody escape (Karim and Karim,

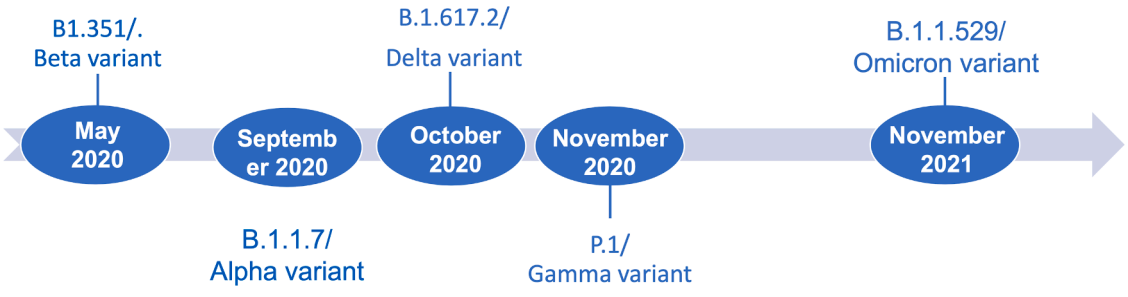

Fig. 4. Timeline of earliest reports of SARS-CoV-2 VOCs reported to date.

2021). However, experimental and clinical data are required to provide evidence of enhanced transmissibility and evasion of the host immune response.

While the S protein has been the major focus for investigating mutations of interest, it is important to note that mutations are scattered throughout the SARS-CoV-2 genome and impact other viral genes. For example, mutations have been identified within genes that encode non-structural proteins that are involved in SARS-CoV-2 replication, such as NSP2 and NSP6, and accessory proteins that are involved in virus-host interactions, such as ORF3a and ORF8 (Alkhatib et al., 2021). Further studies are required to elucidate the role of mutations in such genes and may provide insight into their function in SARS-CoV-2 pathogenesis. However, functional analysis of different mutations identified in SARS-CoV-2 variants on viral replication and pathogenesis remains a challenge, specifically due to the limited convenient mutagenesis methods that can be used for large viral RNA genomes like SARS-CoV-2.

While infectious clones encoding full-length viral cDNA and a suitable promoter are often used to characterize the function of different mutations in smaller positive sense RNA viruses, serious challenges exist when using such a system to study HCoVs due to their large genome size and the instability of some CoV replicase gene sequences during its propagation in bacteria. Nevertheless, reverse genetics systems to engineer full-length cDNA encoding infectious HCoV genomes have been developed since the year 2000 (Almazán et al., 2000) and have been successfully used to study HCoV replication, virus-host interactions, and pathogenesis (Almazán et al., 2014). In regard to SARS-CoV-2, several groups have described alternative reverse genetics-based systems to produce recombinant virus containing mutations of interest for both *in vitro* and *in vivo* studies. For example, Xie et al. (2021) described a bacterium-based system to generate recombinant SARS-CoV-2 infectious clones. Briefly, seven cDNA fragments are designed that span the complete SARS-CoV-2 genome, which are subsequently amplified in *Escherichia coli (E. coli)*. Restriction enzymes are then used to remove the plasmid backbones and ligate the seven cDNA fragments into full genome-length cDNA. The full-length cDNA is subsequently transcribed *in vitro* using the T7 polymerase to generate full-length genomic SARS-CoV-2 RNA, which can be electroporated into cells. Finally, recombinant virus can be collected within several days following electroporation. This approach reduces the risk of off-target mutations that may be incorporated into the recombinant virus since smaller plasmids are manipulated (Xie et al., 2021). Moreover, this method enables for introduction of multiple mutations into different cDNA fragments simultaneously, which can be used to study the combinatory effect of multiple mutations within different viral genes. However, one caveat is that the amplification process of the cDNA fragments in *E. coli* is error-prone and thus sequencing is critical to verify the cDNA plasmids at each stage of amplification (Xie et al., 2021). Thi Nhu Thao et al. (2020) described a similar approach using yeast, specifically *S. cerevisiae,* rather than bacteria where the cDNA fragments are ligated through homologous recombination to generate full-length viral cDNA. Recently, Torii et al. (2021) established a bacterium- and yeast-free method to generate SARS-CoV-2 infectious clones, which not only circumvents the previously described caveats, but also provides a more simple and quick reverse genetics system. Briefly, nine SARS-CoV-2 cDNA fragments are designed that span the full-length viral genome as well as a cDNA linker fragment that encodes a promoter, a polyA signal and a ribozyme sequence, which are amplified by polymerase chain reaction (PCR). The design of each fragment also includes overlapping ends with adjacent fragments such that an additional PCR of the amplified fragments enables the fragments to extend as a circular viral genome containing a suitable promoter. This process, known as circular polymerase extension reactions (SPER), leads to amplified circular viral genome, which can be directly transfected in susceptible cells and infectious virus can subsequently be collected. SPER has previously been used as an effective method to easily generate various mutant flavivirus to study the functions of various viral proteins and the effect of different mutations, further highlighting its feasibility (Setoh et al., 2019).

The origins of the various SARS-CoV-2 VOCs remain unclear. Several case reports have demonstrated that multimutational SARS-CoV-2 variants have arisen in patients with immunosuppression. It is possible that immunocompromised individuals provide conditions in which long-term infection can persist, which enables prolonged viral replication and facilitates adaptation to the host (Avanzato et al., 2020; Choi et al., 2020; Kemp et al., 2021; Truong et al., 2021). Weigang et al. (2021) investigated this hypothesis by studying SARS-CoV-2 evolution in an immunosuppressed kidney transplant recipient with persistent viral infection. Several mutated viral isolates were identified at later stages of persistent infection, one of which had similar mutations to those that are common among the various VOCs. However, lowering immunosuppressive treatment in this patient resulted in the production of S-specific neutralizing antibodies and the infection was resolved (Weigang et al., 2021). Overall, the authors suggest that immunocompromised patients may serve as an important source of SARS-CoV-2 variants. Others have postulated that cross-species transmission of SARS-CoV-2 from humans to non-human hosts has enabled the virus to accumulate mutations and eventually spill back into the human population (Kupferschmidt, 2021). For example, Wei et al. (2021) hypothesized that the Omicron-variant originated in a mammalian host other than humans. In their previous work, the authors demonstrated that the molecular spectrum of *de novo* mutations in RNA genomes that are acquired throughout virus evolution occur in a host-specific manner (Shan et al., 2021). Using this approach, the authors analyzed the molecular spectrum of mutations in the Omicron-variant viral genome to trace its proximal host origin. Their study suggests that the progenitor of the SARS-CoV-2 Omicron-variant originated from a reverse zoonotic event from humans to mice, likely in mid-2020, where it accumulated mutations over an estimated one-year period, and subsequently transmitted back into the human population in late-2021 (Wei et al., 2021). More studies are required to confirm whether either of these possibilities is indeed the source of VOCs.

5. COVID-19 clinical manifestation, treatments, and implications for SARS-CoV-2 variants

SARS-CoV-2 primarily infects the lower respiratory tract and can cause diseases of varying severity. In mild cases, symptoms include fever, fatigue, myalgia, rhinorrhea, coughing and sore throat (Mehta et al., 2021). In severe cases, SARS-CoV-2 can cause acute respiratory distress syndrome (ARDS), septic shock, and multi-organ failure, which in many cases results in life-threatening disease. In some cases of severe COVID-19, SARS-CoV-2 infection can induce an unbalanced inflammatory response that leads to a cytokine storm, which further exacerbates tissue injury, ARDS and multiple organ failure (Song et al., 2020). While SARS-CoV-2 infection is prevalent among all age groups, older age, hypertension, chronic cardiac and pulmonary disease, and immunosuppression are risk factors for severe disease (Salzberger et al., 2021). Emerging evidence has highlighted that certain individuals who have been infected with SARS-CoV-2 continue to have symptoms associated with COVID-19, such as shortness of breath, headaches, chest pain, myalgia, fatigue and cognitive difficulties, week to months after infection (Taquet et al., 2021a). One retrospective cohort study found that about 57% of patients have at least one of these long-term symptoms, now known as long COVID, up to 180 days after SARS-CoV-2 infection (Taquet et al., 2021a).

The limited clinical treatments currently available are an important contributor to the continuous increase in the number of new SARS-CoV-2 infections and deaths from COVID-19. To date, the U.S. Food and Drug Administration (FDA) has granted emergency use authorization for several agents to treat severe COVID-19 (Table 1). Among the approved drugs, many are immunosuppressants including glucocorticoids and JAK inhibitors (M U. S. Food and Drug Administration, 2021) that aim to control the excessive inflammatory response that is observed in patients with severe COVID-19. Although limited, few therapeutic options

Table 1
Summary of US FDA-approved and EUA therapies for COVID-19.

	Drug name	Drug class	Target	Emergency use authorization by FDA	FDA approved
Host-targeted therapies	Baricitinib	Kinase inhibitor	JAK kinase	✓	
	Tocilizumab	Monoclonal antibody	Anti-IL6	✓	
Direct-acting antiviral therapies	Remdesivir	Nucleotide analog	RdRp		✓
	Molnupiravir	Ribonucleoside prodrug	RdRp	✓	
	Paxlovid	Protease inhibitor	3CLpro	✓	
Neutralizing antibodies	Bebtelovimab	Monoclonal antibody	S-ACE2 interaction	✓	
	Tixagevimab + Cilgavimab	Monoclonal antibody	S-ACE2 interaction	✓	
	COVID-19 convalescent plasma	Convalescent plasma	S-ACE2 interactions	✓	

directly targeting SARS-CoV-2 have also been approved by the U.S. FDA for emergency use authorization (M U. S. Food and Drug Administration, 2021). For example, COVID-19 convalescent plasma and monoclonal antibodies bind to the SARS-CoV-2 S protein and interfere with cell entry (M U. S. Food and Drug Administration, 2021). Meanwhile, remdesivir, a nucleotide analog, has received full FDA approval for the treatment of COVID-19 and targets the viral RdRp and aims to interfere with viral replication (M U. S. Food and Drug Administration, 2021). While the antiviral activity of these drugs has been demonstrated using *in vitro* (Wang et al., 2020) and *in vivo* (Williamson et al., 2020) preclinical models, there is conflicting evidence regarding their clinical efficacy in COVID-19 treatment (Ader et al., 2021; Beigel et al., 2020; WHO Solidarity Trial Consortium, 2020; Omrani et al., 2021).

On December 22, 2021, the FDA approved Paxlovid (developed by Pfizer), the first oral antiviral treatment specific for SARS-CoV-2, for emergency use authorization to treat mild-to-moderate COVID-19 in adults and pediatric patients older than 12 years of age who are at high risk for progression to severe COVID-19 (M U. S. Food and Drug Administration, 2021). Paxlovid consists of a combination of SARS-CoV-2 3CL protease inhibitor called nirmatrelvir, and human immunodeficiency virus (HIV)−1 and HIV-2 protease inhibitor ritonavir (Drożdżal et al., 2021). In a randomized, double-blind clinical trial consisting of 3000 participants who tested positive for COVID-19, out of those administered Paxlovid orally every 12 h for five consecutive days within three days of symptom onset, only 0.8% required hospitalization with no deaths (Mahase, 2021). In the placebo group, 7% of participants required hospitalization with seven deaths. Similar results were reported in individuals treated within five days of symptom onset (Mahase, 2021). Overall, Paxlovid was reported to be 89% effective in patients at risk of serious illness. On December 23, 2021, the FDA authorized an additional oral antiviral treatment, molnupiravir (developed by Merck), for emergency use authorization (M U. S. Food and Drug Administration, 2021). Molnupiravir has been authorized to treat mild-to-moderate COVID-19 in adults older than 18 years of age infected with SARS-CoV-2 and who are at high risk for progression to severe COVID-19. However, the FDA has recommended the use of molnupiravir only if alternative COVID-19 treatment options authorized by the FDA are not accessible or clinically appropriate (M U. S. Food and Drug Administration, 2021). Molnupiravir is a prodrug that is converted into a synthetic cytidine nucleoside and leads the viral RdRp to incorporate guanosine or adenosine during viral replication, leading to the accumulation of deleterious mutations until the virus can no longer replicate (Drożdżal et al., 2021). In a randomized, double-blind clinical trial consisting of just over 1433 participants, 7.3% of those who were orally administered molnupiravir twice daily for five days within five days of symptom onset were hospitalized with one death compared to 14.1% hospitalized of those in the placebo group with nine deaths (Jayk Bernal et al., 2021). Overall, molnupiravir was found to reduce hospitalization or death by 30% when administered within five days of symptom onset (Jayk Bernal et al., 2021).

While early studies investigating the effect of the various FDA-approved antiviral therapies appear to be promising and may revolutionize COVID-19 treatment, one concern is the possibility of SARS-CoV-2 variants developing drug resistance. For example, the β− and γ−variants have been reported to be resistant to SARS-CoV-2 monoclonal antibody treatments, Casirivimab and Bamlanivimab, *in vitro* (Hoffmann et al., 2021). In addition to the mutations within the viral S protein that may enable the virus to evade neutralizing monoclonal antibodies, mutations in other viral proteins, including NSPs, are of equal concern of drug resistance for therapeutics that target virus replication. Although resistance to remdesivir has yet to be documented in rhesus macaques or humans infected with SARS-CoV-2, a study using remdesivir to target murine hepatitis virus, another coronavirus, demonstrated that treatment selected for two mutations in the NSP12 polymerase that conferred drug resistance *in vitro* (Agostini et al., 2018). Similarly, introducing these mutations into SARS-CoV, which was subsequently used to infect a preclinical mouse model, was reported to result in resistance to remdesivir, albeit at the expense of viral fitness resulting in attenuated viral replication and pathogenesis (Agostini et al., 2018). One common strategy that is used to reduce the risk of antiviral drug resistance is the use of combination antiviral therapy to reduce the likelihood of the virus developing resistance to multiple antiviral drugs within the same viral genome. This approach has been successfully used to overcome the challenge of drug resistance in the clinical treatment of other RNA viruses such as hepatitis C virus, influenza virus and HIV. Alternatively, therapies that target host factors that are essential to support viral replication diminish the risk of the virus developing mutations that confer drug resistance and likely have more broad-spectrum activity (Prussia et al., 2011), which may be a promising approach to therapeutically target future emerging VOCs and other HCoVs.

6. COVID-19 prevention and implications for SARS-CoV-2 variants

In addition to various antiviral therapies currently under investigation to treat COVID-19, prophylactic COVID-19 vaccines are equally important in controlling the current global pandemic. Within weeks of the SARS-CoV-2 genetic sequence being released, COVID-19 vaccine development was rapidly underway. A variety of vaccine platforms are currently being used for vaccine development including mRNA-, non-replicating viral vector-, protein subunit- and inactivated virus-based vaccines. To date, over 200 vaccines have been tested in preclinical and clinical studies, some of which have been approved for emergency use and some of which have been fully approved in certain countries by their respective regulatory agencies (Table 2) (World Health Organization, 2020). A comprehensive analysis of COVID-19 vaccine trials conducted by Cai et al. (2021) found that mRNA-based vaccines have the highest efficacy of 94.29% in preventing SARS-CoV-2 infection compared to the other vaccine platforms. Protein subunit-, viral vector- and inactivated-based vaccines were found to have efficacies of 89%, 79% and 73%, respectively in preventing SARS-CoV-2 infection (Cai

Table 2
Examples of vaccines approved around the world as of July 2022.

Vaccine platform	Vaccine name	Emergency use authorization by FDA	FDA approved	Other
mRNA-based	Comirnaty (Pfizer-BioNTech)		✓	
	Spikevax (Moderna)		✓	
	GEMCOVAC-19 (Gennova Biopharmaceuticals Limited)			Approved in India only
Non replicating viral vector-based	Janssen (Johnson & Johnson)	✓		
	Covishield (Oxford/AstraZeneca)			Approved in 49 countries including Canada, Egypt, Lebanon and South Africa
	Vaxzevria (Oxford/AstraZeneca)			Approved in 141 countries including Australia, Canada, Mexico, Nigeria and Malaysia
Protein subunit-based	Nuvaxovid (Novavax)	✓		
	COVOVAX (Novavax)			Approved in Bangladesh, India, Indonesia, Philippines and Thailand.
	Abdala (Center for Genetic Engineering and Biotechnology)			Approved in Cuba, Mexico, Nicaragua, Saint Vincent and the Grenadines, Venezuela and Viet Nam
Inactivated virus-based	VLA2001 (Valneva)			Approved in 33 countries including Austria, Denmark, United Kingdom and Spain
	Covaxin (Bharat Biotech)			Approved in 14 countries including India, Mexico, Philippines and Malaysia
	Covilo (Sinopharm Beijing)			Approved in 91 countries including Brazil, South Africa, Venezuela and China.

et al., 2021). Although the level of protection against SARS-CoV-2 infection with the different vaccines varies, the various vaccines substantially protect against severe COVID-19, and more specifically hospitalization and death (Self et al., 2021). The role of COVID-19 vaccination in controlling viral transmission remains unclear. However, empirical evidence to date has demonstrated that vaccination of healthcare workers is associated with reduced COVID-19 cases among members of their households, suggesting that vaccination may reduce SARS-CoV-2 transmission (Shah et al., 2021).

To date, 5 billion COVID-19 vaccine doses have been administered in total (Mathieu et al., 2021). About 54.4% of the global population received at least one dose of a COVID-19 vaccine with about 85% of doses administered in high-income countries (Mathieu et al., 2021). While about 60–70% of individuals in high-income countries have received at least one dose, less than 2% of the population in the entire African continent have received even a single dose (Mathieu et al., 2021). Global vaccine inequity enables continued SARS-CoV-2 infection in low-income countries where a large majority of the population is unvaccinated. These conditions facilitate viral evolution through acquired mutations, some of which may confer selective advantages such as enhanced transmission and immune evasion, potentially giving rise to future VOCs (Asundi et al., 2021).

Despite the efficacy of the COVID-19 vaccines, a small percentage of individuals who have received all the recommended doses and are thus fully vaccinated develop either asymptomatic or symptomatic infections, known as vaccine breakthrough infections. As of April 30, 2021, 10,262 SARS-CoV-2 breakthrough infections were reported in the United States out of approximately 101 million vaccinated individuals in the country, with only a small percentage of reported breakthrough cases resulting in hospitalization (Team CC-VBCI, 2021). In January 2022, the Centers for Diseases Control and Prevention highlighted that vaccine breakthrough infections became much more common when the Omicron-variant rapidly became the predominant circulating strain (Prevention CfDCa, 2022). As SARS-CoV-2 continues to circulate within the population, the virus is exposed to strong selection pressures, such as immunity mediated by both natural infection and vaccines, which may drive the selection of SARS-CoV-2 variants that will likely continue driving the global pandemic. Another important concern is the effectiveness of the current vaccines in protecting against current and future emerging VOCs. A study by Garcia-Beltran et al. (2021) found that sera from individuals who were fully vaccinated with either Pfizer-BioNTech Comirnaty COVID-19 or Moderna Spikevax® COVID-19 vaccines had reduced neutralization against certain VOCs, suggesting that some SARS-CoV-2 variants may escape neutralization by vaccine-induced humoral immunity. Nonetheless, studies have highlighted that the vaccines protect against severe illness and hospitalization in individuals infected with SARS-CoV-2 variants (Hayawi et al., 2021). The COVID-19 vaccines that are currently in use have all been designed to provide protection against the original Wuhan strain of SARS-CoV-2 that was sequenced in early 2020. However, several vaccine technologies currently in use for COVID-19 can be more easily adapted to provide protection against emerging SARS-CoV-2 strains compared to traditional vaccine platforms, such as live-attenuated or protein subunit-based vaccines. For example, mRNA- and viral vector-based vaccines can be modified to encode the SARS-CoV-2 S antigen with the mutations found in any given VOCs. Overall, certain modern vaccine platforms can be more easily adapted to target specific SARS-CoV-2 VOCs.

7. Future perspectives on SARS-CoV-2 and COVID-19

While the future of SARS-CoV-2 and COVID-19 remain largely uncertain, potential possibilities can be drawn from the evolution of influenza A virus (IAV) since the 1918 influenza pandemic. The 1918 IAV, the causative agent of the first well-documented influenza pandemic of the 20th century, contained a novel set of eight minus ssRNA segments likely originating from avian-like viruses that adapted to mammals (Morens et al., 2009). This pandemic is recognized as occurring in three waves, causing an estimated 50 million deaths in total (Johnson and Mueller, 2002). Throughout the last century, interspecies transmission occurred between humans, pigs, and avian species, and continues to occur today, which enables the virus to remain in global circulation (Morens et al., 2009; Nelson et al., 2012). Viral recombination through reassortment of the eight minus ssRNA segments and mixing of these segments in human and animal reservoirs have enabled parts of the 1918 IAV to remain in circulation in both humans and pigs causing pandemic-like events. For example, fourth-generation direct descendants of the 1918 IAV caused the 2009 H1N1 swine flu pandemic. Today, IAV is considered endemic, and remains a major public health concern. Like IAV, the ability of SARS-CoV-2 to undergo “ping-pong” transmission between humans and animals may give rise to novel SARS-CoV-2 strains that may continue to fuel the global pandemic. As reported for the SARS-CoV-2 mink-derived variant that was reintroduced into the human population through reverse zoonosis, the weak species barrier of SARS-CoV-2 may enable the virus to acquire mutations that confer resistance to currently availably vaccines and therapeutics. Also like IAV, albeit through a distinct molecular mechanism, HCoVs

have a high frequency of RNA recombination (Simon-Loriere and Holmes, 2011; Singh and Yi, 2021), particularly during the template-switching process during discontinuous RNA replication. Given the wide species tropism of SARS-CoV-2, genetically distinct viral strains as well as different coronaviruses may co-infect human or animal host cells and undergo recombination giving rise to novel viral strains, variants and perhaps even new HCoVs. This possibility is further supported by the study of Li et al. (2020) demonstrating that the SARS-CoV-2 S proteins' RBD was introduced through recombination with coronaviruses from pangolins, which likely played a critical role in its ability to infect humans. Overall, surveillance and monitoring of CoVs in wildlife will likely be crucial in forecasting and preventing future emerging SARS-CoV-2 strains and novel HCoVs.

Given that four of the seven HCoVs identified to date cause mild respiratory illness, SARS-CoV-2 may possibly become attenuated and cause the common cold. However, as SARS-CoV and MERS-CoV remain highly pathogenic, this possibility seems unlikely, at least in the foreseeable future. As mass vaccination is underway and the currently available COVID-19 vaccines effectively prevent severe disease, a more plausible scenario is one where herd immunity may eventually render the impact of SARS-CoV-2 to be comparable to that of the common cold HCoVs. Whether herd immunity to SARS-CoV-2 will become a reality remains in question for several reasons. Above all, global vaccine inequity had led to large disparities in vaccination rates between high- and low-income countries. Accordingly, high SARS-CoV-2 transmission is sustained in many regions around the world and creates further opportunities for the virus to mutate, which may give rise to emerging variants (Ye et al., 2022). Vaccine hesitancy equally acts as an important barrier to achieving herd immunity (Dhama et al., 2021). Ultimately, collaborative relationships at the international level are required to take urgent action toward reducing vaccine inequity and vaccine hesitancy. Moreover, waning immunity has been reported at around six months post-vaccination in fully vaccinated individuals (Levin et al., 2021; Tartof et al., 2021; Goldberg et al., 2021), which can be circumvented through annual or biannual COVID-19 vaccinations, perhaps with frequently updated composition of the vaccine much like seasonal flu vaccines.

Even with mass vaccination and antiviral therapies available, emerging evidence has highlighted that some individuals who have been infected with SARS-CoV-2 continue to have symptoms associated with COVID-19 weeks to months post-infection (Taquet et al., 2021b), even in vaccinated individuals albeit to a lesser extent than those who are not (UK Health Secruity Agency, 2022). Such long COVID symptoms include shortness of breath, headaches, chest pain, myalgia, fatigue and cognitive difficulties (Taquet et al., 2021b). In fact, a recent study demonstrated that mild SARS-CoV-2 infection is associated with changes in brain structure and cognitive decline (Douaud et al., 2022). Whether these effects can be reversed or whether they will persist long-term remain unknown. Overall, the management and care of individuals with long COVID will continue to burden healthcare systems worldwide.

8. Conclusions

Overall, HCoVs remain an important global health concern, which is emphasized by the emergence of SARS-CoV-2 that has led to the COVID-19 global pandemic. While zoonotic origin is common to the seven HCoVs identified to date, the ability for interspecies and "ping pong" transmission appears to be more unique to SARS-CoV-2 and may have important implications for viral evolution and pandemic persistence. As SARS-CoV-2 continues to infect individuals worldwide, this virus continues to mutate despite its unique proofreading activity and VOCs continue to emerge. Several reverse genetics-based systems are currently being used in efforts to elucidate the impact of mutations of interest in VOCs. To date, limited SARS-CoV-2 antiviral drugs are available, and the rise of SARS-CoV-2 VOCs has raised concern for development of drug resistance. Meanwhile, prophylactic COVID-19 vaccines targeting the viral Spike protein have been developed using various vaccine platforms. Although the currently available vaccines have varying efficacy in preventing SARS-CoV-2 infection, they have shown promising results in preventing severe COVID-19. While there is concern that VOCs may escape vaccine-induced antibodies, certain vaccine platforms, such as mRNA-based vaccines, can feasibly be modified to specifically target the viral Spike protein of VOCs. While the future of SARS-CoV-2 and COVID-19 remain uncertain, it is likely that SARS-CoV-2 will continue to evolve and burden health care systems worldwide for the foreseeable future.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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