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REVIEW

Coronavirus Disease 2019–COVID-19

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SUMMARY In recent decades, several new diseases have emerged in different geographical areas, with pathogens including Ebola virus, Zika virus, Nipah virus, and coronaviruses (CoVs). Recently, a new type of viral infection emerged in Wuhan City, China, and initial genomic sequencing data of this virus do not match with previously sequenced CoVs, suggesting a novel CoV strain (2019-nCoV), which has now been termed severe acute respiratory syndrome CoV-2 (SARS-CoV-2). Although coronavirus disease 2019 (COVID-19) is suspected to originate from an animal host (zoonotic origin) followed by human-to-human transmission, the possibility of other routes should not be ruled out. Compared to diseases caused by previously known

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human CoVs, COVID-19 shows less severe pathogenesis but higher transmission competence, as is evident from the continuously increasing number of confirmed cases globally. Compared to other emerging viruses, such as Ebola virus, avian H7N9, SARS-CoV, and Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-2 has shown relatively low pathogenicity and moderate transmissibility. Codon usage studies suggest that this novel virus has been transferred from an animal source, such as bats. Early diagnosis by real-time PCR and next-generation sequencing has facilitated the identification of the pathogen at an early stage. Since no antiviral drug or vaccine exists to treat or prevent SARS-CoV-2, potential therapeutic strategies that are currently being evaluated predominantly stem from previous experience with treating SARS-CoV, MERS-CoV, and other emerging viral diseases. In this review, we address epidemiological, diagnostic, clinical, and therapeutic aspects, including perspectives of vaccines and preventive measures that have already been globally recommended to counter this pandemic virus.

KEYWORDS COVID-19, emerging coronavirus, SARS-CoV-2, diagnosis, One Health, therapy, vaccines

INTRODUCTION

Over the past 2 decades, coronaviruses (CoVs) have been associated with significant disease outbreaks in East Asia and the Middle East. The severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) began to emerge in 2002 and 2012, respectively. Recently, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19), emerged in late 2019, and it has posed a global health threat, causing an ongoing pandemic in many countries and territories (1).

Health workers worldwide are currently making efforts to control further disease outbreaks caused by the novel CoV (originally named 2019-nCoV), which was first identified in Wuhan City, Hubei Province, China, on 12 December 2019. On 11 February 2020, the World Health Organization (WHO) announced the official designation for the current CoV-associated disease to be COVID-19, caused by SARS-CoV-2. The primary cluster of patients was found to be connected with the Huanan South China Seafood Market in Wuhan (2). CoVs belong to the family *Coronaviridae* (subfamily *Coronavirinae*), the members of which infect a broad range of hosts, producing symptoms and diseases ranging from the common cold to severe and ultimately fatal illnesses, such as SARS, MERS, and, presently, COVID-19. SARS-CoV-2 is considered one of the seven members of the CoV family that infect humans (3), and it belongs to the same lineage of CoVs that causes SARS; however, this novel virus is genetically distinct. Until 2020, six CoVs were known to infect humans, including human CoV 229E (HCoV-229E), HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV, and MERS-CoV. Although SARS-CoV and MERS-CoV have resulted in outbreaks with high mortality, others remain associated with mild upper-respiratory-tract illnesses (4).

Newly evolved CoVs pose a high threat to global public health. The current emergence of COVID-19 is the third CoV outbreak in humans over the past 2 decades (5). It is no coincidence that Fan et al. predicted potential SARS- or MERS-like CoV outbreaks in China following pathogen transmission from bats (6). COVID-19 emerged in China and spread rapidly throughout the country and, subsequently, to other countries. Due to the severity of this outbreak and the potential of spreading on an international scale, the WHO declared a global health emergency on 31 January 2020; subsequently, on 11 March 2020, they declared it a pandemic situation. At present, we are not in a position to effectively treat COVID-19, since neither approved vaccines nor specific antiviral drugs for treating human CoV infections are available (7–9). Most nations are currently making efforts to prevent the further spreading of this potentially deadly virus by implementing preventive and control strategies.

In domestic animals, infections with CoVs are associated with a broad spectrum of

pathological conditions. Apart from infectious bronchitis virus, canine respiratory CoV, and mouse hepatitis virus, CoVs are predominantly associated with gastrointestinal diseases (10). The emergence of novel CoVs may have become possible because of multiple CoVs being maintained in their natural host, which could have favored the probability of genetic recombination (10). High genetic diversity and the ability to infect multiple host species are a result of high-frequency mutations in CoVs, which occur due to the instability of RNA-dependent RNA polymerases along with higher rates of homologous RNA recombination (10, 11). Identifying the origin of SARS-CoV-2 and the pathogen's evolution will be helpful for disease surveillance (12), development of new targeted drugs, and prevention of further epidemics (13). The most common symptoms associated with COVID-19 are fever, cough, dyspnea, expectoration, headache, and myalgia or fatigue.

In contrast, less common signs at the time of hospital admission include diarrhea, hemoptysis, and shortness of breath (14). Recently, individuals with asymptomatic infections were also suspected of transmitting infections, which further adds to the complexity of disease transmission dynamics in COVID-19 infections (1). Such efficient responses require in-depth knowledge regarding the virus, which currently is a novel agent; consequently, further studies are required.

Comparing the genome of SARS-CoV-2 with that of the closely related SARS/SARS-like CoV revealed that the sequence coding for the spike protein, with a total length of 1,273 amino acids, showed 27 amino acid substitutions. Six of these substitutions are in the region of the receptor-binding domain (RBD), and another six substitutions are in the underpinning subdomain (SD) (16). Phylogenetic analyses have revealed that SARS-CoV-2 is closely related (88% similarity) to two SARS-like CoVs derived from bat SARS-like CoVs (bat-SL-CoVZC45 and bat-SL-CoVZXC21) (Fig. 1). Furthermore, SARS-CoV-2 is genetically distinct from SARS-CoV (79% similarity) and MERS-CoV (nearly 50%) (17). COVID-19 is associated with afflictions of the lungs in all cases and generated characteristic chest computer tomography findings, such as the presence of multiple lesions in lung lobes that appear as dense, ground-glass opaque structures that occasionally coexist with consolidation shadows (18).

Some therapeutic options for treating COVID-19 showed efficacy in *in vitro* studies; however, to date, these treatments have not undergone any randomized animal or human clinical trials, which limit their practical applicability in the current pandemic (7, 9, 19–21).

The present comprehensive review describes the various features of SARS-CoV-2/COVID-19 causing the current disease outbreaks and advances in diagnosis and developing vaccines and therapeutics. It also provides a brief comparison with the earlier SARS and MERS CoVs, the veterinary perspective of CoVs and this emerging novel pathogen, and an evaluation of the zoonotic potential of similar CoVs to provide feasible One Health strategies for the management of this fatal virus (22–367).

THE VIRUS (SARS-CoV-2)

Coronaviruses are positive-sense RNA viruses having an extensive and promiscuous range of natural hosts and affect multiple systems (23, 24). Coronaviruses can cause clinical diseases in humans that may extend from the common cold to more severe respiratory diseases like SARS and MERS (17, 279). The recently emerging SARS-CoV-2 has wrought havoc in China and caused a pandemic situation in the worldwide population, leading to disease outbreaks that have not been controlled to date, although extensive efforts are being put in place to counter this virus (25). This virus has been proposed to be designated/named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV), which determined the virus belongs to the *Severe acute respiratory syndrome-related coronavirus* category and found this virus is related to SARS-CoVs (26). SARS-CoV-2 is a member of the order *Nidovirales*, family *Coronaviridae*, subfamily *Orthocoronavirinae*, which is subdivided into four genera, *viz.*, *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus* (3, 27). The genera *Alphacoronavirus* and *Betacoro-*

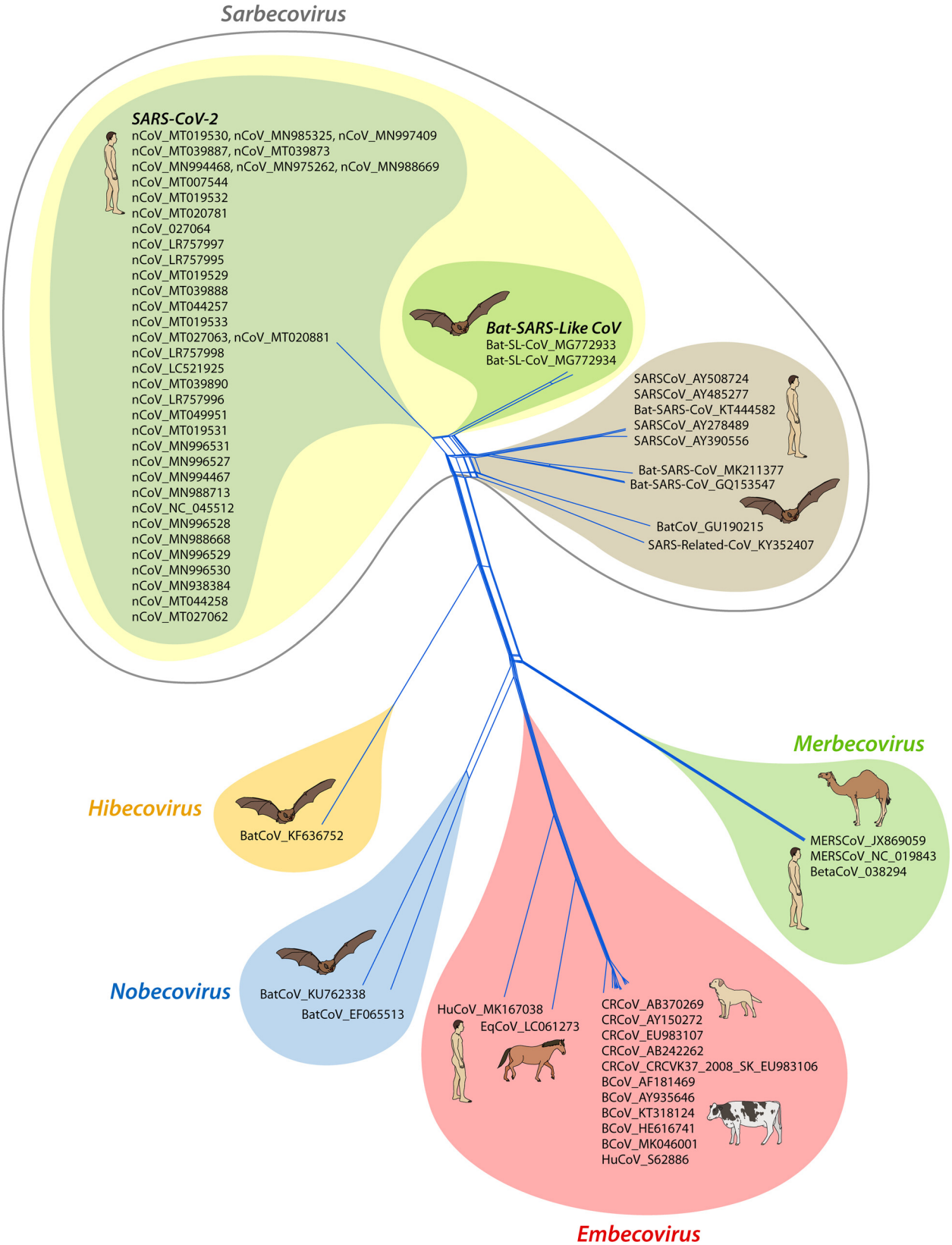

FIG 1 S-gene SplitsTree analysis. Shown is the spike (S) glycoprotein gene-based phylogenetic analysis (SplitsTree 4.0) of SAR-CoV-2 isolates (39 isolates). The SARS-CoV-2 isolates were analyzed with related CoVs from past human outbreaks and of animal origin, including MERS-CoV, bovine coronavirus, canine coronavirus, bat coronaviruses, bat-SL-SARS-CoV, and equine CoV. The analysis includes all five defined subgenera of *Betacoronaviruses*, namely, *Sarbecovirus*, *Embecovirus*, *Merbecovirus*, *Nobecovirus*, and *Hibecovirus*. The isolates in the gray area are from the current outbreak of SARS-CoV-2 from around the world. The nearest neighbors of SARS-CoV-2 are the bat-SL-CoV, encircled in yellow.

navirus originate from bats, while *Gammacoronavirus* and *Deltacoronavirus* have evolved from bird and swine gene pools (24, 28, 29, 275).

Coronaviruses possess an unsegmented, single-stranded, positive-sense RNA genome of around 30 kb, enclosed by a 5′-cap and 3′-poly(A) tail (30). The genome of SARS-CoV-2 is 29,891 bp long, with a G+C content of 38% (31). These viruses are encircled with an envelope containing viral nucleocapsid. The nucleocapsids in CoVs are arranged in helical symmetry, which reflects an atypical attribute in positive-sense RNA viruses (30). The electron micrographs of SARS-CoV-2 revealed a diverging spherical outline with some degree of pleomorphism, virion diameters varying from 60 to 140 nm, and distinct spikes of 9 to 12 nm, giving the virus the appearance of a solar corona (3). The CoV genome is arranged linearly as 5′-leader-UTR-replicase-structural genes (S-E-M-N)-3′ UTR-poly(A) (32). Accessory genes, such as 3a/b, 4a/b, and the hemagglutinin-esterase gene (HE), are also seen intermingled with the structural genes (30). SARS-CoV-2 has also been found to be arranged similarly and encodes several accessory proteins, although it lacks the HE, which is characteristic of some betacoronaviruses (31). The positive-sense genome of CoVs serves as the mRNA and is translated to polyprotein 1a/1ab (pp1a/1ab) (33). A replication-transcription complex (RTC) is formed in double-membrane vesicles (DMVs) by nonstructural proteins (nsps), encoded by the polyprotein gene (34). Subsequently, the RTC synthesizes a nested set of subgenomic RNAs (sgRNAs) via discontinuous transcription (35).

Based on molecular characterization, SARS-CoV-2 is considered a new *Betacoronavirus* belonging to the subgenus *Sarbecovirus* (3). A few other critical zoonotic viruses (MERS-related CoV and SARS-related CoV) belong to the same genus. However, SARS-CoV-2 was identified as a distinct virus based on the percent identity with other *Betacoronavirus*; conserved open reading frame 1a/b (ORF1a/b) is below 90% identity (3). An overall 80% nucleotide identity was observed between SARS-CoV-2 and the original SARS-CoV, along with 89% identity with ZC45 and ZXC21 SARS-related CoVs of bats (2, 31, 36). In addition, 82% identity has been observed between SARS-CoV-2 and human SARS-CoV Tor2 and human SARS-CoV BJ01 2003 (31). A sequence identity of only 51.8% was observed between MERS-related CoV and the recently emerged SARS-CoV-2 (37). Phylogenetic analysis of the structural genes also revealed that SARS-CoV-2 is closer to bat SARS-related CoV. Therefore, SARS-CoV-2 might have originated from bats, while other amplifier hosts might have played a role in disease transmission to humans (31). Of note, the other two zoonotic CoVs (MERS-related CoV and SARS-related CoV) also originated from bats (38, 39). Nevertheless, for SARS and MERS, civet cat and camels, respectively, act as amplifier hosts (40, 41).

Coronavirus genomes and subgenomes encode six ORFs (31). The majority of the 5′ end is occupied by ORF1a/b, which produces 16 nsps. The two polyproteins, pp1a and pp1ab, are initially produced from ORF1a/b by a −1 frameshift between ORF1a and ORF1b (32). The virus-encoded proteases cleave polyproteins into individual nsps (main protease [Mpro], chymotrypsin-like protease [3CLpro], and papain-like proteases [PLPs]) (42). SARS-CoV-2 also encodes these nsps, and their functions have been elucidated recently (31). Remarkably, a difference between SARS-CoV-2 and other CoVs is the identification of a novel short putative protein within the ORF3 band, a secreted protein with an alpha helix and beta-sheet with six strands encoded by ORF8 (31).

Coronaviruses encode four major structural proteins, namely, spike (S), membrane (M), envelope (E), and nucleocapsid (N), which are described in detail below.

S Glycoprotein

Coronavirus S protein is a large, multifunctional class I viral transmembrane protein. The size of this abundant S protein varies from 1,160 amino acids (IBV, infectious bronchitis virus, in poultry) to 1,400 amino acids (FCoV, feline coronavirus) (43). It lies in a trimer on the virion surface, giving the virion a corona or crown-like appearance. Functionally it is required for the entry of the infectious virion particles into the cell through interaction with various host cellular receptors (44).

Furthermore, it acts as a critical factor for tissue tropism and the determination of host range (45). Notably, S protein is one of the vital immunodominant proteins of CoVs capable of inducing host immune responses (45). The ectodomains in all CoVs S proteins have similar domain organizations, divided into two subunits, S1 and S2 (43). The first one, S1, helps in host receptor binding, while the second one, S2, accounts for fusion. The former (S1) is further divided into two subdomains, namely, the N-terminal domain (NTD) and C-terminal domain (CTD). Both of these subdomains act as receptor-binding domains, interacting efficiently with various host receptors (45). The S1 CTD contains the receptor-binding motif (RBM). In each coronavirus spike protein, the trimeric S1 locates itself on top of the trimeric S2 stalk (45). Recently, structural analyses of the S proteins of COVID-19 have revealed 27 amino acid substitutions within a 1,273-amino-acid stretch (16). Six substitutions are located in the RBD (amino acids 357 to 528), while four substitutions are in the RBM at the CTD of the S1 domain (16). Of note, no amino acid change is seen in the RBM, which binds directly to the angiotensin-converting enzyme-2 (ACE2) receptor in SARS-CoV (16, 46). At present, the main emphasis is knowing how many differences would be required to change the host tropism. Sequence comparison revealed 17 nonsynonymous changes between the early sequence of SARS-CoV-2 and the later isolates of SARS-CoV. The changes were found scattered over the genome of the virus, with nine substitutions in ORF1ab, ORF8 (4 substitutions), the spike gene (3 substitutions), and ORF7a (single substitution) (4). Notably, the same nonsynonymous changes were found in a familial cluster, indicating that the viral evolution happened during person-to-person transmission (4, 47). Such adaptive evolution events are frequent and constitute a constantly ongoing process once the virus spreads among new hosts (47). Even though no functional changes occur in the virus associated with this adaptive evolution, close monitoring of the viral mutations that occur during subsequent human-to-human transmission is warranted.

M Protein

The M protein is the most abundant viral protein present in the virion particle, giving a definite shape to the viral envelope (48). It binds to the nucleocapsid and acts as a central organizer of coronavirus assembly (49). Coronavirus M proteins are highly diverse in amino acid contents but maintain overall structural similarity within different genera (50). The M protein has three transmembrane domains, flanked by a short amino terminus outside the virion and a long carboxy terminus inside the virion (50). Overall, the viral scaffold is maintained by M-M interaction. Of note, the M protein of SARS-CoV-2 does not have an amino acid substitution compared to that of SARS-CoV (16).

E Protein

The coronavirus E protein is the most enigmatic and smallest of the major structural proteins (51). It plays a multifunctional role in the pathogenesis, assembly, and release of the virus (52). It is a small integral membrane polypeptide that acts as a viroporin (ion channel) (53). The inactivation or absence of this protein is related to the altered virulence of coronaviruses due to changes in morphology and tropism (54). The E protein consists of three domains, namely, a short hydrophilic amino terminal, a large hydrophobic transmembrane domain, and an efficient C-terminal domain (51). The SARS-CoV-2 E protein reveals a similar amino acid constitution without any substitution (16).

N Protein

The N protein of coronavirus is multipurpose. Among several functions, it plays a role in complex formation with the viral genome, facilitates M protein interaction needed during virion assembly, and enhances the transcription efficiency of the virus (55, 56). It contains three highly conserved and distinct domains, namely, an NTD, an RNA-binding domain or a linker region (LKR), and a CTD (57). The NTD binds with the 3′ end of the viral genome, perhaps via electrostatic interactions, and is highly diverged both in length and sequence (58). The charged LKR is serine and arginine rich and is also known as the SR (serine and arginine) domain (59). The LKR is capable of direct

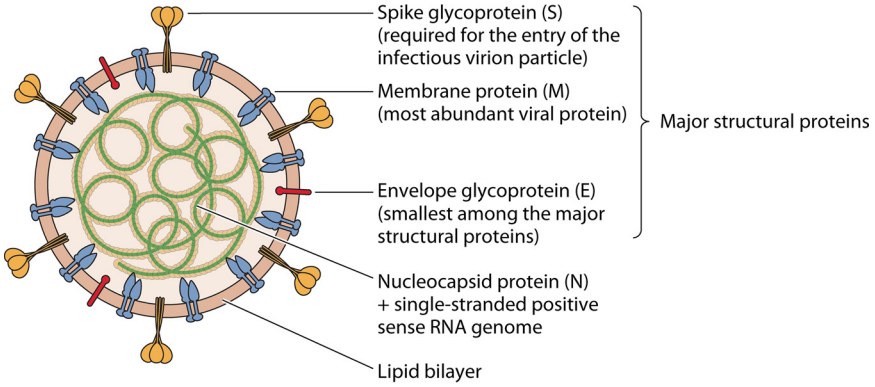

FIG 2 SARS-CoV-2 virus structure.

interaction with *in vitro* RNA interaction and is responsible for cell signaling (60, 61). It also modulates the antiviral response of the host by working as an antagonist for interferon (IFN) and RNA interference (62). Compared to that of SARS-CoV, the N protein of SARS-CoV-2 possess five amino acid mutations, where two are in the intrinsically dispersed region (IDR; positions 25 and 26), one each in the NTD (position 103), LKR (position 217), and CTD (position 334) (16).

nsps and Accessory Proteins

Besides the important structural proteins, the SARS-CoV-2 genome contains 15 nsps, nsp1 to nsp10 and nsp12 to nsp16, and 8 accessory proteins (3a, 3b, p6, 7a, 7b, 8b, 9b, and ORF14) (16). All these proteins play a specific role in viral replication (27). Unlike the accessory proteins of SARS-CoV, SARS-CoV-2 does not contain 8a protein and has a longer 8b and shorter 3b protein (16). The nsp7, nsp13, envelope, matrix, and p6 and 8b accessory proteins have not been detected with any amino acid substitutions compared to the sequences of other coronaviruses (16).

The virus structure of SARS-CoV-2 is depicted in Fig. 2.

SARS-CoV-2 Spike Glycoprotein Gene Analysis

Sequence percent similarity analysis. We assessed the nucleotide percent similarity using the MegAlign software program, where the similarity between the novel SARS-CoV-2 isolates was in the range of 99.4% to 100%. Among the other *Serbecovirus* CoV sequences, the novel SARS-CoV-2 sequences revealed the highest similarity to bat-SL-CoV, with nucleotide percent identity ranges between 88.12 and 89.65%. Meanwhile, earlier reported SARS-CoVs showed 70.6 to 74.9% similarity to SARS-CoV-2 at the nucleotide level. Further, the nucleotide percent similarity was 55.4%, 45.5% to 47.9%, 46.2% to 46.6%, and 45.0% to 46.3% to the other four subgenera, namely, *Hibecovirus*, *Nobecovirus*, *Merbecovirus*, and *Embecovirus*, respectively. The percent similarity index of current outbreak isolates indicates a close relationship between SARS-CoV-2 isolates and bat-SL-CoV, indicating a common origin. However, particular pieces of evidence based on further complete genomic analysis of current isolates are necessary to draw any conclusions, although it was ascertained that the current novel SARS-CoV-2 isolates belong to the subgenus *Sarbecovirus* in the diverse range of betacoronaviruses. Their possible ancestor was hypothesized to be from bat CoV strains, wherein bats might have played a crucial role in harboring this class of viruses.

SplitsTree phylogeny analysis. In the unrooted phylogenetic tree of different betacoronaviruses based on the S protein, virus sequences from different subgenera grouped into separate clusters. SARS-CoV-2 sequences from Wuhan and other countries exhibited a close relationship and appeared in a single cluster (Fig. 1). The CoVs from the subgenus *Sarbecovirus* appeared jointly in SplitsTree and divided into three subclusters, namely, SARS-CoV-2, bat-SARS-like-CoV (bat-SL-CoV), and SARS-CoV (Fig. 1). In the case of other subgenera, like *Merbecovirus*, all of the sequences grouped

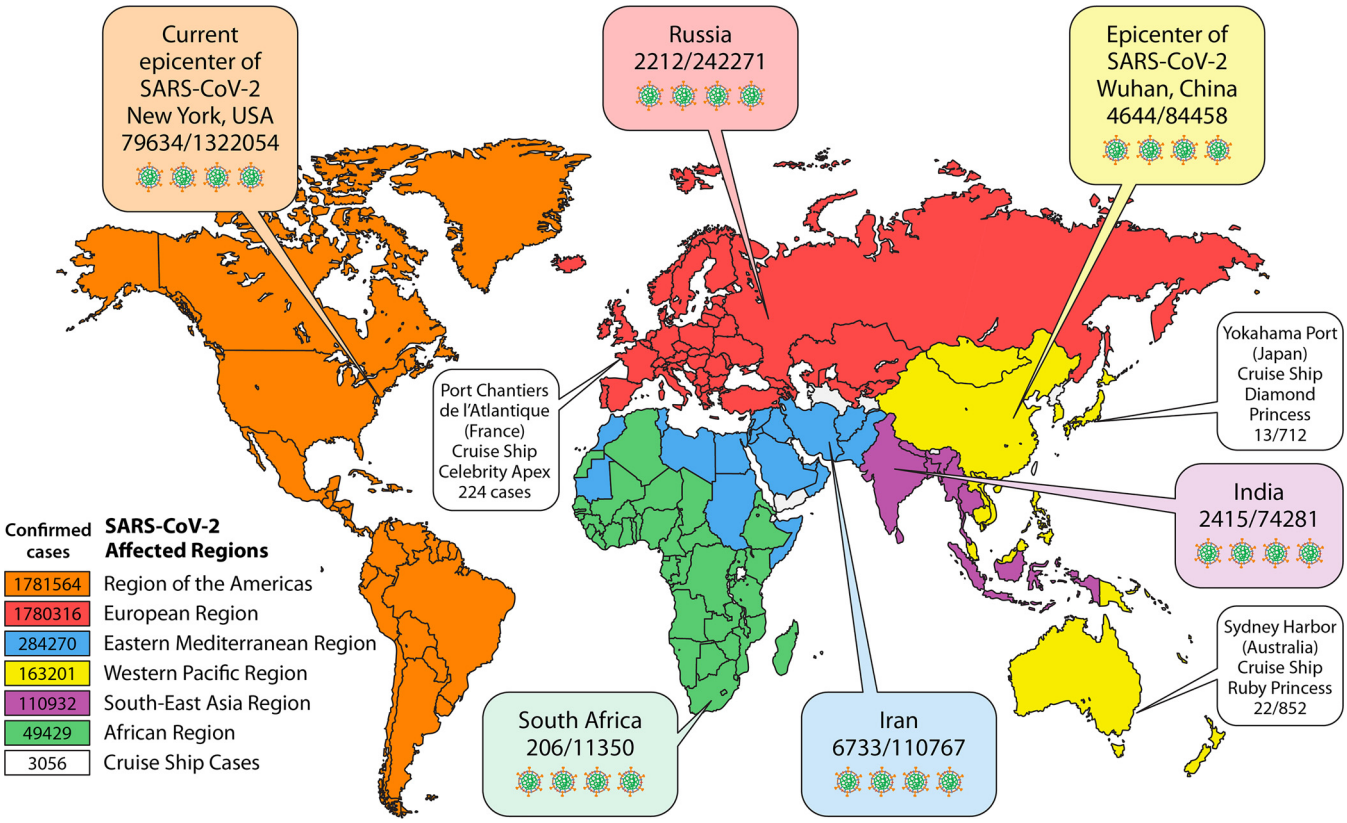

FIG 3 World map depicting the current scenario of COVID-19. Shown are countries, territories, or regions with reported confirmed cases of SARS-CoV-2 as of 13 May 2020. Different colors indicate different WHO designated geographical regions with the number of confirmed cases. The WHO region-wise total number of confirmed cases is depicted in different color strips. The leading information on the confirmed cases and deaths from all six WHO designated regions are depicted in circled balloons. The numbers of COVID-19 cases and fatalities on three major cruise ships are also depicted. (Based on data from the WHO at https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200513-covid-19-sitrep-114.pdf?sfvrsn=17ebbbe_4; updated numbers of cases, deaths, and patients recovered can be found at https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6.)

in a single cluster, whereas in *Embecovirus*, different species, comprised of canine respiratory CoVs, bovine CoVs, equine CoVs, and human CoV strain (OC43), grouped in a common cluster. Isolates in the subgenera *Nobecovorus* and *Hibecovirus* were found to be placed separately away from other reported SARS-CoVs but shared a bat origin.

CURRENT WORLDWIDE SCENARIO OF SARS-CoV-2

This novel virus, SARS-CoV-2, comes under the subgenus *Sarbecovirus* of the *Orthocoronavirinae* subfamily and is entirely different from the viruses responsible for MERS-CoV and SARS-CoV (3). The newly emerged SARS-CoV-2 is a group 2B coronavirus (2). The genome sequences of SARS-CoV-2 obtained from patients share 79.5% sequence similarity to the sequence of SARS-CoV (63).

As of 13 May 2020, a total of 4,170,424 confirmed cases of COVID-19 (with 287,399 deaths) have been reported in more than 210 affected countries worldwide (WHO Situation Report 114 [25]) (Fig. 3).

Initially, the epicenter of the SARS-CoV-2 pandemic was China, which reported a significant number of deaths associated with COVID-19, with 84,458 laboratory-confirmed cases and 4,644 deaths as of 13 May 2020 (Fig. 4). As of 13 May 2020, SARS-CoV-2 confirmed cases have been reported in more than 210 countries apart from China (Fig. 3 and 4) (WHO Situation Report 114) (25, 64). COVID-19 has been reported on all continents except Antarctica. For many weeks, Italy was the focus of concerns regarding the large number of cases, with 221,216 cases and 30,911 deaths, but now, the United States is the country with the largest number of cases, 1,322,054, and 79,634 deaths. Now, the United Kingdom has even more cases (226,4671) and deaths (32,692)

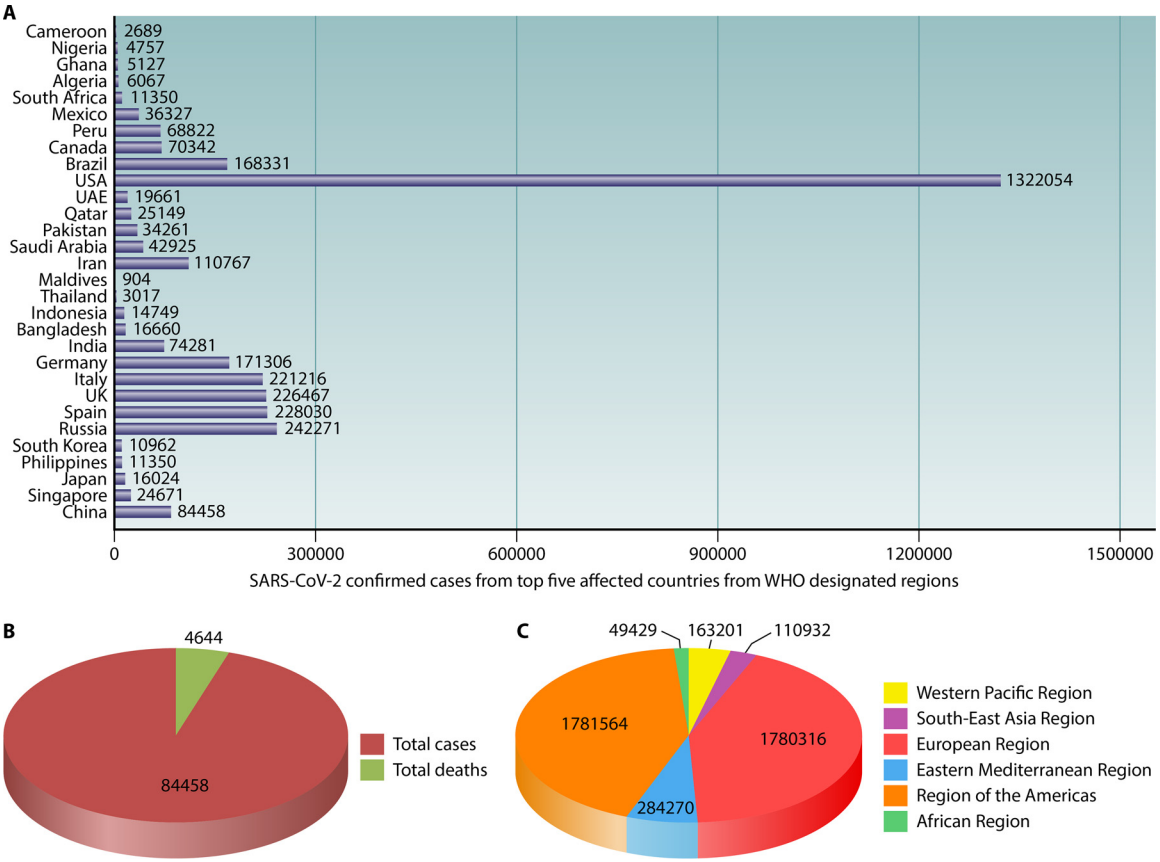

FIG 4 Bar graph and pie chart for cases and deaths. Shown are laboratory-confirmed cases and deaths in China and the rest of the world due to SARS-CoV-2. (A) SARS-CoV-2 confirmed cases in the top five affected countries from each WHO designated region, where maximum casualties were reported to WHO until 13 May 2020. (B) Total numbers of deaths and cases in China only. (C) Total number of cases worldwide by region.

than Italy. A John Hopkins University web platform has provided daily updates on the basic epidemiology of the COVID-19 outbreak (https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6) (238).

COVID-19 has also been confirmed on a cruise ship, named *Diamond Princess*, quarantined in Japanese waters (Port of Yokohama), as well as on other cruise ships around the world (239) (Fig. 3). The significant events of the SARS-CoV-2/COVID-19 virus outbreak occurring since 8 December 2019 are presented as a timeline in Fig. 5.

At the beginning, China experienced the majority of the burden associated with COVID-19 in the form of disease morbidity and mortality (65), but over time the COVID-19 menace moved to Europe, particularly Italy and Spain, and now the United States has the highest number of confirmed cases and deaths. The COVID-19 outbreak has also been associated with severe economic impacts globally due to the sudden interruption of global trade and supply chains that forced multinational companies to make decisions that led to significant economic losses (66). The recent increase in the number of confirmed critically ill patients with COVID-19 has already surpassed the intensive care supplies, limiting intensive care services to only a small portion of critically ill patients (67). This might also have contributed to the increased case fatality rate observed in the COVID-19 outbreak.

Viewpoint on SARS-CoV-2 Transmission, Spread, and Emergence

The novel coronavirus was identified within 1 month (28 days) of the outbreak. This is impressively fast compared to the time taken to identify SARS-CoV reported in Foshan, Guangdong Province, China (125 days) (68). Immediately after the confirmation

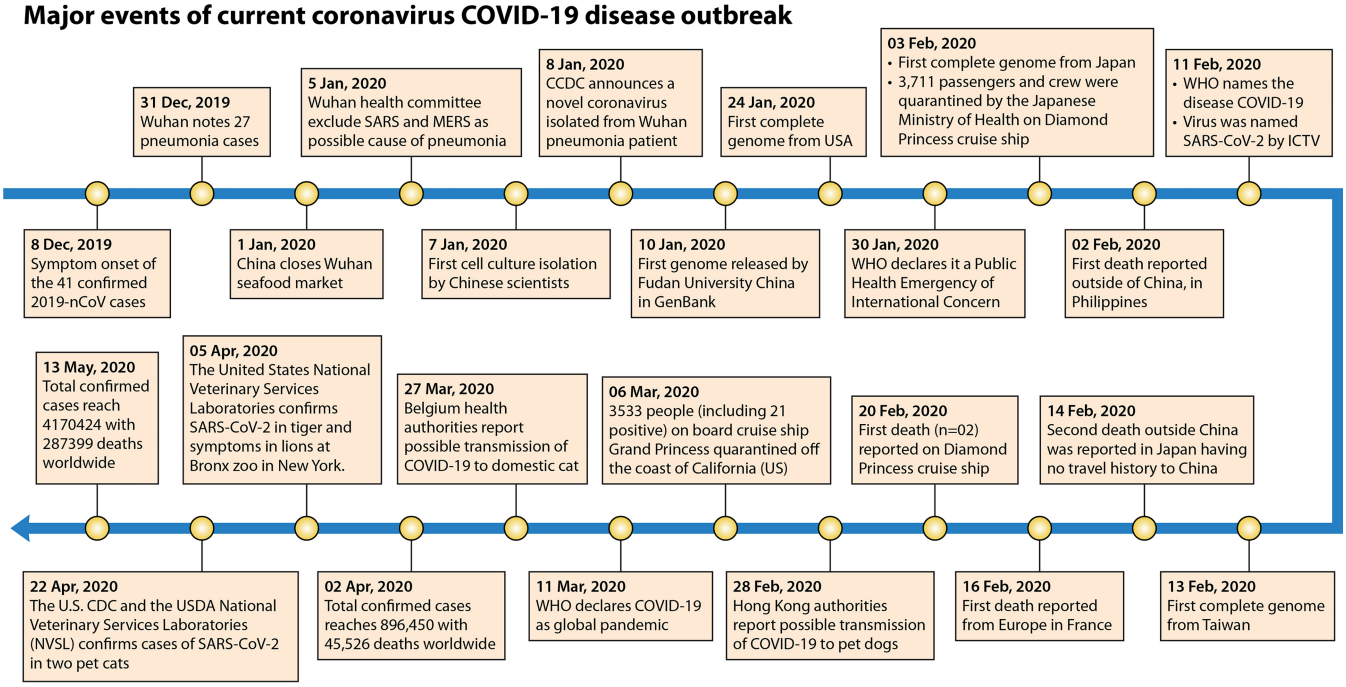

FIG 5 Timeline depicting the significant events that occurred during the SARS-CoV-2/COVID-19 virus outbreak. The timeline describes the significant events during the current SARS-CoV-2 outbreak, from 8 December 2019 to 13 May 2020.

of viral etiology, the Chinese virologists rapidly released the genomic sequence of SARS-CoV-2, which played a crucial role in controlling the spread of this newly emerged novel coronavirus to other parts of the world (69). The possible origin of SARS-CoV-2 and the first mode of disease transmission are not yet identified (70). Analysis of the initial cluster of infections suggests that the infected individuals had a common exposure point, a seafood market in Wuhan, Hubei Province, China (Fig. 6). The restaurants of this market are well-known for providing different types of wild animals for human consumption (71). The Huanan South China Seafood Market also sells live animals, such as poultry, bats, snakes, and marmots (72). This might be the point where zoonotic (animal-to-human) transmission occurred (71). Although SARS-CoV-2 is alleged to have originated from an animal host (zoonotic origin) with further human-to-human transmission (Fig. 6), the likelihood of foodborne transmission should be ruled out with further investigations, since it is a latent possibility (1). Additionally, other

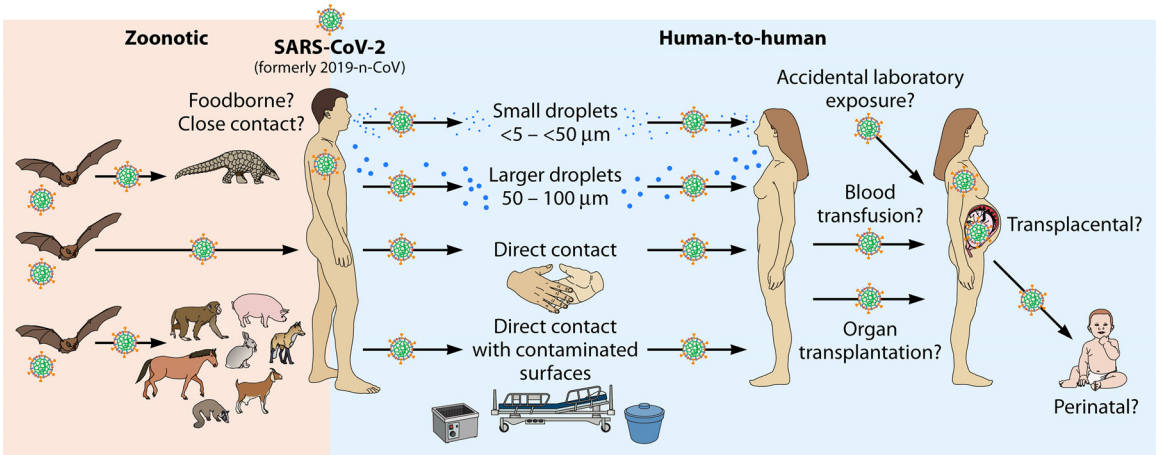

FIG 6 Potential transmission routes for SARS-CoV-2.

potential and expected routes would be associated with transmission, as in other respiratory viruses, by direct contact, such as shaking contaminated hands, or by direct contact with contaminated surfaces (Fig. 6). Still, whether blood transfusion and organ transplantation (276), as well as transplacental and perinatal routes, are possible routes for SARS-CoV-2 transmission needs to be determined (Fig. 6).

From experience with several outbreaks associated with known emerging viruses, higher pathogenicity of a virus is often associated with lower transmissibility. Compared to emerging viruses like Ebola virus, avian H7N9, SARS-CoV, and MERS-CoV, SARS-CoV-2 has relatively lower pathogenicity and moderate transmissibility (15). The risk of death among individuals infected with COVID-19 was calculated using the infection fatality risk (IFR). The IFR was found to be in the range of 0.3% to 0.6%, which is comparable to that of a previous Asian influenza pandemic (1957 to 1958) (73, 277).

Notably, the reanalysis of the COVID-19 pandemic curve from the initial cluster of cases pointed to considerable human-to-human transmission. It is opined that the exposure history of SARS-CoV-2 at the Wuhan seafood market originated from human-to-human transmission rather than animal-to-human transmission (74); however, in light of the zoonotic spillover in COVID-19, is too early to fully endorse this idea (1). Following the initial infection, human-to-human transmission has been observed with a preliminary reproduction number (R_0) estimate of 1.4 to 2.5 (70, 75), and recently it is estimated to be 2.24 to 3.58 (76). In another study, the average reproductive number of COVID-19 was found to be 3.28, which is significantly higher than the initial WHO estimate of 1.4 to 2.5 (77). It is too early to obtain the exact R_0 value, since there is a possibility of bias due to insufficient data. The higher R_0 value is indicative of the more significant potential of SARS-CoV-2 transmission in a susceptible population. This is not the first time where the culinary practices of China have been blamed for the origin of novel coronavirus infection in humans. Previously, the animals present in the live-animal market were identified to be the intermediate hosts of the SARS outbreak in China (78). Several wildlife species were found to harbor potentially evolving coronavirus strains that can overcome the species barrier (79). One of the main principles of Chinese food culture is that live-slaughtered animals are considered more nutritious (5).

After 4 months of struggle that lasted from December 2019 to March 2020, the COVID-19 situation now seems under control in China. The wet animal markets have reopened, and people have started buying bats, dogs, cats, birds, scorpions, badgers, rabbits, pangolins (scaly anteaters), minks, soup from palm civet, ostriches, hamsters, snapping turtles, ducks, fish, Siamese crocodiles, and other animal meats without any fear of COVID-19. The Chinese government is encouraging people to feel they can return to normalcy. However, this could be a risk, as it has been mentioned in advisories that people should avoid contact with live-dead animals as much as possible, as SARS-CoV-2 has shown zoonotic spillover. Additionally, we cannot rule out the possibility of new mutations in the same virus being closely related to contact with both animals and humans at the market (284). In January 2020, China imposed a temporary ban on the sale of live-dead animals in wet markets. However, now hundreds of such wet markets have been reopened without optimizing standard food safety and sanitation practices (286).

With China being the most populated country in the world and due to its domestic and international food exportation policies, the whole world is now facing the menace of COVID-19, including China itself. Wet markets of live-dead animals do not maintain strict food hygienic practices. Fresh blood splashes are present everywhere, on the floor and tabletops, and such food customs could encourage many pathogens to adapt, mutate, and jump the species barrier. As a result, the whole world is suffering from novel SARS-CoV-2, with more than 4,170,424 cases and 287,399 deaths across the globe. There is an urgent need for a rational international campaign against the unhealthy food practices of China to encourage the sellers to increase hygienic food practices or close the crude live-dead animal wet markets. There is a need to modify food policies at national and international levels to avoid further life threats and

economic consequences from any emerging or reemerging pandemic due to close animal-human interaction (285).

Even though individuals of all ages and sexes are susceptible to COVID-19, older people with an underlying chronic disease are more likely to become severely infected (80). Recently, individuals with asymptomatic infection were also found to act as a source of infection to susceptible individuals (81). Both the asymptomatic and symptomatic patients secrete similar viral loads, which indicates that the transmission capacity of asymptomatic or minimally symptomatic patients is very high. Thus, SARS-CoV-2 transmission can happen early in the course of infection (82). Atypical clinical manifestations have also been reported in COVID-19 in which the only reporting symptom was fatigue. Such patients may lack respiratory signs, such as fever, cough, and sputum (83). Hence, the clinicians must be on the look-out for the possible occurrence of atypical clinical manifestations to avoid the possibility of missed diagnosis. The early transmission ability of SARS-CoV-2 was found to be similar to or slightly higher than that of SARS-CoV, reflecting that it could be controlled despite moderate to high transmissibility (84).

Increasing reports of SARS-CoV-2 in sewage and wastewater warrants the need for further investigation due to the possibility of fecal-oral transmission. SARS-CoV-2 present in environmental compartments such as soil and water will finally end up in the wastewater and sewage sludge of treatment plants (328). Therefore, we have to reevaluate the current wastewater and sewage sludge treatment procedures and introduce advanced techniques that are specific and effective against SARS-CoV-2. Since there is active shedding of SARS-CoV-2 in the stool, the prevalence of infections in a large population can be studied using wastewater-based epidemiology. Recently, reverse transcription-quantitative PCR (RT-qPCR) was used to enumerate the copies of SARS-CoV-2 RNA concentrated from wastewater collected from a wastewater treatment plant (327). The calculated viral RNA copy numbers determine the number of infected individuals. The increasing reports of virus shedding via the fecal route warrants the introduction of negative fecal viral nucleic acid test results as one of the additional discharge criteria in laboratory-confirmed cases of COVID-19 (326).

The COVID-19 pandemic does not have any novel factors, other than the genetically unique pathogen and a further possible reservoir. The cause and the likely future outcome are just repetitions of our previous interactions with fatal coronaviruses. The only difference is the time of occurrence and the genetic distinctness of the pathogen involved. Mutations on the RBD of CoVs facilitated their capability of infecting newer hosts, thereby expanding their reach to all corners of the world (85). This is a potential threat to the health of both animals and humans. Advanced studies using Bayesian phylogeographic reconstruction identified the most probable origin of SARS-CoV-2 as the bat SARS-like coronavirus, circulating in the *Rhinolophus* bat family (86).

Phylogenetic analysis of 10 whole-genome sequences of SARS-CoV-2 showed that they are related to two CoVs of bat origin, namely, bat-SL-CoVZC45 and bat-SL-CoVZXC21, which were reported during 2018 in China (17). It was reported that SARS-CoV-2 had been confirmed to use ACE2 as an entry receptor while exhibiting an RBD similar to that of SARS-CoV (17, 87, 254, 255). Several countries have provided recommendations to their people traveling to China (88, 89). Compared to the previous coronavirus outbreaks caused by SARS-CoV and MERS-CoV, the efficiency of SARS-CoV-2 human-to-human transmission was thought to be less. This assumption was based on the finding that health workers were affected less than they were in previous outbreaks of fatal coronaviruses (2). Superspreading events are considered the main culprit for the extensive transmission of SARS and MERS (90, 91). Almost half of the MERS-CoV cases reported in Saudi Arabia are of secondary origin that occurred through contact with infected asymptomatic or symptomatic individuals through human-to-human transmission (92). The occurrence of superspreading events in the COVID-19 outbreak cannot be ruled out until its possibility is evaluated. Like SARS and MERS, COVID-19 can also infect the lower respiratory tract, with milder symptoms (27). The

basic reproduction number of COVID-19 has been found to be in the range of 2.8 to 3.3 based on real-time reports and 3.2 to 3.9 based on predicted infected cases (84).

Coronaviruses in Humans—SARS, MERS, and COVID-19

Coronavirus infection in humans is commonly associated with mild to severe respiratory diseases, with high fever, severe inflammation, cough, and internal organ dysfunction that can even lead to death (92). Most of the identified coronaviruses cause the common cold in humans. However, this changed when SARS-CoV was identified, paving the way for severe forms of the disease in humans (22). Our previous experience with the outbreaks of other coronaviruses, like SARS and MERS, suggests that the mode of transmission in COVID-19 as mainly human-to-human transmission via direct contact, droplets, and fomites (25). Recent studies have demonstrated that the virus could remain viable for hours in aerosols and up to days on surfaces; thus, aerosol and fomite contamination could play potent roles in the transmission of SARS-CoV-2 (257).

The immune response against coronavirus is vital to control and get rid of the infection. However, maladjusted immune responses may contribute to the immunopathology of the disease, resulting in impairment of pulmonary gas exchange. Understanding the interaction between CoVs and host innate immune systems could enlighten our understanding of the lung inflammation associated with this infection (24).

SARS is a viral respiratory disease caused by a formerly unrecognized animal CoV that originated from the wet markets in southern China after adapting to the human host, thereby enabling transmission between humans (90). The SARS outbreak reported in 2002 to 2003 had 8,098 confirmed cases with 774 total deaths (9.6%) (93). The outbreak severely affected the Asia Pacific region, especially mainland China (94). Even though the case fatality rate (CFR) of SARS-CoV-2 (COVID-19) is lower than that of SARS-CoV, there exists a severe concern linked to this outbreak due to its epidemiological similarity to influenza viruses (95, 279). This can fail the public health system, resulting in a pandemic (96).

MERS is another respiratory disease that was first reported in Saudi Arabia during the year 2012. The disease was found to have a CFR of around 35% (97). The analysis of available data sets suggests that the incubation period of SARS-CoV-2, SARS-CoV, and MERS-CoV is in almost the same range. The longest predicted incubation time of SARS-CoV-2 is 14 days. Hence, suspected individuals are isolated for 14 days to avoid the risk of further spread (98). Even though a high similarity has been reported between the genome sequence of the new coronavirus (SARS-CoV-2) and SARS-like CoVs, the comparative analysis recognized a furin-like cleavage site in the SARS-CoV-2 S protein that is missing from other SARS-like CoVs (99). The furin-like cleavage site is expected to play a role in the life cycle of the virus and disease pathogenicity and might even act as a therapeutic target for furin inhibitors. The highly contagious nature of SARS-CoV-2 compared to that of its predecessors might be the result of a stabilizing mutation that occurred in the endosome-associated-protein-like domain of nsp2 protein.

Similarly, the destabilizing mutation near the phosphatase domain of nsp3 proteins in SARS-CoV-2 could indicate a potential mechanism that differentiates it from other CoVs (100). Even though the CFR reported for COVID-19 is meager compared to those of the previous SARS and MERS outbreaks, it has caused more deaths than SARS and MERS combined (101). Possibly related to the viral pathogenesis is the recent finding of an 832-nucleotide (nt) deletion in ORF8, which appears to reduce the replicative fitness of the virus and leads to attenuated phenotypes of SARS-CoV-2 (256).

Coronavirus is the most prominent example of a virus that has crossed the species barrier twice from wild animals to humans during SARS and MERS outbreaks (79, 102). The possibility of crossing the species barrier for the third time has also been suspected in the case of SARS-CoV-2 (COVID-19). Bats are recognized as a possible natural reservoir host of both SARS-CoV and MERS-CoV infection. In contrast, the possible intermediary host is the palm civet for SARS-CoV and the dromedary camel for MERS-CoV infection (102). Bats are considered the ancestral hosts for both SARS and MERS (103). Bats are also considered the reservoir host of human coronaviruses like

HCoV-229E and HCoV-NL63 (104). In the case of COVID-19, there are two possibilities for primary transmission: it can be transmitted either through intermediate hosts, similar to that of SARS and MERS, or directly from bats (103). The emergence paradigm put forward in the SARS outbreak suggests that SARS-CoV originated from bats (reservoir host) and later jumped to civets (intermediate host) and incorporated changes within the receptor-binding domain (RBD) to improve binding to civet ACE2. This civet-adapted virus, during their subsequent exposure to humans at live markets, promoted further adaptations that resulted in the epidemic strain (104). Transmission can also occur directly from the reservoir host to humans without RBD adaptations. The bat coronavirus that is currently in circulation maintains specific "poised" spike proteins that facilitate human infection without the requirement of any mutations or adaptations (105). Altogether, different species of bats carry a massive number of coronaviruses around the world (106).

The high plasticity in receptor usage, along with the feasibility of adaptive mutation and recombination, may result in frequent interspecies transmission of coronavirus from bats to animals and humans (106). The pathogenesis of most bat coronaviruses is unknown, as most of these viruses are not isolated and studied (4). Hedgehog coronavirus HKU31, a *Betacoronavirus*, has been identified from amur hedgehogs in China. Studies show that hedgehogs are the reservoir of *Betacoronavirus*, and there is evidence of recombination (107).

The current scientific evidence available on MERS infection suggests that the significant reservoir host, as well as the animal source of MERS infection in humans, is the dromedary camels (97). The infected dromedary camels may not show any visible signs of infection, making it challenging to identify animals actively excreting MERS-CoV that has the potential to infect humans. However, they may shed MERS-CoV through milk, urine, feces, and nasal and eye discharge and can also be found in the raw organs (108). In a study conducted to evaluate the susceptibility of animal species to MERS-CoV infection, llamas and pigs were found to be susceptible, indicating the possibility of MERS-CoV circulation in animal species other than dromedary camels (109).

Following the outbreak of SARS in China, SARS-CoV-like viruses were isolated from Himalayan palm civets (*Paguma larvata*) and raccoon dogs (*Nyctereutes procyonoides*) found in a live-animal market in Guangdong, China. The animal isolates obtained from the live-animal market retained a 29-nucleotide sequence that was not present in most of the human isolates (78). These findings were critical in identifying the possibility of interspecies transmission in SARS-CoV. The higher diversity and prevalence of bat coronaviruses in this region compared to those in previous reports indicate a host/pathogen coevolution. SARS-like coronaviruses also have been found circulating in the Chinese horseshoe bat (*Rhinolophus sinicus*) populations. The *in vitro* and *in vivo* studies carried out on the isolated virus confirmed that there is a potential risk for the reemergence of SARS-CoV infection from the viruses that are currently circulating in the bat population (105).

CLINICAL PATHOLOGY OF SARS-CoV-2 (COVID-19)

The disease caused by SARS-CoV-2 is also named severe specific contagious pneumonia (SSCP), Wuhan pneumonia, and, recently, COVID-19 (110). Compared to SARS-CoV, SARS-CoV-2 has less severe pathogenesis but has superior transmission capability, as evidenced by the rapidly increasing number of COVID-19 cases (111). The incubation period of SARS-CoV-2 in familial clusters was found to be 3 to 6 days (112). The mean incubation period of COVID-19 was found to be 6.4 days, ranging from 2.1 to 11.1 days (113). Among an early affected group of 425 patients, 59 years was the median age, of which more males were affected (114). Similar to SARS and MERS, the severity of this nCoV is high in age groups above 50 years (2, 115). Symptoms of COVID-19 include fever, cough, myalgia or fatigue, and, less commonly, headache, hemoptysis, and diarrhea (116, 282). Compared to the SARS-CoV-2-infected patients in Wuhan during

the initial stages of the outbreak, only mild symptoms were noticed in those patients that are infected by human-to-human transmission (14).

The initial trends suggested that the mortality associated with COVID-19 was less than that of previous outbreaks of SARS (101). The updates obtained from countries like China, Japan, Thailand, and South Korea indicated that the COVID-19 patients had relatively mild manifestations compared to those with SARS and MERS (4). Regardless of the coronavirus type, immune cells, like mast cells, that are present in the submucosa of the respiratory tract and nasal cavity are considered the primary barrier against this virus (92). Advanced in-depth analysis of the genome has identified 380 amino acid substitutions between the amino acid sequences of SARS-CoV-2 and the SARS/SARS-like coronaviruses. These differences in the amino acid sequences might have contributed to the difference in the pathogenic divergence of SARS-CoV-2 (16). Further research is required to evaluate the possible differences in tropism, pathogenesis, and transmission of this novel agent associated with this change in the amino acid sequence. With the current outbreak of COVID-19, there is an expectancy of a significant increase in the number of published studies about this emerging coronavirus, as occurred with SARS and MERS (117).

SARS-CoV-2 invades the lung parenchyma, resulting in severe interstitial inflammation of the lungs. This is evident on computed tomography (CT) images as ground-glass opacity in the lungs. This lesion initially involves a single lobe but later expands to multiple lung lobes (118). The histological assessment of lung biopsy samples obtained from COVID-19-infected patients revealed diffuse alveolar damage, cellular fibromyxoid exudates, hyaline membrane formation, and desquamation of pneumocytes, indicative of acute respiratory distress syndrome (119). It was also found that the SARS-CoV-2-infected patients often have lymphocytopenia with or without leukocyte abnormalities. The degree of lymphocytopenia gives an idea about disease prognosis, as it is found to be positively correlated with disease severity (118). Pregnant women are considered to have a higher risk of getting infected by COVID-19. The coronaviruses can cause adverse outcomes for the fetus, such as intrauterine growth restriction, spontaneous abortion, preterm delivery, and perinatal death.

Nevertheless, the possibility of intrauterine maternal-fetal transmission (vertical transmission) of CoVs is low and was not seen during either the SARS- or MERS-CoV outbreak (120). However, there has been concern regarding the impact of SARS-CoV-2/COVID-19 on pregnancy. Researchers have mentioned the probability of *in utero* transmission of novel SARS-CoV-2 from COVID-19-infected mothers to their neonates in China based upon the rise in IgM and IgG antibody levels and cytokine values in the blood obtained from newborn infants immediately postbirth; however, RT-PCR failed to confirm the presence of SARS-CoV-2 genetic material in the infants (283). Recent studies show that at least in some cases, preterm delivery and its consequences are associated with the virus. Nonetheless, some cases have raised doubts for the likelihood of vertical transmission (240–243).

COVID-19 infection was associated with pneumonia, and some developed acute respiratory distress syndrome (ARDS). The blood biochemistry indexes, such as albumin, lactate dehydrogenase, C-reactive protein, lymphocytes (percent), and neutrophils (percent) give an idea about the disease severity in COVID-19 infection (121). During COVID-19, patients may present leukocytosis, leukopenia with lymphopenia (244), hypoalbuminemia, and an increase of lactate dehydrogenase, aspartate transaminase, alanine aminotransferase, bilirubin, and, especially, D-dimer (244). Middle-aged and elderly patients with primary chronic diseases, especially high blood pressure and diabetes, were found to be more susceptible to respiratory failure and, therefore, had poorer prognoses. Providing respiratory support at early stages improved the disease prognosis and facilitated recovery (18). The ARDS in COVID-19 is due to the occurrence of cytokine storms that results in exaggerated immune response, immune regulatory network imbalance, and, finally, multiple-organ failure (122). In addition to the exaggerated inflammatory response seen in patients with COVID-19 pneumonia, the bile

duct epithelial cell-derived hepatocytes upregulate ACE2 expression in liver tissue by compensatory proliferation that might result in hepatic tissue injury (123).

CORONAVIRUSES IN ANIMALS AND ZOONOTIC LINKS—A BRIEF VIEWPOINT

Coronavirus can cause disease in several species of domestic and wild animals, as well as humans (23). The different animal species that are infected with CoV include horses, camels, cattle, swine, dogs, cats, rodents, birds, ferrets, minks, bats, rabbits, snakes, and various other wild animals (20, 30, 79, 93, 124, 125, 287). Coronavirus infection is linked to different kinds of clinical manifestations, varying from enteritis in cows and pigs, upper respiratory disease in chickens, and fatal respiratory infections in humans (30).

Among the CoV genera, *Alphacoronavirus* and *Betacoronavirus* infect mammals, while *Gammacoronavirus* and *Deltacoronavirus* mainly infect birds, fishes, and, sometimes, mammals (27, 29, 106). Several novel coronaviruses that come under the genus *Deltacoronavirus* have been discovered in the past from birds, like Wigeon coronavirus HKU20, Bulbul coronavirus HKU11, Munia coronavirus HKU13, white-eye coronavirus HKU16, night-heron coronavirus HKU19, and common moorhen coronavirus HKU21, as well as from pigs (porcine coronavirus HKU15) (6, 29). Transmissible gastroenteritis virus (TGEV), porcine epidemic diarrhea virus (PEDV), and porcine hemagglutinating encephalomyelitis virus (PHEV) are some of the coronaviruses of swine. Among them, TGEV and PEDV are responsible for causing severe gastroenteritis in young piglets with noteworthy morbidity and mortality. Infection with PHEV also causes enteric infection but can cause encephalitis due to its ability to infect the nervous system (30).

Bovine coronaviruses (BoCoVs) are known to infect several domestic and wild ruminants (126). BoCoV inflicts neonatal calf diarrhea in adult cattle, leading to bloody diarrhea (winter dysentery) and respiratory disease complex (shipping fever) in cattle of all age groups (126). BoCoV-like viruses have been noted in humans, suggesting its zoonotic potential as well (127). Feline enteric and feline infectious peritonitis (FIP) viruses are the two major feline CoVs (128), where feline CoVs can affect the gastrointestinal tract, abdominal cavity (peritonitis), respiratory tract, and central nervous system (128). Canines are also affected by CoVs that fall under different genera, namely, canine enteric coronavirus in *Alphacoronavirus* and canine respiratory coronavirus in *Betacoronavirus*, affecting the enteric and respiratory tract, respectively (129, 130). IBV, under *Gammacoronavirus*, causes diseases of respiratory, urinary, and reproductive systems, with substantial economic losses in chickens (131, 132). In small laboratory animals, mouse hepatitis virus, rat sialodacryoadenitis coronavirus, and guinea pig and rabbit coronaviruses are the major CoVs associated with disease manifestations like enteritis, hepatitis, and respiratory infections (10, 133).

Swine acute diarrhea syndrome coronavirus (SADS-CoV) was first identified in suckling piglets having severe enteritis and belongs to the genus *Alphacoronavirus* (106). The outbreak was associated with considerable scale mortality of piglets (24,693 deaths) across four farms in China (134). The virus isolated from the piglets was almost identical to and had 95% genomic similarity with horseshoe bat (*Rhinolophus* species) coronavirus HKU2, suggesting a bat origin of the pig virus (106, 134, 135). It is also imperative to note that the SADS-CoV outbreak started in Guangdong province, near the location of the SARS pandemic origin (134). Before this outbreak, pigs were not known to be infected with bat-origin coronaviruses. This indicates that the bat-origin coronavirus jumped to pig by breaking the species barrier. The next step of this jump might not end well, since pigs are considered the mixing vessel for influenza A viruses due to their ability to be infected by both human and avian influenza A viruses (136).

Similarly, they may act as the mixing vessel for coronaviruses, since they are in frequent contact with both humans and multiple wildlife species. Additionally, pigs are also found to be susceptible to infection with human SARS-CoV and MERS-CoV, making this scenario a nightmare (109, 137). It is only a matter of time before another zoonotic coronavirus results in an epidemic by jumping the so-called species barrier (287).

The host spectrum of coronavirus increased when a novel coronavirus, namely, SW1,

was recognized in the liver tissue of a captive beluga whale (*Delphinapterus leucas*) (138). In recent decades, several novel coronaviruses were identified from different animal species. Bats can harbor these viruses without manifesting any clinical disease but are persistently infected (30). They are the only mammals with the capacity for self-powered flight, which enables them to migrate long distances, unlike land mammals. Bats are distributed worldwide and also account for about a fifth of all mammalian species (6). This makes them the ideal reservoir host for many viral agents and also the source of novel coronaviruses that have yet to be identified. It has become a necessity to study the diversity of coronavirus in the bat population to prevent future outbreaks that could jeopardize livestock and public health. The repeated outbreaks caused by bat-origin coronaviruses calls for the development of efficient molecular surveillance strategies for studying *Betacoronavirus* among animals (12), especially in the *Rhinolophus* bat family (86). Chinese bats have high commercial value, since they are used in traditional Chinese medicine (TCM). Therefore, the handling of bats for trading purposes poses a considerable risk of transmitting zoonotic CoV epidemics (139).

Due to the possible role played by farm and wild animals in SARS-CoV-2 infection, the WHO, in their novel coronavirus (COVID-19) situation report, recommended the avoidance of unprotected contact with both farm and wild animals (25). The live-animal markets, like the one in Guangdong, China, provides a setting for animal coronaviruses to amplify and to be transmitted to new hosts, like humans (78). Such markets can be considered a critical place for the origin of novel zoonotic diseases and have enormous public health significance in the event of an outbreak. Bats are the reservoirs for several viruses; hence, the role of bats in the present outbreak cannot be ruled out (140). In a qualitative study conducted for evaluating the zoonotic risk factors among rural communities of southern China, the frequent human-animal interactions along with the low levels of environmental biosecurity were identified as significant risks for the emergence of zoonotic disease in local communities (141, 142).

The comprehensive sequence analysis of the SARS-CoV-2 RNA genome identified that the CoV from Wuhan is a recombinant virus of the bat coronavirus and another coronavirus of unknown origin. The recombination was found to have happened within the viral spike glycoprotein, which recognizes the cell surface receptor. Further analysis of the genome based on codon usage identified the snake as the most probable animal reservoir of SARS-CoV-2 (143). Contrary to these findings, another genome analysis proposed that the genome of SARS-CoV-2 is 96% identical to bat coronavirus, reflecting its origin from bats (63). The involvement of bat-derived materials in causing the current outbreak cannot be ruled out. High risk is involved in the production of bat-derived materials for TCM practices involving the handling of wild bats. The use of bats for TCM practices will remain a severe risk for the occurrence of zoonotic coronavirus epidemics in the future (139).

Furthermore, the pangolins are an endangered species of animals that harbor a wide variety of viruses, including coronaviruses (144). The coronavirus isolated from Malayan pangolins (*Manis javanica*) showed a very high amino acid identity with COVID-19 at E (100%), M (98.2%), N (96.7%), and S genes (90.4%). The RBD of S protein in CoV isolated from pangolin was almost identical (one amino acid difference) to that of SARS-CoV-2. A comparison of the genomes suggests recombination between pangolin-CoV-like viruses with the bat-CoV-RaTG13-like virus. All this suggests the potential of pangolins to act as the intermediate host of SARS-CoV-2 (145).

Human-wildlife interactions, which are increasing in the context of climate change (142), are further considered high risk and responsible for the emergence of SARS-CoV. COVID-19 is also suspected of having a similar mode of origin. Hence, to prevent the occurrence of another zoonotic spillover (1), exhaustive coordinated efforts are needed to identify the high-risk pathogens harbored by wild animal populations, conducting surveillance among the people who are susceptible to zoonotic spillover events (12), and to improve the biosecurity measures associated with the wildlife trade (146). The serological surveillance studies conducted in people living in proximity to bat caves had earlier identified the serological confirmation of SARS-related CoVs in humans. People

living at the wildlife-human interface, mainly in rural China, are regularly exposed to SARS-related CoVs (147). These findings will not have any significance until a significant outbreak occurs due to a virus-like SARS-CoV-2.

There is a steady increase in the reports of COVID-19 in companion and wild animals around the world. Further studies are required to evaluate the potential of animals (especially companion animals) to serve as an efficient reservoir host that can further alter the dynamics of human-to-human transmission (330). To date, two pet dogs (Hong Kong) and four pet cats (one each from Belgium and Hong Kong, two from the United States) have tested positive for SARS-CoV-2 (335). The World Organization for Animal Health (OIE) has confirmed the diagnosis of COVID-19 in both dogs and cats due to human-to-animal transmission (331). The similarity observed in the gene sequence of SARS-CoV-2 from an infected pet owner and his dog further confirms the occurrence of human-to-animal transmission (333). Even though asymptomatic, feline species should be considered a potential transmission route from animals to humans (326). However, currently, there are no reports of SARS-CoV-2 transmission from felines to human beings. Based on the current evidence, we can conclude that cats are susceptible to SARS-CoV-2 and can get infected by human beings. However, evidence of cat-to-human transmission is lacking and requires further studies (332). Rather than waiting for firmer evidence on animal-to-human transmission, necessary preventive measures are advised, as well as following social distancing practices among companion animals of different households (331). One of the leading veterinary diagnostic companies, IDEXX, has conducted large-scale testing for COVID-19 in specimens collected from dogs and cats. However, none of the tests turned out to be positive (334).

In a study conducted to investigate the potential of different animal species to act as the intermediate host of SARS-CoV-2, it was found that both ferrets and cats can be infected via experimental inoculation of the virus. In addition, infected cats efficiently transmitted the disease to naive cats (329). SARS-CoV-2 infection and subsequent transmission in ferrets were found to recapitulate the clinical aspects of COVID-19 in humans. The infected ferrets also shed virus via multiple routes, such as saliva, nasal washes, feces, and urine, postinfection, making them an ideal animal model for studying disease transmission (337). Experimental inoculation was also done in other animal species and found that the dogs have low susceptibility, while the chickens, ducks, and pigs are not at all susceptible to SARS-CoV-2 (329).

Similarly, the National Veterinary Services Laboratories of the USDA have reported COVID-19 in tigers and lions that exhibited respiratory signs like dry cough and wheezing. The zoo animals are suspected to have been infected by an asymptomatic zookeeper (335). The total number of COVID-19-positive cases in human beings is increasing at a high rate, thereby creating ideal conditions for viral spillover to other species, such as pigs. The evidence obtained from SARS-CoV suggests that pigs can get infected with SARS-CoV-2 (336). However, experimental inoculation with SARS-CoV-2 failed to infect pigs (329).

Further studies are required to identify the possible animal reservoirs of SARS-CoV-2 and the seasonal variation in the circulation of these viruses in the animal population. Research collaboration between human and animal health sectors is becoming a necessity to evaluate and identify the possible risk factors of transmission between animals and humans. Such cooperation will help to devise efficient strategies for the management of emerging zoonotic diseases (12).

DIAGNOSIS OF SARS-CoV-2 (COVID-19)

RNA tests can confirm the diagnosis of SARS-CoV-2 (COVID-19) cases with real-time RT-PCR or next-generation sequencing (148, 149, 245, 246). At present, nucleic acid detection techniques, like RT-PCR, are considered an effective method for confirming the diagnosis in clinical cases of COVID-19 (148). Several companies across the world are currently focusing on developing and marketing SARS-CoV-2-specific nucleic acid detection kits. Multiple laboratories are also developing their own in-house RT-PCR. One of them is the SARS-CoV-2 nucleic acid detection kit produced by Shuoshi

TABLE 1 FDA-approved *in vitro* Emergency Use Authorization diagnostics available for SARS-CoV-2 as of 30 March 2020[a]

Developer	Diagnostic platform
Centers for Disease Control and Prevention (CDC)	CDC 2019-nCoV real-time RT-PCR diagnostic panel
Wadsworth Center, New York State Department of Public Health (CDC)	New York SARS-CoV-2 real-time reverse transcriptase (RT)-PCR diagnostic panel
Roche Molecular Systems, Inc. (RMS)	cobas SARS-CoV-2
Thermo Fisher Scientific, Inc.	TaqPath COVID-19 combo kit
Laboratory Corporation of America (LabCorp)	COVID-19 RT-PCR test
Hologic, Inc.	Panther fusion SARS-CoV-2
Quest Diagnostics Infectious Disease, Inc.	Quest SARS-CoV-2 rRT-PCR
Quidel Corporation	Lyra SARS-CoV-2 assay
Abbott Molecular	Abbott RealTime SARS-CoV-2 assay
GenMark Diagnostics, Inc.	ePlex SARS-CoV-2 test
DiaSorin Molecular, LLC	Simplexa COVID-19 direct assay
Cepheid	Xpert Xpress SARS-CoV-2 test
Primerdesign, Ltd.	COVID-19 Genesig real-time PCR assay
Mesa Biotech, Inc.	Accula SARS-Cov-2 test
BioFire Defense, LLC	BioFire COVID-19 test
PerkinElmer, Inc.	PerkinElmer new coronavirus nucleic acid detection kit
Avellino Lab USA, Inc.	AvellinoCoV2 test
BGI Genomics, Co. Ltd.	Real-time fluorescent RT-PCR kit for detecting SARS-2019-nCoV
Luminex Molecular Diagnostics, Inc.	NxTAG CoV extended panel assay
Abbott Diagnostics Scarborough, Inc.	ID Now COVID-19
Qiagen GmbH	QIAstat-Dx respiratory SARS-CoV-2 panel
NeuMoDx Molecular, Inc.	NeuMoDx SARS-CoV-2 assay

[a]Data are from references 258 and 259.

Biotechnology (double fluorescence PCR method) (150). Up to 30 March 2020, the U.S. Food and Drug Administration (FDA) had granted 22 *in vitro* diagnostics Emergency Use Authorizations (EUAs), including for the RT-PCR diagnostic panel for the universal detection of SARS-like betacoronaviruses and specific detection of SARS-CoV-2, developed by the U.S. CDC (Table 1) (258, 259).

Recently, 95 full-length genomic sequences of SARAS-CoV-2 strains available in the National Center for Biotechnology Information and GISAID databases were subjected to multiple-sequence alignment and phylogenetic analyses for studying variations in the viral genome (260). All the viral strains revealed high homology of 99.99% (99.91% to 100%) at the nucleotide level and 99.99% (99.79% to 100%) at the amino acid level. Overall variation was found to be low in ORF regions, with 13 variation sites recognized in 1a, 1b, S, 3a, M, 8, and N regions. Mutation rates of 30.53% (29/95) and 29.47% (28/95) were observed at nt 28144 (ORF8) and nt 8782 (ORF1a) positions, respectively. Owing to such selective mutations, a few specific regions of SARS-CoV-2 should not be considered for designing primers and probes. The SARS-CoV-2 reference sequence could pave the way to study molecular biology and pathobiology, along with developing diagnostics and appropriate prevention and control strategies for countering SARS-CoV-2 (260).

Nucleic acids of SARS-CoV-2 can be detected from samples (64) such as bronchoalveolar lavage fluid, sputum, nasal swabs, fiber bronchoscope brush biopsy specimen, pharyngeal swabs, feces, blood, and urine, with different levels of diagnostic performance (Table 2) (80, 245, 246). The viral loads of SARS-CoV-2 were measured using N-gene-specific quantitative RT-PCR in throat swab and sputum samples collected from COVID-19-infected individuals. The results indicated that the viral load peaked at around 5 to 6 days following the onset of symptoms, and it ranged from 10^4 to 10^7 copies/ml during this time (151). In another study, the viral load was found to be higher in the nasal swabs than the throat swabs obtained from COVID-19 symptomatic patients (82). Although initially it was thought that viral load would be associated with poor outcomes, some case reports have shown asymptomatic individuals with high viral loads (247). Recently, the viral load in nasal and throat swabs of 17 symptomatic patients was determined, and higher viral loads were recorded soon after the onset of symptoms, particularly in the nose compared to the throat. The pattern of viral nucleic

TABLE 2 Clinical specimens for detection of SARS CoV-2

Sample	Recommendation[a]
Bronchoalveolar lavage fluid	+++
Sputum	+++
Nasal swabs	+++
Fibrobronchoscope brush biopsy	++
Pharyngeal swabs	++
Feces	+
Blood	+
Urine	+

[a]Recommendations are based on references 245 and 246. +++, strong; ++, moderate; +, weak.

acid shedding of SARS-CoV-2-infected patients was similar to that of influenza patients but seemed to be different from that of SARS-CoV patients. The viral load detected in asymptomatic patients resembled that of symptomatic patients as studied in China, which reflects the transmission perspective of asymptomatic or symptomatic patients having minimum signs and symptoms (82). Another study, conducted in South Korea, related to SARS-CoV-2 viral load, opined that SARS-CoV-2 kinetics were significantly different from those of earlier reported CoV infections, including SARS-CoV (253). SARS-CoV-2 transmission can occur early in the viral infection phase; thus, diagnosing cases and isolation attempts for this virus warrant different strategies than those needed to counter SARS-CoV. Studies are required to establish any correlation between SARS-CoV-2 viral load and cultivable virus. Recognizing patients with fewer or no symptoms, along with having modest detectable viral RNA in the oropharynx for 5 days, indicates the requirement of data for assessing SARS-CoV-2 transmission dynamics and updating the screening procedures in the clinics (82).

The results of the studies related to SARS-CoV-2 viral loads reflect active replication of this virus in the upper respiratory tract and prolonged viral shedding after symptoms disappear, including via stool. Thus, the current case definition needs to be updated along with a reassessment of the strategies to be adopted for restraining the SARS-CoV-2 outbreak spread (248). In some cases, the viral load studies of SARS-CoV-2 have also been useful to recommend precautionary measures when handling specific samples, e.g., feces. In a recent survey from 17 confirmed cases of SARS-CoV-2 infection with available data (representing days 0 to 13 after onset), stool samples from nine cases (53%; days 0 to 11 after onset) were positive on RT-PCR analysis. Although the viral loads were lower than those of respiratory samples (range, 550 copies per ml to 1.21×10^5 copies per ml), this has essential biosafety implications (151).

The samples from 18 SARS-CoV-2-positive patients in Singapore who had traveled from Wuhan to Singapore showed the presence of viral RNA in stool and whole blood but not in urine by real-time RT-PCR (288). Further, novel SARS-CoV-2 infections have been detected in a variety of clinical specimens, like bronchoalveolar lavage fluid, sputum, nasal swabs, fibrobronchoscope brush biopsy specimens, pharyngeal swabs, feces, and blood (246).

The presence of SARS-CoV-2 in fecal samples has posed grave public health concerns. In addition to the direct transmission mainly occurring via droplets of sneezing and coughing, other routes, such as fecal excretion and environmental and fomite contamination, are contributing to SARS-CoV-2 transmission and spread (249–252). Fecal excretion has also been documented for SARS-CoV and MERS-CoV, along with the potential to stay viable in situations aiding fecal-oral transmission. Thus, SARS-CoV-2 has every possibility to be transmitted through this mode. Fecal-oral transmission of SARS-CoV-2, particularly in regions having low standards of hygiene and poor sanitation, may have grave consequences with regard to the high spread of this virus. Ethanol and disinfectants containing chlorine or bleach are effective against coronaviruses (249–252). Appropriate precautions need to be followed strictly while handling the stools of patients infected with SARS-CoV-2. Biowaste materials and sewage from hospitals must be adequately disinfected, treated, and disposed of properly. The

significance of frequent and good hand hygiene and sanitation practices needs to be given due emphasis (249–252). Future explorative research needs to be conducted with regard to the fecal-oral transmission of SARS-CoV-2, along with focusing on environmental investigations to find out if this virus could stay viable in situations and atmospheres facilitating such potent routes of transmission. The correlation of fecal concentrations of viral RNA with disease severity needs to be determined, along with assessing the gastrointestinal symptoms and the possibility of fecal SARS-CoV-2 RNA detection during the COVID-19 incubation period or convalescence phases of the disease (249–252).

The lower respiratory tract sampling techniques, like bronchoalveolar lavage fluid aspirate, are considered the ideal clinical materials, rather than the throat swab, due to their higher positive rate on the nucleic acid test (148). The diagnosis of COVID-19 can be made by using upper-respiratory-tract specimens collected using nasopharyngeal and oropharyngeal swabs. However, these techniques are associated with unnecessary risks to health care workers due to close contact with patients (152). Similarly, a single patient with a high viral load was reported to contaminate an entire endoscopy room by shedding the virus, which may remain viable for at least 3 days and is considered a great risk for uninfected patients and health care workers (289). Recently, it was found that the anal swabs gave more positive results than oral swabs in the later stages of infection (153). Hence, clinicians have to be cautious while discharging any COVID-19-infected patient based on negative oral swab test results due to the possibility of fecal-oral transmission. Even though the viral loads in stool samples were found to be less than those of respiratory samples, strict precautionary measures have to be followed while handling stool samples of COVID-19 suspected or infected patients (151). Children infected with SARS-CoV-2 experience only a mild form of illness and recover immediately after treatment. It was recently found that stool samples of SARS-CoV-2-infected children that gave negative throat swab results were positive within ten days of negative results. This could result in the fecal-oral transmission of SARS-CoV-2 infections, especially in children (290). Hence, to prevent the fecal-oral transmission of SARS-CoV-2, infected COVID-19 patients should only be considered negative when they test negative for SARS-CoV-2 in the stool sample.

A suspected case of COVID-19 infection is said to be confirmed if the respiratory tract aspirate or blood samples test positive for SARS-CoV-2 nucleic acid using RT-PCR or by the identification of SARS-CoV-2 genetic sequence in respiratory tract aspirate or blood samples (80). The patient will be confirmed as cured when two subsequent oral swab results are negative (153). Recently, the live virus was detected in the self-collected saliva of patients infected with COVID-19. These findings were confirmative of using saliva as a noninvasive specimen for the diagnosis of COVID-19 infection in suspected individuals (152). It has also been observed that the initial screening of COVID-19 patients infected with RT-PCR may give negative results even if they have chest CT findings that are suggestive of infection. Hence, for the accurate diagnosis of COVID-19, a combination of repeated swab tests using RT-PCR and CT scanning is required to prevent the possibility of false-negative results during disease screening (154). RT-PCR is the most widely used test for diagnosing COVID-19. However, it has some significant limitations from the clinical perspective, since it will not give any clarity regarding disease progression. Droplet digital PCR (ddPCR) can be used for the quantification of viral load in the samples obtained from lower respiratory tracts. Hence, based on the viral load, we can quickly evaluate the progression of infection (291). In addition to all of the above findings, sequencing and phylogenetics are critical in the correct identification and confirmation of the causative viral agent and useful to establish relationships with previous isolates and sequences, as well as to know, especially during an epidemic, the nucleotide and amino acid mutations and the molecular divergence. The rapid development and implementation of diagnostic tests against emerging novel diseases like COVID-19 pose significant challenges due to the lack of resources and logistical limitations associated with an outbreak (155).

SARS-CoV-2 infection can also be confirmed by isolation and culturing. The

human airway epithelial cell culture was found to be useful in isolating SARS-CoV-2 (3). The efficient control of an outbreak depends on the rapid diagnosis of the disease. Recently, in response to the COVID-19 outbreak, 1-step quantitative real-time reverse transcription-PCR assays were developed that detect the ORF1b and N regions of the SARS-CoV-2 genome (156). That assay was found to achieve the rapid detection of SARS-CoV-2. Nucleic acid-based assays offer high accuracy in the diagnosis of SARS-CoV-2, but the current rate of spread limits its use due to the lack of diagnostic assay kits. This will further result in the extensive transmission of COVID-19, since only a portion of suspected cases can be diagnosed. In such situations, conventional serological assays, like enzyme-linked immunosorbent assay (ELISA), that are specific to COVID-19 IgM and IgG antibodies can be used as a high-throughput alternative (149). At present, there is no diagnostic kit available for detecting the SARS-CoV-2 antibody (150). The specific antibody profiles of COVID-19 patients were analyzed, and it was found that the IgM level lasted more than 1 month, indicating a prolonged stage of virus replication in SARS-CoV-2-infected patients. The IgG levels were found to increase only in the later stages of the disease. These findings indicate that the specific antibody profiles of SARS-CoV-2 and SARS-CoV were similar (325). These findings can be utilized for the development of specific diagnostic tests against COVID-19 and can be used for rapid screening. Even though diagnostic test kits are already available that can detect the genetic sequences of SARS-CoV-2 (95), their availability is a concern, as the number of COVID-19 cases is skyrocketing (155, 157). A major problem associated with this diagnostic kit is that it works only when the test subject has an active infection, limiting its use to the earlier stages of infection. Several laboratories around the world are currently developing antibody-based diagnostic tests against SARS-CoV-2 (157).

Chest CT is an ideal diagnostic tool for identifying viral pneumonia. The sensitivity of chest CT is far superior to that of X-ray screening. The chest CT findings associated with COVID-19-infected patients include characteristic patchy infiltration that later progresses to ground-glass opacities (158). Early manifestations of COVID-19 pneumonia might not be evident in X-ray chest radiography. In such situations, a chest CT examination can be performed, as it is considered highly specific for COVID-19 pneumonia (118). Those patients having COVID-19 pneumonia will exhibit the typical ground-glass opacity in their chest CT images (154). The patients infected with COVID-19 had elevated plasma angiotensin 2 levels. The level of angiotensin 2 was found to be linearly associated with viral load and lung injury, indicating its potential as a diagnostic biomarker (121). The chest CT imaging abnormalities associated with COVID-19 pneumonia have also been observed even in asymptomatic patients. These abnormalities progress from the initial focal unilateral to diffuse bilateral ground-glass opacities and will further progress to or coexist with lung consolidation changes within 1 to 3 weeks (159). The role played by radiologists in the current scenario is very important. Radiologists can help in the early diagnosis of lung abnormalities associated with COVID-19 pneumonia. They can also help in the evaluation of disease severity, identifying its progression to acute respiratory distress syndrome and the presence of secondary bacterial infections (160). Even though chest CT is considered an essential diagnostic tool for COVID-19, the extensive use of CT for screening purposes in the suspected individuals might be associated with a disproportionate risk-benefit ratio due to increased radiation exposure as well as increased risk of cross-infection. Hence, the use of CT for early diagnosis of SARS-CoV-2 infection in high-risk groups should be done with great caution (292).

More recently, other advanced diagnostics have been designed and developed for the detection of SARS-CoV-2 (345, 347, 350–352). A reverse transcriptional loop-mediated isothermal amplification (RT-LAMP), namely, iLACO, has been developed for rapid and colorimetric detection of this virus (354). RT-LAMP serves as a simple, rapid, and sensitive diagnostic method that does not require sophisticated equipment or skilled personnel (349). An interactive web-based dashboard for tracking SARS-CoV-2 in a real-time mode has been designed (238). A smartphone-integrated home-based point-of-care testing (POCT) tool, a paper-based POCT combined with LAMP, is a useful

point-of-care diagnostic (353). An Abbott ID Now COVID-19 molecular POCT-based test, using isothermal nucleic acid amplification technology, has been designed as a point-of-care test for very rapid detection of SARS-CoV-2 in just 5 min (344). A CRISPR-based SHERLOCK (specific high-sensitivity enzymatic reporter unlocking) diagnostic for rapid detection of SARS-CoV-2 without the requirement of specialized instrumentation has been reported to be very useful in the clinical diagnosis of COVID-19 (360). A CRISPR-Cas12-based lateral flow assay also has been developed for rapid detection of SARS-CoV-2 (346). Artificial intelligence, by means of a three-dimensional deep-learning model, has been developed for sensitive and specific diagnosis of COVID-19 via CT images (332).

Tracking and mapping of the rising incidence rates, disease outbreaks, community spread, clustered transmission events, hot spots, and superspreader potential of SARS-CoV-2/COVID warrant full exploitation of real-time disease mapping by employing geographical information systems (GIS), such as the GIS software Kosmo 3.1, web-based real-time tools and dashboards, apps, and advances in information technology (356–359). Researchers have also developed a few prediction tools/models, such as the prediction model risk of bias assessment tool (PROBAST) and critical appraisal and data extraction for systematic reviews of prediction modeling studies (CHARMS), which could aid in assessing the possibility of getting infection and estimating the prognosis in patients; however, such models may suffer from bias issues and, hence, cannot be considered completely trustworthy, which necessitates the development of new and reliable predictors (360).

VACCINES, THERAPEUTICS, AND DRUGS

Recently emerged viruses, such as Zika, Ebola, and Nipah viruses, and their grave threats to humans have begun a race in exploring the designing and developing of advanced vaccines, prophylactics, therapeutics, and drug regimens to counter emerging viruses (161–163, 280). Several attempts are being made to design and develop vaccines for CoV infection, mostly by targeting the spike glycoprotein. Nevertheless, owing to extensive diversity in antigenic variants, cross-protection rendered by the vaccines is significantly limited, even within the strains of a phylogenetic subcluster (104). Due to the lack of effective antiviral therapy and vaccines in the present scenario, we need to depend solely on implementing effective infection control measures to lessen the risk of possible nosocomial transmission (68). Recently, the receptor for SARS-CoV-2 was established as the human angiotensin-converting enzyme 2 (hACE2), and the virus was found to enter the host cell mainly through endocytosis. It was also found that the major components that have a critical role in viral entry include PIKfyve, TPC2, and cathepsin L. These findings are critical, since the components described above might act as candidates for vaccines or therapeutic drugs against SARS-CoV-2 (293).

The majority of the treatment options and strategies that are being evaluated for SARS-CoV-2 (COVID-19) have been taken from our previous experiences in treating SARS-CoV, MERS-CoV, and other emerging viral diseases. Several therapeutic and preventive strategies, including vaccines, immunotherapeutics, and antiviral drugs, have been exploited against the previous CoV outbreaks (SARS-CoV and MERS-CoV) (8, 104, 164–167). These valuable options have already been evaluated for their potency, efficacy, and safety, along with several other types of current research that will fuel our search for ideal therapeutic agents against COVID-19 (7, 9, 19, 21, 36). The primary cause of the unavailability of approved and commercial vaccines, drugs, and therapeutics to counter the earlier SARS-CoV and MERS-CoV seems to owe to the lesser attention of the biomedicine and pharmaceutical companies, as these two CoVs did not cause much havoc, global threat, and panic like those posed by the SARS-CoV-2 pandemic (19). Moreover, for such outbreak situations, the requirement for vaccines and therapeutics/drugs exists only for a limited period, until the outbreak is controlled. The proportion of the human population infected with SARS-CoV and MERS-CoV was also much lower across the globe, failing to attract drug and vaccine manufacturers and

producers. Therefore, by the time an effective drug or vaccine is designed against such disease outbreaks, the virus would have been controlled by adopting appropriate and strict prevention and control measures, and patients for clinical trials will not be available. The newly developed drugs cannot be marketed due to the lack of end users.

Vaccines

The S protein plays a significant role in the induction of protective immunity against SARS-CoV by mediating T-cell responses and neutralizing antibody production (168). In the past few decades, we have seen several attempts to develop a vaccine against human coronaviruses by using S protein as the target (168, 169). However, the developed vaccines have minimal application, even among closely related strains of the virus, due to a lack of cross-protection. That is mainly because of the extensive diversity existing among the different antigenic variants of the virus (104). The contributions of the structural proteins, like spike (S), matrix (M), small envelope (E), and nucleocapsid (N) proteins, of SARS-CoV to induce protective immunity has been evaluated by expressing them in a recombinant parainfluenza virus type 3 vector (BHPIV3). Of note, the result was conclusive that the expression of M, E, or N proteins without the presence of S protein would not confer any noticeable protection, with the absence of detectable serum SARS-CoV-neutralizing antibodies (170). Antigenic determinant sites present over S and N structural proteins of SARS-CoV-2 can be explored as suitable vaccine candidates (294). In the Asian population, S, E, M, and N proteins of SARS-CoV-2 are being targeted for developing subunit vaccines against COVID-19 (295).

The identification of the immunodominant region among the subunits and domains of S protein is critical for developing an effective vaccine against the coronavirus. The C-terminal domain of the S1 subunit is considered the immunodominant region of the porcine deltacoronavirus S protein (171). Similarly, further investigations are needed to determine the immunodominant regions of SARS-CoV-2 for facilitating vaccine development.

However, our previous attempts to develop a universal vaccine that is effective for both SARS-CoV and MERS-CoV based on T-cell epitope similarity pointed out the possibility of cross-reactivity among coronaviruses (172). That can be made possible by selected potential vaccine targets that are common to both viruses. SARS-CoV-2 has been reported to be closely related to SARS-CoV (173, 174). Hence, knowledge and understanding of S protein-based vaccine development in SARS-CoV will help to identify potential S protein vaccine candidates in SARS-CoV-2. Therefore, vaccine strategies based on the whole S protein, S protein subunits, or specific potential epitopes of S protein appear to be the most promising vaccine candidates against coronaviruses. The RBD of the S1 subunit of S protein has a superior capacity to induce neutralizing antibodies. This property of the RBD can be utilized for designing potential SARS-CoV vaccines either by using RBD-containing recombinant proteins or recombinant vectors that encode RBD (175). Hence, the superior genetic similarity existing between SARS-CoV-2 and SARS-CoV can be utilized to repurpose vaccines that have proven *in vitro* efficacy against SARS-CoV to be utilized for SARS-CoV-2. The possibility of cross-protection in COVID-19 was evaluated by comparing the S protein sequences of SARS-CoV-2 with that of SARS-CoV. The comparative analysis confirmed that the variable residues were found concentrated on the S1 subunit of S protein, an important vaccine target of the virus (150). Hence, the possibility of SARS-CoV-specific neutralizing antibodies providing cross-protection to COVID-19 might be lower. Further genetic analysis is required between SARS-CoV-2 and different strains of SARS-CoV and SARS-like (SL) CoVs to evaluate the possibility of repurposed vaccines against COVID-19. This strategy will be helpful in the scenario of an outbreak, since much time can be saved, because preliminary evaluation, including *in vitro* studies, already would be completed for such vaccine candidates.

Multiepitope subunit vaccines can be considered a promising preventive strategy against the ongoing COVID-19 pandemic. *In silico* and advanced immunoinformatic tools can be used to develop multiepitope subunit vaccines. The vaccines that are

engineered by this technique can be further evaluated using docking studies and, if found effective, then can be further evaluated in animal models (365). Identifying epitopes that have the potential to become a vaccine candidate is critical to developing an effective vaccine against COVID-19. The immunoinformatics approach has been used for recognizing essential epitopes of cytotoxic T lymphocytes and B cells from the surface glycoprotein of SARS-CoV-2. Recently, a few epitopes have been recognized from the SARS-CoV-2 surface glycoprotein. The selected epitopes explored targeting molecular dynamic simulations, evaluating their interaction with corresponding major histocompatibility complex class I molecules. They potentially induce immune responses (176). The recombinant vaccine can be designed by using rabies virus (RV) as a viral vector. RV can be made to express MERS-CoV S1 protein on its surface so that an immune response is induced against MERS-CoV. The RV vector-based vaccines against MERS-CoV can induce faster antibody response as well as higher degrees of cellular immunity than the Gram-positive enhancer matrix (GEM) particle vector-based vaccine. However, the latter can induce a very high antibody response at lower doses (167). Hence, the degree of humoral and cellular immune responses produced by such vaccines depends upon the vector used.

Dual vaccines have been getting more popular recently. Among them, the rabies virus-based vectored vaccine platform is used to develop vaccines against emerging infectious diseases. The dual vaccine developed from inactivated rabies virus particles that express the MERS-CoV S1 domain of S protein was found to induce immune responses for both MERS-CoV and rabies virus. The vaccinated mice were found to be completely protected from challenge with MERS-CoV (169). The intranasal administration of the recombinant adenovirus-based vaccine in BALB/c mice was found to induce long-lasting neutralizing immunity against MERS spike pseudotyped virus, characterized by the induction of systemic IgG, secretory IgA, and lung-resident memory T-cell responses (177). Immunoinformatics methods have been employed for the genome-wide screening of potential vaccine targets among the different immunogens of MERS-CoV (178). The N protein and the potential B-cell epitopes of MERS-CoV E protein have been suggested as immunoprotective targets inducing both T-cell and neutralizing antibody responses (178, 179).

The collaborative effort of the researchers of Rocky Mountain Laboratories and Oxford University is designing a chimpanzee adenovirus-vectored vaccine to counter COVID-19 (180). The Coalition for Epidemic Preparedness Innovations (CEPI) has initiated three programs to design SARS-CoV-2 vaccines (181). CEPI has a collaborative project with Inovio for designing a MERS-CoV DNA vaccine that could potentiate effective immunity. CEPI and the University of Queensland are designing a molecular clamp vaccine platform for MERS-CoV and other pathogens, which could assist in the easier identification of antigens by the immune system (181). CEPI has also funded Moderna to develop a vaccine for COVID-19 in partnership with the Vaccine Research Center (VRC) of the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH) (182). By employing mRNA vaccine platform technology, a vaccine candidate expressing SARS-CoV-2 spike protein is likely to go through clinical testing in the coming months (180). On 16 March 2020, Jennifer Haller became the first person outside China to receive an experimental vaccine, developed by Moderna, against this pandemic virus. Moderna, along with China's CanSino Biologics, became the first research group to launch small clinical trials of vaccines against COVID-19. Their study is evaluating the vaccine's safety and ability to trigger immune responses (296).

Scientists from all over the world are trying hard to develop working vaccines with robust protective immunity against COVID-19. Vaccine candidates, like mRNA-1273 SARS-CoV-2 vaccine, INO-4800 DNA coronavirus vaccine, and adenovirus type 5 vector vaccine candidate (Ad5-nCoV), are a few examples under phase I clinical trials, while self-amplifying RNA vaccine, oral recombinant COVID-19 vaccine, BNT162, plant-based COVID-19 vaccine, and Ii-Key peptide COVID-19 vaccine are under preclinical trials (297). Similarly, the WHO, on its official website, has mentioned a detailed list of

COVID-19 vaccine agents that are under consideration. Different phases of trials are ongoing for live attenuated virus vaccines, formaldehyde alum inactivated vaccine, adenovirus type 5 vector vaccine, LNP-encapsulated mRNA vaccine, DNA plasmid vaccine, and S protein, S-trimer, and Ii-Key peptide as a subunit protein vaccine, among others (298). The process of vaccine development usually takes approximately ten years, in the case of inactivated or live attenuated vaccines, since it involves the generation of long-term efficacy data. However, this was brought down to 5 years during the Ebola emergency for viral vector vaccines. In the urgency associated with the COVID-19 outbreaks, we expect a vaccine by the end of this year (343). The development of an effective vaccine against COVID-19 with high speed and precision is the combined result of advancements in computational biology, gene synthesis, protein engineering, and the invention of advanced manufacturing platforms (342).

The recurring nature of the coronavirus outbreaks calls for the development of a pan-coronavirus vaccine that can produce cross-reactive antibodies. However, the success of such a vaccine relies greatly on its ability to provide protection not only against present versions of the virus but also the ones that are likely to emerge in the future. This can be achieved by identifying antibodies that can recognize relatively conserved epitopes that are maintained as such even after the occurrence of considerable variations (362). Even though several vaccine clinical trials are being conducted around the world, pregnant women have been completely excluded from these studies. Pregnant women are highly vulnerable to emerging diseases such as COVID-19 due to alterations in the immune system and other physiological systems that are associated with pregnancy. Therefore, in the event of successful vaccine development, pregnant women will not get access to the vaccines (361). Hence, it is recommended that pregnant women be included in the ongoing vaccine trials, since successful vaccination in pregnancy will protect the mother, fetus, and newborn.

The heterologous immune effects induced by Bacillus Calmette Guérin (BCG) vaccination is a promising strategy for controlling the COVID-19 pandemic and requires further investigations. BCG is a widely used vaccine against tuberculosis in high-risk regions. It is derived from a live attenuated strain of *Mycobacterium bovis*. At present, three new clinical trials have been registered to evaluate the protective role of BCG vaccination against SARS-CoV-2 (363). Recently, a cohort study was conducted to evaluate the impact of childhood BCG vaccination in COVID-19 PCR positivity rates. However, childhood BCG vaccination was found to be associated with a rate of COVID-19-positive test results similar to that of the nonvaccinated group (364). Further studies are required to analyze whether BCG vaccination in childhood can induce protective effects against COVID-19 in adulthood. Population genetic studies conducted on 103 genomes identified that the SARS-CoV-2 virus has evolved into two major types, L and S. Among the two types, L type is expected to be the most prevalent (~70%), followed by the S type (~30%) (366). This finding has a significant impact on our race to develop an ideal vaccine, since the vaccine candidate has to target both strains to be considered effective. At present, the genetic differences between the L and S types are very small and may not affect the immune response. However, we can expect further genetic variations in the coming days that could lead to the emergence of new strains (367).

Therapeutics and Drugs

There is no currently licensed specific antiviral treatment for MERS- and SARS-CoV infections, and the main focus in clinical settings remains on lessening clinical signs and providing supportive care (183–186). Effective drugs to manage COVID-19 patients include remdesivir, lopinavir/ritonavir alone or in a blend with interferon beta, convalescent plasma, and monoclonal antibodies (MAbs); however, efficacy and safety issues of these drugs require additional clinical trials (187, 281). A controlled trial of ritonavir-boosted lopinavir and interferon alpha 2b treatment was performed on COVID-19 hospitalized patients (ChiCTR2000029308) (188). In addition, the use of hydroxychloroquine and tocilizumab for their potential role in modulating inflammatory responses

in the lungs and antiviral effect has been proposed and discussed in many research articles. Still, no fool-proof clinical trials have been published (194, 196, 197, 261–272). Recently, a clinical trial conducted on adult patients suffering from severe COVID-19 revealed no benefit of lopinavir-ritonavir treatment over standard care (273).

The efforts to control SARS-CoV-2 infection utilize defined strategies as followed against MERS and SARS, along with adopting and strengthening a few precautionary measures owing to the unknown nature of this novel virus (36, 189). Presently, the main course of treatment for severely affected SARS-CoV-2 patients admitted to hospitals includes mechanical ventilation, intensive care unit (ICU) admittance, and symptomatic and supportive therapies. Additionally, RNA synthesis inhibitors (lamivudine and tenofovir disoproxil fumarate), remdesivir, neuraminidase inhibitors, peptide (EK1), anti-inflammatory drugs, abidol, and Chinese traditional medicine (Lianhuaqingwen and ShuFengJieDu capsules) could aid in COVID-19 treatment. However, further clinical trials are being carried out concerning their safety and efficacy (7). It might require months to a year(s) to design and develop effective drugs, therapeutics, and vaccines against COVID-19, with adequate evaluation and approval from regulatory bodies and moving to the bulk production of many millions of doses at commercial levels to meet the timely demand of mass populations across the globe (9). Continuous efforts are also warranted to identify and assess viable drugs and immunotherapeutic regimens that revealed proven potency in combating other viral agents similar to SARS-CoV-2.

COVID-19 patients showing severe signs are treated symptomatically along with oxygen therapy. In such cases where the patients progress toward respiratory failure and become refractory to oxygen therapy, mechanical ventilation is necessitated. The COVID-19-induced septic shock can be managed by providing adequate hemodynamic support (299). Several classes of drugs are currently being evaluated for their potential therapeutic action against SARS-CoV-2. Therapeutic agents that have anti-SARS-CoV-2 activity can be broadly classified into three categories: drugs that block virus entry into the host cell, drugs that block viral replication as well as its survival within the host cell, and drugs that attenuate the exaggerated host immune response (300). An inflammatory cytokine storm is commonly seen in critically ill COVID-19 patients. Hence, they may benefit from the use of timely anti-inflammation treatment. Anti-inflammatory therapy using drugs like glucocorticoids, cytokine inhibitors, JAK inhibitors, and chloroquine/hydroxychloroquine should be done only after analyzing the risk/benefit ratio in COVID-19 patients (301). There have not been any studies concerning the application of nonsteroidal anti-inflammatory drugs (NSAID) to COVID-19-infected patients. However, reasonable pieces of evidence are available that link NSAID uses with the occurrence of respiratory and cardiovascular adverse effects. Hence, as a cautionary approach, it is better to recommend the use of NSAIDs as the first-line option for managing COVID-19 symptoms (302). The use of corticosteroids in COVID-19 patients is still a matter of controversy and requires further systematic clinical studies. The guidelines that were put forward to manage critically ill adults suggest the use of systemic corticosteroids in mechanically ventilated adults with ARDS (303). The generalized use of corticosteroids is not indicated in COVID-19, since there are some concerns associated with the use of corticosteroids in viral pneumonia. Stem cell therapy using mesenchymal stem cells (MSCs) is another hopeful strategy that can be used in clinical cases of COVID-19 owing to its potential immunomodulatory capacity. It may have a beneficial role in attenuating the cytokine storm that is observed in severe cases of SARS-CoV-2 infection, thereby reducing mortality. Among the different types of MSCs, expanded umbilical cord MSCs can be considered a potential therapeutic agent that requires further validation for managing critically ill COVID-19 patients (304).

Repurposed broad-spectrum antiviral drugs having proven uses against other viral pathogens can be employed for SARS-CoV-2-infected patients. These possess benefits of easy accessibility and recognized pharmacokinetic and pharmacodynamic activities, stability, doses, and side effects (9). Repurposed drugs have been studied for treating CoV infections, like lopinavir/ritonavir, and interferon-1β revealed *in vitro* anti-MERS-CoV action. The *in vivo* experiment carried out in the nonhuman primate model of

common marmosets treated with lopinavir/ritonavir and interferon beta showed superior protective results in treated animals than in the untreated ones (190). A combination of these drugs is being evaluated to treat MERS in humans (MIRACLE trial) (191). These two protease inhibitors (lopinavir and ritonavir), in combination with ribavirin, gave encouraging clinical outcomes in SARS patients, suggesting their therapeutic values (165). However, in the current scenario, due to the lack of specific therapeutic agents against SARS-CoV-2, hospitalized patients confirmed for the disease are given supportive care, like oxygen and fluid therapy, along with antibiotic therapy for managing secondary bacterial infections (192). Patients with novel coronavirus or COVID-19 pneumonia who are mechanically ventilated often require sedatives, analgesics, and even muscle relaxation drugs to prevent ventilator-related lung injury associated with human-machine incoordination (122). The result obtained from a clinical study of four patients infected with COVID-19 claimed that combination therapy using lopinavir/ritonavir, arbidol, and Shufeng Jiedu capsules (traditional Chinese medicine) was found to be effective in managing COVID-19 pneumonia (193). It is difficult to evaluate the therapeutic potential of a drug or a combination of drugs for managing a disease based on such a limited sample size. Before choosing the ideal therapeutic agent for the management of COVID-19, randomized clinical control studies should be performed with a sufficient study population.

Antiviral Drugs

Several classes of routinely used antiviral drugs, like oseltamivir (neuraminidase inhibitor), acyclovir, ganciclovir, and ribavirin, do not have any effect on COVID-19 and, hence, are not recommended (187). Oseltamivir, a neuraminidase inhibitor, has been explored in Chinese hospitals for treating suspected COVID-19 cases, although proven efficacy against SARS-CoV-2 is still lacking for this drug (7). The *in vitro* antiviral potential of FAD-approved drugs, *viz.*, ribavirin, penciclovir, nitazoxanide, nafamostat, and chloroquine, tested in comparison to remdesivir and favipiravir (broad-spectrum antiviral drugs) revealed remdesivir and chloroquine to be highly effective against SARS-CoV-2 infection *in vitro* (194). Ribavirin, penciclovir, and favipiravir might not possess noteworthy *in vivo* antiviral actions for SARS-CoV-2, since higher concentrations of these nucleoside analogs are needed *in vitro* to lessen the viral infection. Both remdesivir and chloroquine are being used in humans to treat other diseases, and such safer drugs can be explored for assessing their effectiveness in COVID-19 patients.

Several therapeutic agents, such as lopinavir/ritonavir, chloroquine, and hydroxychloroquine, have been proposed for the clinical management of COVID-19 (299). A molecular docking study, conducted in the RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2 using different commercially available antipolymerase drugs, identified that drugs such as ribavirin, remdesivir, galidesivir, tenofovir, and sofosbuvir bind RdRp tightly, indicating their vast potential to be used against COVID-19 (305). A broad-spectrum antiviral drug that was developed in the United States, tilorone dihydrochloride (tilorone), was previously found to possess potent antiviral activity against MERS, Marburg, Ebola, and Chikungunya viruses (306). Even though it had broad-spectrum activity, it was neglected for an extended period. Tilorone is another antiviral drug that might have activity against SARS-CoV-2.

Remdesivir, a novel nucleotide analog prodrug, was developed for treating Ebola virus disease (EVD), and it was also found to inhibit the replication of SARS-CoV and MERS-CoV in primary human airway epithelial cell culture systems (195). Recently, *in vitro* study has proven that remdesivir has better antiviral activity than lopinavir and ritonavir. Further, *in vivo* studies conducted in mice also identified that treatment with remdesivir improved pulmonary function and reduced viral loads and lung pathology both in prophylactic and therapeutic regimens compared to lopinavir/ritonavir-IFN-γ treatment in MERS-CoV infection (8). Remdesivir also inhibits a diverse range of coronaviruses, including circulating human CoV, zoonotic bat CoV, and prepandemic zoonotic CoV (195). Remdesivir is also considered the only therapeutic drug that significantly reduces pulmonary pathology (8). All these findings indicate that remde-

sivir has to be further evaluated for its efficacy in the treatment of COVID-19 infection in humans. The broad-spectrum activity exhibited by remdesivir will help control the spread of disease in the event of a new coronavirus outbreak.

Chloroquine is an antimalarial drug known to possess antiviral activity due to its ability to block virus-cell fusion by raising the endosomal pH necessary for fusion. It also interferes with virus-receptor binding by interfering with the terminal glycosylation of SARS-CoV cellular receptors, such as ACE2 (196). In a recent multicenter clinical trial that was conducted in China, chloroquine phosphate was found to exhibit both efficacy and safety in the therapeutic management of SARS-CoV-2-associated pneumonia (197). This drug is already included in the treatment guidelines issued by the National Health Commission of the People's Republic of China. The preliminary clinical trials using hydroxychloroquine, another aminoquinoline drug, gave promising results. The COVID-19 patients received 600 mg of hydroxychloroquine daily along with azithromycin as a single-arm protocol. This protocol was found to be associated with a noteworthy reduction in viral load. Finally, it resulted in a complete cure (271); however, the study comprised a small population and, hence, the possibility of misinterpretation could arise. However, in another case study, the authors raised concerns over the efficacy of hydroxychloroquine-azithromycin in the treatment of COVID-19 patients, since no observable effect was seen when they were used. In some cases, the treatment was discontinued due to the prolongation of the QT interval (307). Hence, further randomized clinical trials are required before concluding this matter.

Recently, another FDA-approved drug, ivermectin, was reported to inhibit the *in vitro* replication of SARS-CoV-2. The findings from this study indicate that a single treatment of this drug was able to induce an ~5,000-fold reduction in the viral RNA at 48 h in cell culture. (308). One of the main disadvantages that limit the clinical utility of ivermectin is its potential to cause cytotoxicity. However, altering the vehicles used in the formulations, the pharmacokinetic properties can be modified, thereby having significant control over the systemic concentration of ivermectin (338). Based on the pharmacokinetic simulation, it was also found that ivermectin may have limited therapeutic utility in managing COVID-19, since the inhibitory concentration that has to be achieved for effective anti-SARS-CoV-2 activity is far higher than the maximum plasma concentration achieved by administering the approved dose (340). However, ivermectin, being a host-directed agent, exhibits antiviral activity by targeting a critical cellular process of the mammalian cell. Therefore, the administration of ivermectin, even at lower doses, will reduce the viral load at a minor level. This slight decrease will provide a great advantage to the immune system for mounting a large-scale antiviral response against SARS-CoV-2 (341). Further, a combination of ivermectin and hydroxychloroquine might have a synergistic effect, since ivermectin reduces viral replication, while hydroxychloroquine inhibits the entry of the virus in the host cell (339). Further, *in vivo* studies and randomized clinical control trials are required to understand the mechanism as well as the clinical utility of this promising drug.

Nafamostat is a potent inhibitor of MERS-CoV that acts by preventing membrane fusion. Nevertheless, it does not have any sort of inhibitory action against SARS-CoV-2 infection (194). Recently, several newly synthesized halogenated triazole compounds were evaluated, using fluorescence resonance energy transfer (FRET)-based helicase assays, for their ability to inhibit helicase activity.

Among the evaluated compounds, 4-(cyclopent-1-en-3-ylamino)-5-[2-(4-iodophenyl) hydrazinyl]-4H-1,2,4-triazole-3-thiol and 4-(cyclopent-1-en-3-ylamino)-5-[2-(4-chlorophenyl) hydrazinyl]-4H-1,2,4-triazole-3-thiol were found to be the most potent. These compounds were used for *in silico* studies, and molecular docking was accomplished into the active binding site of MERS-CoV helicase nsp13 (21). Further studies are required for evaluating the therapeutic potential of these newly identified compounds in the management of COVID-19 infection.

Passive Immunization/Antibody Therapy/MAb

Monoclonal antibodies (MAbs) may be helpful in the intervention of disease in

CoV-exposed individuals. Patients recovering from SARS showed robust neutralizing antibodies against this CoV infection (164). A set of MAbs aimed at the MERS-CoV S protein-specific domains, comprising six specific epitope groups interacting with receptor-binding, membrane fusion, and sialic acid-binding sites, make up crucial entry tasks of S protein (198, 199). Passive immunization employing weaker and strongly neutralizing antibodies provided considerable protection in mice against a MERS-CoV lethal challenge. Such antibodies may play a crucial role in enhancing protective humoral responses against the emerging CoVs by aiming appropriate epitopes and functions of the S protein. The cross-neutralization ability of SARS-CoV RBD-specific neutralizing MAbs considerably relies on the resemblance between their RBDs; therefore, SARS-CoV RBD-specific antibodies could cross-neutralized SL CoVs, i.e., bat-SL-CoV strain WIV1 (RBD with eight amino acid differences from SARS-CoV) but not bat-SL-CoV strain SHC014 (24 amino acid differences) (200).

Appropriate RBD-specific MAbs can be recognized by a relative analysis of RBD of SARS-CoV-2 to that of SARS-CoV, and cross-neutralizing SARS-CoV RBD-specific MAbs could be explored for their effectiveness against COVID-19 and further need to be assessed clinically. The U.S. biotechnology company Regeneron is attempting to recognize potent and specific MAbs to combat COVID-19. An ideal therapeutic option suggested for SARS-CoV-2 (COVID-19) is the combination therapy comprised of MAbs and the drug remdesivir (COVID-19) (201). The SARS-CoV-specific human MAb CR3022 is found to bind with SARS-CoV-2 RBD, indicating its potential as a therapeutic agent in the management of COVID-19. It can be used alone or in combination with other effective neutralizing antibodies for the treatment and prevention of COVID-19 (202). Furthermore, SARS-CoV-specific neutralizing antibodies, like m396 and CR3014, failed to bind the S protein of SARS-CoV-2, indicating that a particular level of similarity is mandatory between the RBDs of SARS-CoV and SARS-CoV-2 for the cross-reactivity to occur.

Further assessment is necessary before confirming the effectiveness of such combination therapy. In addition, to prevent further community and nosocomial spread of COVID-19, the postprocedure risk management program should not be neglected (309). Development of broad-spectrum inhibitors against the human coronaviral pathogens will help to facilitate clinical trials on the effectiveness of such inhibitors against endemic and emerging coronaviruses (203). A promising animal study revealed the protective effect of passive immunotherapy with immune serum from MERS-immune camels on mice infected with MERS-CoV (204). Passive immunotherapy using convalescent plasma is another strategy that can be used for treating COVID-19-infected, critically ill patients (205).

The exploration of fully human antibodies (human single-chain antibodies; HuscFvs) or humanized nanobodies (single-domain antibodies; sdAb, VH/VHH) could aid in blocking virus replication, as these agents can traverse the virus-infected cell membranes (transbodies) and can interfere with the biological characteristics of the replicating virus proteins. Such examples include transbodies to the influenza virus, hepatitis C virus, Ebola virus, and dengue virus (206). Producing similar transbodies against intracellular proteins of coronaviruses, such as papain-like proteases (PLpro), cysteine-like protease (3CLpro), or other nsps, which are essential for replication and transcription of the virus, might formulate a practical move forward for a safer and potent passive immunization approach for virus-exposed persons and rendering therapy to infected patients.

In a case study on five grimly sick patients having symptoms of severe pneumonia due to COVID-19, convalescent plasma administration was found to be helpful in patients recovering successfully. The convalescent plasma containing a SARS-CoV-2-specific ELISA (serum) antibody titer higher than 1:1,000 and neutralizing antibody titer more significant than 40 was collected from the recovered patients and used for plasma transfusion twice in a volume of 200 to 250 ml on the day of collection (310). At present, treatment for sepsis and ARDS mainly involves antimicrobial therapy, source control, and supportive care. Hence, the use of therapeutic plasma exchange can be considered

an option in managing such severe conditions. Further randomized trials can be designed to investigate its efficacy (311).

Potential Therapeutic Agents

Potent therapeutics to combat SARS-CoV-2 infection include virus binding molecules, molecules or inhibitors targeting particular enzymes implicated in replication and transcription process of the virus, helicase inhibitors, vital viral proteases and proteins, protease inhibitors of host cells, endocytosis inhibitors, short interfering RNA (siRNA), neutralizing antibodies, MAbs against the host receptor, MAbs interfering with the S1 RBD, antiviral peptide aimed at S2, and natural drugs/medicines (7, 166, 186). The S protein acts as the critical target for developing CoV antivirals, like inhibitors of S protein and S cleavage, neutralizing antibodies, RBD-ACE2 blockers, siRNAs, blockers of the fusion core, and proteases (168).

All of these therapeutic approaches have revealed both *in vitro* and *in vivo* anti-CoV potential. Although *in vitro* research carried out with these therapeutics showed efficacy, most need appropriate support from randomized animal or human trials. Therefore, they might be of limited applicability and require trials against SARS-CoV-2 to gain practical usefulness. The binding of SARS-CoV-2 with ACE2 leads to the exacerbation of pneumonia as a consequence of the imbalance in the renin-angiotensin system (RAS). The virus-induced pulmonary inflammatory responses may be reduced by the administration of ACE inhibitors (ACEI) and angiotensin type-1 receptor (AT1R) (207).

Several investigations have suggested the use of small-molecule inhibitors for the potential control of SARS-CoV infections. Drugs of the FDA-approved compound library were screened to identify four small-molecule inhibitors of MERS-CoV (chlorpromazine, chloroquine, loperamide, and lopinavir) that inhibited viral replication. These compounds also hinder SARS-CoV and human CoVs (208). Therapeutic strategies involving the use of specific antibodies or compounds that neutralize cytokines and their receptors will help to restrain the host inflammatory responses. Such drugs acting specifically in the respiratory tract will help to reduce virus-triggered immune pathologies in COVID-19 (209). The later stages of coronavirus-induced inflammatory cascades are characterized by the release of proinflammatory interleukin-1 (IL-1) family members, such as IL-1 and IL-33. Hence, there exists a possibility that the inflammation associated with coronavirus can be inhibited by utilizing anti-inflammatory cytokines that belong to the IL-1 family (92). It has also been suggested that the actin protein is the host factor that is involved in cell entry and pathogenesis of SARS-CoV-2. Hence, those drugs that modulate the biological activity of this protein, like ibuprofen, might have some therapeutic application in managing the disease (174). The plasma angiotensin 2 level was found to be markedly elevated in COVID-19 infection and was correlated with viral load and lung injury. Hence, drugs that block angiotensin receptors may have potential for treating COVID-19 infection (121). A scientist from Germany, named Rolf Hilgenfeld, has been working on the identification of drugs for the treatment of coronaviral infection since the time of the first SARS outbreak (19).

The SARS-CoV S2 subunit has a significant function in mediating virus fusion that provides entry into the host cell. Heptad repeat 1 (HR1) and heptad repeat 2 (HR2) can interact and form a six-helix bundle that brings the viral and cellular membranes in close proximity, facilitating its fusion. The sequence alignment study conducted between COVID-19 and SARS-CoV identified that the S2 subunits are highly conserved in these CoVs. The HR1 and HR2 domains showed 92.6% and 100% overall identity, respectively (210). From these findings, we can confirm the significance of COVID-19 HR1 and HR2 and their vital role in host cell entry. Hence, fusion inhibitors target the HR1 domain of S protein, thereby preventing viral fusion and entry into the host cell. This is another potential therapeutic strategy that can be used in the management of COVID-19. Other than the specific therapy directed against COVID-19, general treatments play a vital role in the enhancement of host immune responses against the viral agent. Inadequate nutrition is linked to the weakening of the host immune response,

making the individual more susceptible. The role played by nutrition in disease susceptibility should be measured by evaluating the nutritional status of patients with COVID-19 (205).

Animal Models and Cell Cultures

For evaluating the potential of vaccines and therapeutics against CoVs, including SARS-CoV, MERS-CoVs, and the presently emerging SARS-CoV-2, suitable animal models that can mimic the clinical disease are needed (211, 212). Various animal models were assessed for SARS- and MERS-CoVs, such as mice, guinea pigs, golden Syrian hamsters, ferrets, rabbits, nonhuman primates like rhesus macaques and marmosets, and cats (185, 213–218). The specificity of the virus to hACE2 (receptor of SARS-CoV) was found to be a significant barrier in developing animal models. Consequently, a SARS-CoV transgenic mouse model has been developed by inserting the hACE2 gene into the mouse genome (219). The inability of MERS-CoV to replicate in the respiratory tracts of animals (mice, hamsters, and ferrets) is another limiting factor. However, with genetic engineering, a 288-330$^{+/+}$ MERS-CoV genetically modified mouse model was developed and now is in use for the assessment of novel drugs and vaccines against MERS-CoV (220). In the past, small animals (mice or hamsters) have been targeted for being closer to a humanized structure, such as mouse DPP4 altered with human DPP4 (hDPP4), hDPP4-transduced mice, and hDPP4-Tg mice (transgenic for expressing hDPP4) for MERS-CoV infection (221). The CRISPR-Cas9 gene-editing tool has been used for inserting genomic alterations in mice, making them susceptible to MERS-CoV infection (222). Efforts are under way to recognize suitable animal models for SARS-CoV2/COVID-19, identify the receptor affinity of this virus, study pathology in experimental animal models, and explore virus-specific immune responses and protection studies, which together would increase the pace of efforts being made for developing potent vaccines and drugs to counter this emerging virus. Cell lines, such as monkey epithelial cell lines (LLC-MK2 and Vero-B4), goat lung cells, alpaca kidney cells, dromedary umbilical cord cells, and advanced *ex vivo* three-dimensional tracheobronchial tissue, have been explored to study human CoVs (MERS-CoV) (223, 224). Vero and Huh-7 cells (human liver cancer cells) have been used for isolating SARS-CoV-2 (194).

Recently, an experimental study with rhesus monkeys as animal models revealed the absence of any viral loads in nasopharyngeal and anal swabs, and no viral replication was recorded in the primary tissues at a time interval of 5 days post-reinfection in reexposed monkeys (274). The subsequent virological, radiological, and pathological observations indicated that the monkeys with reexposure had no recurrence of COVID-19, like the SARS-CoV-2-infected monkeys without rechallenge. These findings suggest that primary infection with SARS-CoV-2 could protect from later exposures to the virus, which could help in defining disease prognosis and crucial inferences for designing and developing potent vaccines against COVID-19 (274).

PREVENTION, CONTROL, AND MANAGEMENT

In contrast to their response to the 2002 SARS outbreak, China has shown immense political openness in reporting the COVID-19 outbreak promptly. They have also performed rapid sequencing of COVID-19 at multiple levels and shared the findings globally within days of identifying the novel virus (225). The move made by China opened a new chapter in global health security and diplomacy. Even though complete lockdown was declared following the COVID-19 outbreak in Wuhan, the large-scale movement of people has resulted in a radiating spread of infections in the surrounding provinces as well as to several other countries. Large-scale screening programs might help us to control the spread of this virus. However, this is both challenging as well as time-consuming due to the present extent of infection (226). The current scenario demands effective implementation of vigorous prevention and control strategies owing to the prospect of COVID-19 for nosocomial infections (68). Follow-ups of infected patients by telephone on day 7 and day 14 are advised to avoid any further unintentional spread or nosocomial transmission (312). The availability of public data sets

provided by independent analytical teams will act as robust evidence that would guide us in designing interventions against the COVID-19 outbreak. Newspaper reports and social media can be used to analyze and reconstruct the progression of an outbreak. They can help us to obtain detailed patient-level data in the early stages of an outbreak (227). Immediate travel restrictions imposed by several countries might have contributed significantly to preventing the spread of SARS-CoV-2 globally (89, 228). Following the outbreak, a temporary ban was imposed on the wildlife trade, keeping in mind the possible role played by wild animal species in the origin of SARS-CoV-2/COVID-19 (147). Making a permanent and bold decision on the trade of wild animal species is necessary to prevent the possibility of virus spread and initiation of an outbreak due to zoonotic spillover (1).

Personal protective equipment (PPE), like face masks, will help to prevent the spread of respiratory infections like COVID-19. Face masks not only protect from infectious aerosols but also prevent the transmission of disease to other susceptible individuals while traveling through public transport systems (313). Another critical practice that can reduce the transmission of respiratory diseases is the maintenance of hand hygiene. However, the efficacy of this practice in reducing the transmission of respiratory viruses like SARS-CoV-2 is much dependent upon the size of droplets produced. Hand hygiene will reduce disease transmission only if the virus is transmitted through the formation of large droplets (314). Hence, it is better not to overemphasize that hand hygiene will prevent the transmission of SARS-CoV-2, since it may produce a false sense of safety among the general public that further contributes to the spread of COVID-19. Even though airborne spread has not been reported in SARS-CoV-2 infection, transmission can occur through droplets and fomites, especially when there is close, unprotected contact between infected and susceptible individuals. Hence, hand hygiene is equally as important as the use of appropriate PPE, like face masks, to break the transmission cycle of the virus; both hand hygiene and face masks help to lessen the risk of COVID-19 transmission (315).

Medical staff are in the group of individuals most at risk of getting COVID-19 infection. This is because they are exposed directly to infected patients. Hence, proper training must be given to all hospital staff on methods of prevention and protection so that they become competent enough to protect themselves and others from this deadly disease (316). As a preventive measure, health care workers caring for infected patients should take extreme precautions against both contact and airborne transmission. They should use PPE such as face masks (N95 or FFP3), eye protection (goggles), gowns, and gloves to nullify the risk of infection (299).

The human-to-human transmission reported in SARS-CoV-2 infection occurs mainly through droplet or direct contact. Due to this finding, frontline health care workers should follow stringent infection control and preventive measures, such as the use of PPE, to prevent infection (110). The mental health of the medical/health workers who are involved in the COVID-19 outbreak is of great importance, because the strain on their mental well-being will affect their attention, concentration, and decision-making capacity. Hence, for control of the COVID-19 outbreak, rapid steps should be taken to protect the mental health of medical workers (229).

Since the living mammals sold in the wet market are suspected to be the intermediate host of SARS-CoV-2, there is a need for strengthening the regulatory mechanism for wild animal trade (13). The total number of COVID-19 confirmed cases is on a continuous rise and the cure rate is relatively low, making disease control very difficult to achieve. The Chinese government is making continuous efforts to contain the disease by taking emergency control and prevention measures. They have already built a hospital for patients affected by this virus and are currently building several more for accommodating the continuously increasing infected population (230). The effective control of SARS-CoV-2/COVID-19 requires high-level interventions like intensive contact tracing, as well as the quarantine of people with suspected infection and the isolation of infected individuals. The implementation of rigorous control and preventive measures together might control the R_0 number and reduce the transmission risk (228).

Considering the zoonotic links associated with SARS-CoV-2, the One Health approach may play a vital role in the prevention and control measures being followed to restrain this pandemic virus (317–319). The substantial importation of COVID-19 presymptomatic cases from Wuhan has resulted in independent, self-sustaining outbreaks across major cities both within the country and across the globe. The majority of Chinese cities are now facing localized outbreaks of COVID-19 (231). Hence, deploying efficient public health interventions might help to cut the spread of this virus globally.

The occurrence of COVID-19 infection on several cruise ships gave us a preliminary idea regarding the transmission pattern of the disease. Cruise ships act as a closed environment and provide an ideal setting for the occurrence of respiratory disease outbreaks. Such a situation poses a significant threat to travelers, since people from different countries are on board, which favors the introduction of the pathogen (320). Although nearly 30 cruise ships from different countries have been found harboring COVID-19 infection, the major cruise ships that were involved in the COVID-19 outbreaks are the *Diamond Princess*, *Grand Princess*, *Celebrity Apex*, and *Ruby Princess*. The number of confirmed COVID-19 cases around the world is on the rise. The success of preventive measures put forward by every country is mainly dependent upon their ability to anticipate the approaching waves of patients. This will help to properly prepare the health care workers and increase the intensive care unit (ICU) capacity (321). Instead of entirely relying on lockdown protocols, countries should focus mainly on alternative intervention strategies, such as large-scale testing, contract tracing, and localized quarantine of suspected cases for limiting the spread of this pandemic virus. Such intervention strategies will be useful either at the beginning of the pandemic or after lockdown relaxation (322). Lockdown should be imposed only to slow down disease progression among the population so that the health care system is not overloaded.

The reproduction number (R_0) of COVID-19 infection was earlier estimated to be in the range of 1.4 to 2.5 (70); recently, it was estimated to be 2.24 to 3.58 (76). Compared to its coronavirus predecessors, COVID-19 has an R_0 value that is greater than that of MERS ($R_0 < 1$) (108) but less than that of SARS (R_0 value of 2 to 5) (93). Still, to prevent further spread of disease at mass gatherings, functions remain canceled in the affected cities, and persons are asked to work from home (232). Hence, it is a relief that the current outbreak of COVID-19 infection can be brought under control with the adoption of strategic preventive and control measures along with the early isolation of subsequent cases in the coming days. Studies also report that since air traffic between China and African countries increased many times over in the decade after the SARS outbreak, African countries need to be vigilant to prevent the spread of novel coronavirus in Africa (225). Due to fear of virus spread, Wuhan City was completely shut down (233). The immediate control of the ongoing COVID-19 outbreaks appears a mammoth task, especially for developing countries, due to their inability to allocate quarantine stations that could screen infected individuals' movements (234). Such underdeveloped countries should divert their resources and energy to enforcing the primary level of preventive measures, like controlling the entry of individuals from China or countries where the disease has flared up, isolating the infected individuals, and quarantining individuals with suspected infection. Most of the sub-Saharan African countries have a fragile health system that can be crippled in the event of an outbreak. Effective management of COVID-19 would be difficult for low-income countries due to their inability to respond rapidly due to the lack of an efficient health care system (65). Controlling the imported cases is critical in preventing the spread of COVID-19 to other countries that have not reported the disease until now. The possibility of an imported case of COVID-19 leading to sustained human-to-human transmission was estimated to be 0.41. This can be reduced to a value of 0.012 by decreasing the mean time from the onset of symptoms to hospitalization and can only be made possible by using intense disease surveillance systems (235). The silent importations of infected individuals (before the manifestation of clinical signs) also contributed significantly to the spread of disease across the major cities of the world. Even though the travel ban was

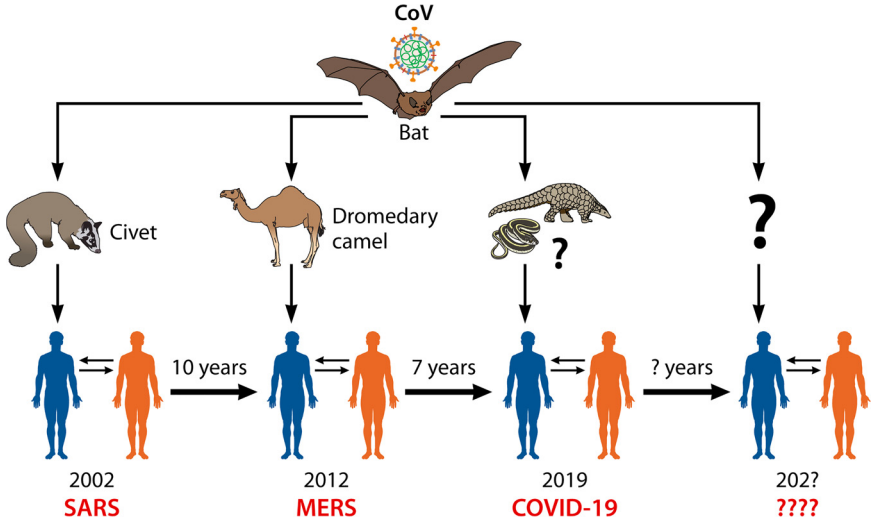

FIG 7 Coronavirus origins. Coronavirus is the most prominent example of an emerging virus that has crossed the species barrier from wild animals to humans, like SARS and MERS. The origin of SARS-CoV-2 is also suspected to be from an intermediate animal host. The possibility of crossing the species barrier again for the fourth time cannot be ruled out.

implemented in Wuhan (89), infected persons who traveled out of the city just before the imposition of the ban might have remained undetected and resulted in local outbreaks (236). Emerging novel diseases like COVID-19 are difficult to contain within the country of origin, since globalization has led to a world without borders. Hence, international collaboration plays a vital role in preventing the further spread of this virus across the globe (237).

We also predict the possibility of another outbreak, as predicted by Fan et al. (6). Indeed, the present outbreak caused by SARS-CoV-2 (COVID-19) was expected. Similar to previous outbreaks, the current outbreak also will be contained shortly. However, the real issue is how we are planning to counter the next zoonotic CoV epidemic that is likely to occur within the next 5 to 10 years or even sooner (Fig. 7).

CONCLUDING REMARKS

Several years after the global SARS epidemic, the current SARS-CoV-2/COVID-19 pandemic has served as a reminder of how novel pathogens can rapidly emerge and spread through the human population and eventually cause severe public health crises. Further research should be conducted to establish animal models for SARS-CoV-2 to investigate replication, transmission dynamics, and pathogenesis in humans. This may help develop and evaluate potential therapeutic strategies against zoonotic CoV epidemics. Present trends suggest the occurrence of future outbreaks of CoVs due to changes in the climate, and ecological conditions may be associated with human-animal contact. Live-animal markets, such as the Huanan South China Seafood Market, represent ideal conditions for interspecies contact of wildlife with domestic birds, pigs, and mammals, which substantially increases the probability of interspecies transmission of CoV infections and could result in high risks to humans due to adaptive genetic recombination in these viruses (323–325).

The COVID-19-associated symptoms are fever, cough, expectoration, headache, and myalgia or fatigue. Individuals with asymptomatic and atypical clinical manifestations were also identified recently, further adding to the complexity of disease transmission dynamics. Atypical clinical manifestations may only express symptoms such as fatigue instead of respiratory signs such as fever, cough, and sputum. In such cases, the clinician must be vigilant for the possible occurrence of asymptomatic and atypical clinical manifestations to avoid the possibility of missed diagnoses.

The present outbreak caused by SARS-CoV-2 was, indeed, expected. Similar to

previous outbreaks, the current pandemic also will be contained shortly. However, the real question is, how are we planning to counter the next zoonotic CoV epidemic that is likely to occur within the next 5 to 10 years or perhaps sooner? Our knowledge of most of the bat CoVs is scarce, as these viruses have not been isolated and studied, and extensive studies on such viruses are typically only conducted when they are associated with specific disease outbreaks. The next step following the control of the COVID-19 outbreak in China should be focused on screening, identification, isolation, and characterization of CoVs present in wildlife species of China, particularly in bats. Both *in vitro* and *in vivo* studies (using suitable animal models) should be conducted to evaluate the risk of future epidemics. Presently, licensed antiviral drugs or vaccines against SARS-CoV, MERS-CoV, and SARS-CoV-2 are lacking. However, advances in designing antiviral drugs and vaccines against several other emerging diseases will help develop suitable therapeutic agents against COVID-19 in a short time. Until then, we must rely exclusively on various control and prevention measures to prevent this new disease from becoming a pandemic.

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REFERENCES

1. Rodriguez-Morales AJ, Bonilla-Aldana DK, Balbin-Ramon GJ, Rabaan AA, Sah R, Paniz-Mondolfi A, Pagliano P, Esposito S. 2020. History is repeating itself: probable zoonotic spillover as the cause of the 2019 novel coronavirus epidemic. Infez Med 28:3–5.
2. Gralinski LE, Menachery VD. 2020. Return of the coronavirus: 2019-nCoV. Viruses 12:135. https://doi.org/10.3390/v12020135.
3. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W, China Novel Coronavirus Investigating and Research Team. 2020. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 382:727–733. https://doi.org/10.1056/NEJMoa2001017.
4. Wei X, Li X, Cui J. 2020. Evolutionary perspectives on novel coronaviruses identified in pneumonia cases in China. Natl Sci Rev 7:239–242. https://doi.org/10.1093/nsr/nwaa009.
5. Munster VJ, Koopmans M, van Doremalen N, van Riel D, de Wit E. 2020. A novel coronavirus emerging in China-key questions for impact assessment. N Engl J Med 382:692–694. https://doi.org/10.1056/NEJMp2000929.
6. Fan Y, Zhao K, Shi ZL, Zhou P. 2019. Bat coronaviruses in China. Viruses 11:210. https://doi.org/10.3390/v11030210.
7. Lu H. 2020. Drug treatment options for the 2019-new coronavirus (2019-nCoV). Biosci Trends 14:69–71. https://doi.org/10.5582/bst.2020.01020.
8. Sheahan TP, Sims AC, Leist SR, Schäfer A, Won J, Brown AJ, Montgomery SA, Hogg A, Babusis D, Clarke MO, Spahn JE, Bauer L, Sellers S, Porter D, Feng JY, Cihlar T, Jordan R, Denison MR, Baric RS. 2020. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun 11:222. https://doi.org/10.1038/s41467-019-13940-6.
9. Pillaiyar T, Meenakshisundaram S, Manickam M. 2020. Recent discovery and development of inhibitors targeting coronaviruses. Drug Discov Today 25:668–688. https://doi.org/10.1016/j.drudis.2020.01.015.
10. Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, Liu W, Bi Y, Gao GF. 2016. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol 24:490–502. https://doi.org/10.1016/j.tim.2016.03.003.
11. Ng OW, Tan YJ. 2017. Understanding bat SARS-like coronaviruses for the preparation of future corona virus outbreaks–implications for coronavirus vaccine development. Hum Vaccin Immunother 13:186–189. https://doi.org/10.1080/21645515.2016.1228500.
12. Bonilla-Aldana DK, Holguin-Rivera Y, Cortes-Bonilla I, Cardona-Trujillo MC, García-Barco A, Bedoya-Arias HA, Rabaan AA, Sah R, Rodriguez-Morales AJ. 6 February 2020. Coronavirus infections reported by ProMED, February 2000–January 2020. Travel Med Infect Dis https://doi.org/10.1016/j.tmaid.2020.101575.
13. Zhang L, Shen FM, Chen F, Lin Z. 3 February 2020. Origin and evolution of the 2019 novel coronavirus. Clin Infect Dis https://doi.org/10.1093/cid/ciaa112.
14. Xu XW, Wu XX, Jiang XG, Xu KJ, Ying LJ, Ma CL, Li SB, Wang HY, Zhang S, Gao HN, Sheng JF, Cai HL, Qiu YQ, Li LJ. 2020. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. BMJ 368:m606. https://doi.org/10.1136/bmj.m606.
15. Chen J. 2020. Pathogenicity and transmissibility of 2019-nCoV-A quick overview and comparison with other emerging viruses. Microbes Infect 22:69–71. https://doi.org/10.1016/j.micinf.2020.01.004.
16. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, Meng J, Zhu Z, Zhang Z, Wang J, Sheng J, Quan L, Xia Z, Tan W, Cheng G, Jiang T. 2020. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe 27:325–328. https://doi.org/10.1016/j.chom.2020.02.001.
17. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W. 2020. Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395:565–574. https://doi.org/10.1016/S0140-6736(20)30251-8.
18. Kui L, Fang YY, Deng Y, Liu W, Wang MF, Ma JP, Xiao W, Wang YN, Zhong MH, Li CH, Li GC, Liu HG. 2020. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. Chin Med J 133:1025–1031. https://doi.org/10.1097/CM9.0000000000000744.
19. Cyranoski D. 2020. This scientist hopes to test coronavirus drugs on animals in locked-down Wuhan. Nature 577:607. https://doi.org/10.1038/d41586-020-00190-6.
20. Dhama K, Pawaiya RVS, Chakrabort S, Tiwari R, Saminathan M, Verma

AK. 2014. Coronavirus infection in equines: a review. Asian J Anim Vet Adv 9:164–176. https://doi.org/10.3923/ajava.2014.164.176.
21. Zaher NH, Mostafa MI, Altaher AY. 2020. Design, synthesis and molecular docking of novel triazole derivatives as potential CoV helicase inhibitors. Acta Pharm 70:145–159. https://doi.org/10.2478/acph-2020-0024.
22. Bonilla-Aldana DK, Villamil-Gómez WE, Rabaan AA, Rodriguez-Morales AJ. 2020. Una nueva zoonosis viral de preocupación global: COVID-19, enfermedad por coronavirus 2019. Iatreia 33:107–110.
23. Weiss SR, Leibowitz JL. 2011. Coronavirus pathogenesis. Adv Virus Res 81:85–164. https://doi.org/10.1016/B978-0-12-385885-6.00009-2.
24. Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P, Pan P, Wang W, Hu D, Liu X, Zhang Q, Wu J. 2020. Coronavirus infections and immune responses. J Med Virol 92:424–432. https://doi.org/10.1002/jmv.25685.
25. WHO. 2020. Coronavirus disease 2019 (COVID-19) situation report–114 (13th May, 2020). https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200513-covid-19-sitrep-114.pdf?sfvrsn=17ebbbe_4. Accessed on 13 May 2020.
26. Gorbalenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA, Haagmans BL, Lauber C, Leontovich AM, Neuman BW, Penzar D. 2020. Severe acute respiratory syndrome-related coronavirus: the species and its viruses–a statement of the Coronavirus Study Group. bioRxiv https://doi.org/10.1101/2020.02.07.937862.
27. Chen Y, Liu Q, Guo D. 2020. Emerging coronaviruses: genome structure, replication, and pathogenesis. J Med Virol 92:418–423. https://doi.org/10.1002/jmv.25681.
28. Lai MMC, Holmes KV. 2001. Coronaviridae: the viruses and their replication, p 1163–1185. *In* Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE (ed), Fields virology, 4th ed. Lippincott-Raven, Philadephia, PA.
29. Woo PC, Lau SK, Lam CS, Lau CC, Tsang AK, Lau JH, Bai R, Teng JL, Tsang CC, Wang M, Zheng BJ, Chan KH, Yuen KY. 2012. Discovery of seven novel mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. J Virol 86:3995–4008. https://doi.org/10.1128/JVI.06540-11.
30. Fehr AR, Perlman S. 2015. Coronaviruses: an overview of their replication and pathogenesis. Methods Mol Biol 1282:1–23. https://doi.org/10.1007/978-1-4939-2438-7_1.
31. Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, Yuen KY. 2020. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect 9:221–236. https://doi.org/10.1080/22221751.2020.1719902.
32. Brian DA, Baric RS. 2005. Coronavirus genome structure and replication. Curr Topics Microbiol Immunol 287:1–30. https://doi.org/10.1007/3-540-26765-4_1.
33. Nakagawa K, Lokugamage KG, Makino S. 2016. Viral and cellular mRNA translation in coronavirus-infected cells. Adv Virus Res 96:165–192. https://doi.org/10.1016/bs.aivir.2016.08.001.
34. Snijder EJ, van der Meer Y, Zevenhoven-Dobbe J, Onderwater JJM, van der Meulen J, Koerten HK, Mommaas AM. 2006. Ultrastructure and origin of membrane vesicles associated with the severe acute respiratory syndrome coronavirus replication complex. J Virol 80:5927–5940. https://doi.org/10.1128/JVI.02501-05.
35. Hussain S, Pan J, Chen Y, Yang Y, Xu J, Peng Y, Wu Y, Li Z, Zhu Y, Tien P, Guo D. 2005. Identification of novel subgenomic RNAs and noncanonical transcription initiation signals of severe acute respiratory syndrome coronavirus. J Virol 79:5288–5295. https://doi.org/10.1128/JVI.79.9.5288-5295.2005.
36. Malik YS, Sircar S, Bhat S, Sharun K, Dhama K, Dadar M, Tiwari R, Chaicumpa W. 2020. Emerging novel coronavirus (2019-nCoV)—current scenario, evolutionary perspective based on genome analysis and recent developments. Vet Q 40:68–76. https://doi.org/10.1080/01652176.2020.1727993.
37. Ren LL, Wang YM, Wu ZQ, Xiang ZC, Guo L, Xu T, Jiang YZ, Xiong Y, Li YJ, Li H, Fan GH, Gu XY, Xiao Y, Gao H, Xu JY, Yang F, Wang XM, Wu C, Chen L, Liu YW, Liu B, Yang J, Wang XR, Dong J, Li L, Huang CL, Zhao JP, Hu Y, Cheng ZS, Liu LL, Qian ZH, Qin C, Jin Q, Cao B, Wang JW. 2020. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. Chin Med J 133:1015–1024. https://doi.org/10.1097/CM9.0000000000000722.
38. Hu B, Ge X, Wang LF, Shi Z. 2015. Bat origin of human coronaviruses. Virol J 12:221. https://doi.org/10.1186/s12985-015-0422-1.
39. Li B, Si HR, Zhu Y, Yang XL, Anderson DE, Shi ZL, Wang LF, Zhou P. 2020. Discovery of bat coronaviruses through surveillance and probe capture-based next-generation sequencing. mSphere 5:e00807-19. https://doi.org/10.1128/mSphere.00807-19.
40. Wang LF, Eaton BT. 2007. Bats, civets and the emergence of SARS, p 325–344. *In* Wildlife and emerging zoonotic diseases: the biology, circumstances and consequences of cross-species transmission. Springer, Berlin, Germany.
41. Hemida MG. 2019. Middle East respiratory syndrome coronavirus and the One Health concept. Peer J 7:e7556. https://doi.org/10.7717/peerj.7556.
42. Masters PS. 2006. The molecular biology of coronaviruses. Adv Virus Res 66:193–292. https://doi.org/10.1016/S0065-3527(06)66005-3.
43. Belouzard S, Millet JK, Licitra BN, Whittaker GR. 2012. Mechanisms of coronavirus cell entry mediated by the viral spike protein. Viruses 4:1011–1033. https://doi.org/10.3390/v4061011.
44. Beniac DR, Andonov A, Grudeski E, Booth TF. 2006. Architecture of the SARS coronavirus prefusion spike. Nat Struct Mol Biol 13:751–752. https://doi.org/10.1038/nsmb1123.
45. Li F. 2016. Structure, function, and evolution of coronavirus spike proteins. Annu Rev Virol 3:237–261. https://doi.org/10.1146/annurev-virology-110615-042301.
46. Ge XY, Li JL, Yang XL, Chmura AA, Zhu G, Epstein JH, Mazet JK, Hu B, Zhang W, Peng C, Zhang YJ, Luo CM, Tan B, Wang N, Zhu Y, Crameri G, Zhang SY, Wang LF, Daszak P, Shi ZL. 2013. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature 503:535–538. https://doi.org/10.1038/nature12711.
47. Li X, Song Y, Wong G, Cui J. 2020. Bat origin of a new human coronavirus: there and back again. Sci China Life Sci 63:461–462. https://doi.org/10.1007/s11427-020-1645-7.
48. Neuman BW, Kiss G, Kunding AH, Bhella D, Baksh MF, Connelly S, Droese B, Klaus JP, Makino S, Sawicki SG, Siddell SG, Stamou DG, Wilson IA, Kuhn P, Buchmeier MJ. 2011. A structural analysis of M protein in coronavirus assembly and morphology. J Struct Biol 174:11–22. https://doi.org/10.1016/j.jsb.2010.11.021.
49. Nal B, Chan C, Kien F, Siu L, Tse J, Chu K, Kam J, Staropoli I, Crescenzo-Chaigne B, Escriou N, van der Werf S, Yuen K-Y, Altmeyer R. 2005. Differential maturation and subcellular localization of severe acute respiratory syndrome coronavirus surface proteins S, M and E. J Gen Virol 86:1423–1434. https://doi.org/10.1099/vir.0.80671-0.
50. Arndt AL, Larson BJ, Hogue BG. 2010. A conserved domain in the coronavirus membrane protein tail is important for virus assembly. J Virol 84:11418–11428. https://doi.org/10.1128/JVI.01131-10.
51. Schoeman D, Fielding BC. 2019. Coronavirus envelope protein: current knowledge. Virol J 16:69. https://doi.org/10.1186/s12985-019-1182-0.
52. Nieto-Torres JL, DeDiego ML, Verdiá-Báguena C, Jimenez-Guardeño JM, Regla-Nava JA, Fernandez-Delgado R, Castaño-Rodriguez C, Alcaraz A, Torres J, Aguilella VM, Enjuanes L. 2014. Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. PLoS Pathog 10:e1004077. https://doi.org/10.1371/journal.ppat.1004077.
53. Pervushin K, Tan E, Parthasarathy K, Lin X, Jiang FL, Yu D, Vararattanavech A, Soong TW, Liu DX, Torres J. 2009. Structure and inhibition of the SARS coronavirus envelope protein ion channel. PLoS Pathog 5:e1000511. https://doi.org/10.1371/journal.ppat.1000511.
54. DeDiego ML, Alvarez E, Almazán F, Rejas MT, Lamirande E, Roberts A, Shieh WJ, Zaki SR, Subbarao K, Enjuanes L. 2007. A severe acute respiratory syndrome corona virus that lacks the E gene is attenuated in vitro and in vivo. J Virol 81:1701–1713. https://doi.org/10.1128/JVI.01467-06.
55. Chang C-K, Sue S-C, Yu T-H, Hsieh C-M, Tsai C-K, Chiang Y-C, Lee S-J, Hsiao H-H, Wu W-J, Chang W-L, Lin C-H, Huang T-H. 2006. Modular organization of SARS coronavirus nucleocapsid protein. J Biomed Sci 13:59–72. https://doi.org/10.1007/s11373-005-9035-9.
56. Sheikh A, Al-Taher A, Al-Nazawi M, Al-Mubarak AI, Kandeel M. 2020. Analysis of preferred codon usage in the coronavirus N genes and their implications for genome evolution and vaccine design. J Virol Methods 277:113806. https://doi.org/10.1016/j.jviromet.2019.113806.
57. McBride R, van Zyl M, Fielding BC. 2014. The coronavirus nucleocapsid is a multifunctional protein. Viruses 6:2991–3018. https://doi.org/10.3390/v6082991.
58. Fan H, Ooi A, Tan YW, Wang S, Fang S, Liu DX, Lescar J. 2005. The

nucleocapsid protein of coronavirus infectious bronchitis virus: crystal structure of its N-terminal domain and multimerization properties. Structure 13:1859–1868. https://doi.org/10.1016/j.str.2005.08.021.
59. Hurst KR, Koetzner CA, Masters PS. 2009. Identification of in vivo-interacting domains of the murine coronavirus nucleocapsid protein. J Virol 83:7221–7234. https://doi.org/10.1128/JVI.00440-09.
60. Stohlman SA, Baric RS, Nelson GN, Soe LH, Welter LM, Deans RJ. 1988. Specific interaction between coronavirus leader RNA and nucleocapsid protein. J Virol 62:4288–4295. https://doi.org/10.1128/JVI.62.11.4288-4295.1988.
61. You J, Dove BK, Enjuanes L, DeDiego ML, Alvarez E, Howell G, Heinen P, Zambon M, Hiscox JA. 2005. Subcellular localization of the severe acute respiratory syndrome coronavirus nucleocapsid protein. J Gen Virol 86:3303–3310. https://doi.org/10.1099/vir.0.81076-0.
62. Cui L, Wang H, Ji Y, Yang J, Xu S, Huang X, Wang Z, Qin L, Tien P, Zhou X, Guo D, Chen Y. 2015. The nucleocapsid protein of coronaviruses acts as a viral suppressor of RNA silencing in mammalian cells. J Virol 89:9029–9043. https://doi.org/10.1128/JVI.01331-15.
63. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Shi ZL. 2020. Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin. bioRxiv https://doi.org/10.1101/2020.01.22.914952.
64. Bastola A, Sah R, Rodriguez-Morales AJ, Lal BK, Jha R, Ojha HC, Shrestha B, Chu DKW, Poon LLM, Costello A, Morita K, Pandey BD. 2020. The first 2019 novel coronavirus case in Nepal. Lancet Infect Dis 20:279–280. https://doi.org/10.1016/S1473-3099(20)30067-0.
65. Velavan TP, Meyer CG. 2020. The Covid-19 epidemic. Trop Med Int Health 25:278–280. https://doi.org/10.1111/tmi.13383.
66. Ayittey FK, Ayittey MK, Chiwero NB, Kamasah JS, Dzuvor C. 2020. Economic impacts of Wuhan 2019-nCoV on China and the world. J Med Virol 92:473–475. https://doi.org/10.1002/jmv.25706.
67. Qiu H, Tong Z, Ma P, Hu M, Peng Z, Wu W, Du B, China Critical Care Clinical Trials Group (CCCCTG). 2020. Intensive care during the coronavirus epidemic. Intensive Care Med 46:576–578. https://doi.org/10.1007/s00134-020-05966-y.
68. Cheng VCC, Wong SC, To KKW, Ho PL, Yuen KY. 2020. Preparedness and proactive infection control measures against the emerging Wuhan coronavirus pneumonia in China. J Hosp Infect 104:254–255. https://doi.org/10.1016/j.jhin.2020.01.010.
69. Liu SL, Saif L. 2020. Emerging viruses without borders: the Wuhan coronavirus. Viruses 12:130. https://doi.org/10.3390/v12020130.
70. Mahase E. 2020. China coronavirus: what do we know so far? BMJ 368:m308. https://doi.org/10.1136/bmj.m308.
71. Hui DS, I Azhar E, Madani TA, Ntoumi F, Kock R, Dar O, Ippolito G, Mchugh TD, Memish ZA, Drosten C, Zumla A, Petersen E. 2020. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health—the latest 2019 novel coronavirus outbreak in Wuhan, China. Int J Infect Dis 91:264–266. https://doi.org/10.1016/j.ijid.2020.01.009.
72. Lu H, Stratton CW, Tang YW. 2020. Outbreak of pneumonia of unknown etiology in Wuhan China: the mystery and the miracle. J Med Virol 92:401–402. https://doi.org/10.1002/jmv.25678.
73. Nishiura H, Kobayashi T, Yang Y, Hayashi K, Miyama T, Kinoshita R, Linton NM, Jung SM, Yuan B, Suzuki A, Akhmetzhanov AR. 2020. The rate of underascertainment of novel corona virus (2019-nCoV) infection: estimation using Japanese passengers data on evacuation flights. J Clin Med 9:419. https://doi.org/10.3390/jcm9020419.
74. Nishiura H, Linton NM, Akhmetzhanov AR. 2020. Initial cluster of novel coronavirus (2019-nCoV) infections in Wuhan, China, is consistent with substantial human-to-human transmission. J Clin Med 9:488. https://doi.org/10.3390/jcm9020488.
75. Parry J. 2020. China coronavirus: cases surge as official admits human to human transmission. BMJ 368:m236. https://doi.org/10.1136/bmj.m236.
76. Zhao S, Lin Q, Ran J, Musa SS, Yang G, Wang W, Lou Y, Gao D, Yang L, He D, Wang MH. 2020. Preliminary estimation of the basic reproduction number of novel coronavirus (2019-nCoV) in China, from 2019 to 2020: a data-driven analysis in the early phase of the outbreak. Int J Infect Dis 92:214–217. https://doi.org/10.1016/j.ijid.2020.01.050.
77. Liu Y, Gayle AA, Wilder-Smith A, Rocklöv J. 13 February 2020. The reproductive number of COVID-19 is higher compared to SARS coronavirus. J Travel Med 27:taaa021. https://doi.org/10.1093/jtm/taaa021.
78. Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, Cheung CL, Luo SW, Li PH, Zhang LJ, Guan YJ, Butt KM, Wong KL, Chan KW, Lim W, Shortridge KF, Yuen KY, Peiris JS, Poon LL. 2003. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science 302:276–278. https://doi.org/10.1126/science.1087139.
79. Monchatre-Leroy E, Boue F, Boucher JM, Renault C, Moutou F, Ar Gouilh M, Umhang G. 2017. Identification of alpha and beta coronavirus in wildlife species in France: bats, rodents, rabbits, and hedgehogs. Viruses 9:364. https://doi.org/10.3390/v9120364.
80. Shen K, Yang Y, Wang T, Zhao D, Jiang Y, Jin R, Zheng Y, Xu B, Xie Z, Lin L, Shang Y, Lu X, Shu S, Bai Y, Deng J, Lu M, Ye L, Wang X, Wang Y, Gao L. 2020. Diagnosis, treatment, and prevention of 2019 novel coronavirus infection in children: experts' consensus statement. World J Pediatr 7:1–9. https://doi.org/10.1007/s12519-020-00343-7.
81. Lin L, Li TS. 2020. Interpretation of guidelines for the diagnosis and treatment of novel coronavirus (2019-nCoV) infection by the National Health Commission (trial version 5). Zhonghua Yi Xue Za Zhi 100:E001. https://doi.org/10.3760/cma.j.issn.0376-2491.2020.0001.
82. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, Yu J, Kang M, Song Y, Xia J, Guo Q, Song T, He J, Yen HL, Peiris M, Wu J. 2020. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med 382:1177–1179. https://doi.org/10.1056/NEJMc2001737.
83. Hao W, Li M, Huang X. 19 February 2020. First atypical case of 2019 novel coronavirus in Yan'an, China. Clin Microbiol Infect https://doi.org/10.1016/j.cmi.2020.02.011.
84. Zhou T, Liu Q, Yang Z, Liao J, Yang K, Bai W, Lu X, Zhang W. 2020. Preliminary prediction of the basic reproduction number of the Wuhan novel coronavirus 2019-nCoV. J Evid Based Med 13:3–7. https://doi.org/10.1111/jebm.12376.
85. Wang L, Su S, Bi Y, Wong G, Gao GF. 2018. Bat-origin coronaviruses expand their host range to pigs. Trends Microbiol 26:466–470. https://doi.org/10.1016/j.tim.2018.03.001.
86. Benvenuto D, Giovanetti M, Salemi M, Prosperi M, De Flora C, Jr, Alcantara LC, Angeletti S, Ciccozzi M. 2020. The global spread of 2019-nCoV: a molecular evolutionary analysis. Pathog Glob Health 114:64–67. https://doi.org/10.1080/20477724.2020.1725339.
87. Wan Y, Shang J, Graham R, Baric RS, Li F. 2020. Receptor recognition by novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS. J Virol 94:e00127-20. https://doi.org/10.1128/JVI.00127-20.
88. Biscayart C, Angeleri P, Lloveras S, Chaves T, Schlagenhauf P, Rodríguez-Morales AJ. 2020. The next big threat to global health? 2019 novel coronavirus (2019-nCoV): what advice can we give to travellers? Interim recommendations January 2020, from the Latin-American Society for Travel Medicine (SLAMVI). Travel Med Infect Dis 33:101567. https://doi.org/10.1016/j.tmaid.2020.101567.
89. Rodriguez-Morales AJ, MacGregor K, Kanagarajah S, Patel D, Schlagenhauf P. 2020. Going global—travel and the 2019 novel coronavirus. Travel Med Infect Dis 33:101578. https://doi.org/10.1016/j.tmaid.2020.101578.
90. Peiris JS, Guan Y, Yuen KY. 2004. Severe acute respiratory syndrome. Nat Med 10:S88–S97. https://doi.org/10.1038/nm1143.
91. Hui DS, Azhar EI, Kim YJ, Memish ZA, Oh MD, Zumla A. 2018. Middle East respiratory syndrome coronavirus: risk factors and determinants of primary, household, and nosocomial transmission. Lancet Infect Dis 18:e217–e227. https://doi.org/10.1016/S1473-3099(18)30127-0.
92. Kritas SK, Ronconi G, Caraffa A, Gallenga CE, Ross R, Conti P. 4 February 2020. Mast cells contribute to coronavirus-induced inflammation: new anti-inflammatory strategy. J Biol Regul Homeost Agents https://doi.org/10.23812/20-Editorial-Kritas.
93. WHO. 2003. Consensus document on the epidemiology of severe acute respiratory syndrome (SARS). https://www.who.int/csr/sars/en/WHOconsensus.pdf. Accessed 29 January 2020.
94. Donnelly CA, Ghani AC, Leung GM, Hedley AJ, Fraser C, Riley S, Abu-Raddad LJ, Ho LM, Thach TQ, Chau P, Chan KP, Lam TH, Tse LY, Tsang T, Liu SH, Kong JH, Lau EM, Ferguson NM, Anderson RM. 2003. Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in Hong Kong. Lancet 361:1761–1766. https://doi.org/10.1016/S0140-6736(03)13410-1.
95. Millan-Oñate J, Rodríguez-Morales AJ, Camacho-Moreno G, Mendoza-Ramírez H, Rodríguez-Sabogal IA, Álvarez-Moreno C. 2020. A new emerging zoonotic virus of concern: the 2019 novel coronavirus (COVID-19). Infect 24:187. https://doi.org/10.22354/in.v24i3.848.
96. Wilder-Smith A, Freedman DO. 2020. Isolation, quarantine, social distancing and community containment: pivotal role for old-style public

health measures in the novel coronavirus (2019-nCoV) outbreak. J Travel Med 27:taaa020. https://doi.org/10.1093/jtm/taaa020.
97. WHO. 2019. Middle East respiratory syndrome coronavirus (MERS-CoV). https://www.who.int/en/news-room/fact-sheets/detail/middle-east-respiratory-syndromecoronavirus-(mers-cov). Accessed 29 January 2020.
98. Jiang X, Rayner S, Luo MH. 2020. Does SARS-CoV-2 has a longer incubation period than SARS and MERS? J Med Virol 92:476–478. https://doi.org/10.1002/jmv.25708.
99. Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E. 2020. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral Res 176:104742. https://doi.org/10.1016/j.antiviral.2020.104742.
100. Angeletti S, Benvenuto D, Bianchi M, Giovanetti M, Pascarella S, Ciccozzi M. 2020. COVID-2019: the role of the nsp2 and nsp3 in its pathogenesis. J Med Virol 92:584–588. https://doi.org/10.1002/jmv.25719.
101. Mahase E. 2020. Coronavirus Covid-19 has killed more people than SARS and MERS combined, despite lower case fatality rate. BMJ 368:m641. https://doi.org/10.1136/bmj.m641.
102. Song Z, Xu Y, Bao L, Zhang L, Yu P, Qu Y, Zhu H, Zhao W, Han Y, Qin C. 2019. From SARS to MERS, thrusting coronaviruses into the spotlight. Viruses 11:59. https://doi.org/10.3390/v11010059.
103. Perlman S. 2020. Another decade, another coronavirus. N Engl J Med 382:10.1056/NEJMe2001126-762. https://doi.org/10.1056/NEJMe2001126.
104. Graham RL, Donaldson EF, Baric RS. 2013. A decade after SARS: strategies for controlling emerging coronaviruses. Nat Rev Microbiol 11:836–848. https://doi.org/10.1038/nrmicro3143.
105. Menachery VD, Yount BL, Jr, Debbink K, Agnihothram S, Gralinski LE, Plante JA, Graham RL, Scobey T, Ge XY, Donaldson EF, Randell SH, Lanzavecchia A, Marasco WA, Shi ZL, Baric RS. 2015. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med 21:1508–1513. https://doi.org/10.1038/nm.3985.
106. Cui J, Li F, Shi ZL. 2019. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 17:181–192. https://doi.org/10.1038/s41579-018-0118-9.
107. Lau SKP, Luk HKH, Wong ACP, Fan RYY, Lam CSF, Li KSM, Ahmed SS, Chow FWN, Cai JP, Zhu X, Chan JFW, Lau TCK, Cao K, Li M, Woo PCY, Yuen KY. 2019. Identification of a novel betacoronavirus (Merbecovirus) in amur hedgehogs from China. Viruses 11:980. https://doi.org/10.3390/v11110980.
108. WHO. 2018. WHO MERS global summary and assessment of risk, August 2018. https://www.who.int/csr/disease/coronavirus_infections/risk-assessment-august-2018.pdf. Accessed 29 January 2020.
109. Vergara-Alert J, van den Brand JM, Widagdo W, Muñoz M, V, Raj S, Schipper D, Solanes D, Cordón I, Bensaid A, Haagmans BL, Segalés J. 2017. Livestock susceptibility to infection with Middle East respiratory syndrome coronavirus. Emerg Infect Dis 23:232–240. https://doi.org/10.3201/eid2302.161239.
110. Wu YC, Chen CS, Chan YJ. 2020. Overview of the 2019 novel coronavirus (2019-nCoV): the pathogen of severe specific contagious pneumonia (SSCP). J Chin Med Assoc 83:217–220. https://doi.org/10.1097/JCMA.0000000000000270.
111. Tian HY. 2020. 2019-nCoV: new challenges from coronavirus. Zhonghua Yu Fang Yi Xue Za Zhi 54:E001. https://doi.org/10.3760/cma.j.issn.0253-9624.2020.0001.
112. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, Tsoi HW, Lo SK, Chan KH, Poon VK, Chan WM, Ip JD, Cai JP, Cheng VC, Chen H, Hui CK, Yuen KY. 2020. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 395:514–523. https://doi.org/10.1016/S0140-6736(20)30154-9.
113. Backer JA, Klinkenberg D, Wallinga J. 2020. Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20–28 January 2020. Euro Surveill 25:2000062. https://doi.org/10.2807/1560-7917.ES.2020.25.5.2000062.
114. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KSM, Lau EHY, Wong JY, Xing X, Xiang N, Wu Y, Li C, Chen Q, Li D, Liu T, Zhao J, Li M, Tu W, Chen C, Jin L, Yang R, Wang Q, Zhou S, Wang R, Liu H, Luo Y, Liu Y, Shao G, Li H, Tao Z, Yang Y, Deng Z, Liu B, Ma Z, Zhang Y, Shi G, Lam TTY, Wu JTK, Gao GF, Cowling BJ, Yang B, Leung GM, Feng Z. 2020. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med 382:1199–1207. https://doi.org/10.1056/NEJMoa2001316.
115. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, Al-Rabiah FA, Al-Hajjar S, Al-Barrak A, Flemban H, Al-Nassir WN, Balkhy HH, Al-Hakeem RF, Makhdoom HQ, Zumla AI, Memish ZA. 2013. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. Lancet Infect Dis 13:752–761. https://doi.org/10.1016/S1473-3099(13)70204-4.
116. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395:497–506. https://doi.org/10.1016/S0140-6736(20)30183-5.
117. Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Ramirez-Ocampo S, Paniz-Mondolfi A, Rabaan AA, Sah R, Rodriguez-Morales AJ. 2020. SARS-CoV, MERS-CoV and now the 2019-novel CoV: have we investigated enough about coronaviruses? A bibliometric analysis. Travel Med Infect Dis 33:101566. https://doi.org/10.1016/j.tmaid.2020.101566.
118. Zhou L, Liu HG. 2020. Early detection and disease assessment of patients with novel coronavirus pneumonia. Zhonghua Jie He He Hu Xi Za Zhi 43:E003. https://doi.org/10.3760/cma.j.issn.1001-0939.2020.0003.
119. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, Wang F-S. 2020. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 8:420–422. https://doi.org/10.1016/S2213-2600(20)30076-X.
120. Schwartz DA, Graham AL. 2020. Potential maternal and infant outcomes from (Wuhan) coronavirus 2019-nCoV infecting pregnant women: lessons from SARS, MERS, and other human coronavirus infections. Viruses 12:194. https://doi.org/10.3390/v12020194.
121. Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, Wang Z, Li J, Li J, Feng C, Zhang Z, Wang L, Peng L, Chen L, Qin Y, Zhao D, Tan S, Yin L, Xu J, Zhou C, Jiang C, Liu L. 2020. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. 63:364–374. Sci China Life Sci https://doi.org/10.1007/s11427-020-1643-8.
122. Qiu HB, Li XY, Du B, Kang HYJ, Wang YS, Wang F, Sun B, Tong ZH. 2020. The keypoints in treatment of the critical novel coronavirus pneumonia patient. Zhonghua Jie He He Hu Xi Za Zhi 43:E022. https://doi.org/10.3760/cma.j.cn112147-20200222-00151.
123. Guan GW, Gao L, Wang JW, Wen XJ, Mao TH, Peng SW, Zhang T, Chen XM, Lu FM. 2020. Exploring the mechanism of liver enzyme abnormalities in patients with novel coronavirus-infected pneumonia. Zhonghua Gan Zang Bing Za Zhi 28:E002. https://doi.org/10.3760/cma.j.issn.1007-3418.2020.02.002.
124. MacLachlan NJ, Dubovi EJ. 2017. Fenner's veterinary virology, 5th ed. Academic Press, New York, NY.
125. Gouilh M, Puechmaille SJ, Diancourt L, Vandenbogaert M, Serra-Cobo J, Lopez Roïg M, Brown P, Moutou F, Caro V, Vabret A, Manuguerra J-C, EPICOREM Consortium. 2018. SARS-CoV related Betacoronavirus and diverse Alphacoronavirus members found in western old-world. Virology 517:88–97. https://doi.org/10.1016/j.virol.2018.01.014.
126. Suzuki T, Otake Y, Uchimoto S, Hasebe A, Goto Y. 2020. Genomic characterization and phylogenetic classification of bovine coronaviruses through whole genome sequence analysis. Viruses 12:183. https://doi.org/10.3390/v12020183.
127. Zhang XM, Herbst W, Kousoulas KG, Storz J. 1994. Biological and genetic characterization of a hemagglutinating coronavirus isolated from a diarrhoeic child. J Med Virol 44:152–161. https://doi.org/10.1002/jmv.1890440207.
128. Tekes G, Thiel HJ. 2016. Feline coronaviruses: pathogenesis of feline infectious peritonitis. Adv Virus Res 96:193–218. https://doi.org/10.1016/bs.aivir.2016.08.002.
129. Licitra BN, Duhamel GE, Whittaker GR. 2014. Canine enteric coronaviruses: emerging viral pathogens with distinct recombinant spike proteins. Viruses 6:3363–3376. https://doi.org/10.3390/v6083363.
130. Erles K, Brownlie J. 2008. Canine respiratory coronavirus: an emerging pathogen in the canine infectious respiratory disease complex. Vet Clin North Am Small Anim Pract 38:815–825. https://doi.org/10.1016/j.cvsm.2008.02.008.
131. Dhama K, Singh SD, Barathidasan R, Desingu PA, Chakraborty S, Tiwari R, Kumar MA. 2014. Emergence of avian infectious bronchitis virus and its variants need better diagnosis, prevention and control strategies: a

global perspective. Pak J Biol Sci 17:751–767. https://doi.org/10.3923/pjbs.2014.751.767.
132. Bande F, Arshad SS, Omar AR, Bejo MH, Abubakar MS, Abba Y. 2016. Pathogenesis and diagnostic approaches of avian infectious bronchitis. Adv Virol 2016:4621659. https://doi.org/10.1155/2016/4621659.
133. Wege H, Siddell S, ter Meulen V. 1982. The biology and pathogenesis of coronaviruses. Curr Top Microbiol Immunol 99:165–200. https://doi.org/10.1007/978-3-642-68528-6_5.
134. Zhou P, Fan H, Lan T, Yang XL, Shi WF, Zhang W, Zhu Y, Zhang YW, Xie QM, Mani S, Zheng XS, Li B, Li JM, Guo H, Pei GQ, An XP, Chen JW, Zhou L, Mai KJ, Wu ZX, Li D, Anderson DE, Zhang LB, Li SY, Mi ZQ, He TT, Cong F, Guo PJ, Huang R, Luo Y, Liu XL, Chen J, Huang Y, Sun Q, Zhang XL, Wang YY, Xing SZ, Chen YS, Sun Y, Li J, Daszak P, Wang LF, Shi ZL, Tong YG, Ma JY. 2018. Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. Nature 556:255–258. https://doi.org/10.1038/s41586-018-0010-9.
135. Lau SK, Woo PC, Li KS, Huang Y, Wang M, Lam CS, Xu H, Guo R, Chan KH, Zheng BJ, Yuen KY. 2007. Complete genome sequence of bat coronavirus HKU2 from Chinese horseshoe bats revealed a much smaller spike gene with a different evolutionary lineage from the rest of the genome. Virology 367:428–439. https://doi.org/10.1016/j.virol.2007.06.009.
136. Brown IH. 2001. The pig as an intermediate host for influenza A viruses between birds and humans. Int Congr Ser 1219:173–178. https://doi.org/10.1016/S0531-5131(01)00666-5.
137. Chen W, Yan M, Yang L, Ding B, He B, Wang Y, Liu X, Liu C, Zhu H, You B, Huang S, Zhang J, Mu F, Xiang Z, Feng X, Wen J, Fang J, Yu J, Yang H, Wang J. 2005. SARS-associated coronavirus transmitted from human to pig. Emerg Infect Dis 11:446–448. https://doi.org/10.3201/eid1103.040824.
138. Mihindukulasuriya KA, Wu G, St Leger J, Nordhausen RW, Wang D. 2008. Identification of a novel coronavirus from a beluga whale by using a panviral microarray. J Virol 82:5084–5088. https://doi.org/10.1128/JVI.02722-07.
139. Wassenaar TM, Zou Y. 14 February 2020. 2019_nCoV: rapid classification of betacoronaviruses and identification of traditional Chinese medicine as potential origin of zoonotic coronaviruses. Lett Appl Microbiol 70:342–348. https://doi.org/10.1111/lam.13285.
140. Phan T. 2020. Novel coronavirus: from discovery to clinical diagnostics. Infect Genet Evol 79:104211. https://doi.org/10.1016/j.meegid.2020.104211.
141. Li HY, Zhu GJ, Zhang YZ, Zhang LB, Hagan EA, Martinez S, Chmura AA, Francisco L, Tai H, Miller M, Daszak P. 2020. A qualitative study of zoonotic risk factors among rural communities in southern China. Int Health 12:77–85. https://doi.org/10.1093/inthealth/ihaa001.
142. Bonilla-Aldana DK, Suárez JA, Franco-Paredes C, Vilcarromero S, Mattar S, Gómez-Marín JE, Villamil-Gómez WE, Ruíz-Sáenz J, Cardona-Ospina JA, Idarraga-Bedoya SE, García-Bustos JJ, Jimenez-Posada EV, Rodríguez-Morales AJ. 2019. Brazil burning! What is the potential impact of the Amazon wildfires on vector-borne and zoonotic emerging diseases? A statement from an international experts meeting. Travel Med Infect Dis 31:101474. https://doi.org/10.1016/j.tmaid.2019.101474.
143. Ji W, Wang W, Zhao X, Zai J, Li X. 2020. Homologous recombination within the spike glycoprotein of the newly identified coronavirus may boost cross-species transmission from snake to human. J Med Virol 92:433–440. https://doi.org/10.1002/jmv.25682.
144. Liu P, Chen W, Chen JP. 2019. Viral metagenomics revealed Sendai virus and coronavirus infection of Malayan pangolins (Manisjavanica). Viruses 11:979. https://doi.org/10.3390/v11110979.
145. Xiao K, Zhai J, Feng Y, Zhou N, Zhang X, Zou JJ, Li N, Guo Y, Li X, Shen X, Zhang Z, Shu F, Huang W, Li Y, Zhang Z, Chen R-A, Wu Y-J, Peng S-M, Huang M, Xie W-J, Cai Q-H, Hou F-H, Liu Y, Chen W, Xiao L, Shen Y. 2020. Isolation and characterization of 2019-nCoV-like coronavirus from Malayan pangolins. bioRxiv https://doi.org/10.1101/2020.02.17.951335.
146. Daszak P, Olival KJ, Li H. 2020. A strategy to prevent future epidemics similar to the 2019-nCoV outbreak. Biosafety Health 2:6–8. https://doi.org/10.1016/j.bsheal.2020.01.003.
147. Wang N, Li SY, Yang XL, Huang HM, Zhang YJ, Guo H, Luo CM, Miller M, Zhu G, Chmura AA, Hagan E, Zhou JH, Zhang YZ, Wang LF, Daszak P, Shi ZL. 2018. Serological evidence of bat SARS-related coronavirus infection in humans, China. Virol Sin 33:104–107. https://doi.org/10.1007/s12250-018-0012-7.
148. Gao ZC. 2020. Efficient management of novel coronavirus pneumonia by efficient prevention and control in scientific manner. Zhonghua Jie He He Hu Xi Za Zhi 43:E001. https://doi.org/10.3760/cma.j.issn.1001-0939.2020.0001.
149. Xiao SY, Wu Y, Liu H. 2020. Evolving status of the 2019 novel coronavirus infection: proposal of conventional serologic assays for disease diagnosis and infection monitoring. J Med Virol 92:464–467. https://doi.org/10.1002/jmv.25702.
150. Yu F, Du L, Ojcius DM, Pan C, Jiang S. 2020. Measures for diagnosing and treating infections by a novel coronavirus responsible for a pneumonia outbreak originating in Wuhan, China. Microbes Infect 22:74–79. https://doi.org/10.1016/j.micinf.2020.01.003.
151. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. 2020. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis 20:411–412. https://doi.org/10.1016/S1473-3099(20)30113-4.
152. To KK, Tsang OT, Chik-Yan Yip C, Chan KH, Wu TC, Chan JMC, Leung WS, Chik TS, Choi CY, Kandamby DH, Lung DC, Tam AR, Poon RW, Fung AY, Hung IF, Cheng VC, Chan JF, Yuen KY. 12 February 2020. Consistent detection of 2019 novel coronavirus in saliva. Clin Infect Dis https://doi.org/10.1093/cid/ciaa149.
153. Zhang W, Du RH, Li B, Zheng XS, Yang XL, Hu B, Wang YY, Xiao GF, Yan B, Shi ZL, Zhou P. 2020. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect 9:386–389. https://doi.org/10.1080/22221751.2020.1729071.
154. Xie X, Zhong Z, Zhao W, Zheng C, Wang F, Liu J. 12 February 2020. Chest CT for typical 2019-nCoV pneumonia: relationship to negative RT-PCR testing. Radiology https://doi.org/10.1148/radiol.2020200343.
155. Binnicker MJ. 2020. Emergence of a novel coronavirus disease (COVID-19) and the importance of diagnostic testing: why partnership between clinical laboratories, public health agencies, and industry is essential to control the outbreak. Clin Chem 66:664–666. https://doi.org/10.1093/clinchem/hvaa071.
156. Chu DKW, Pan Y, Cheng SMS, Hui KPY, Krishnan P, Liu Y, Ng DYM, Wan CKC, Yang P, Wang Q, Peiris M, Poon L. 2020. Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. Clin Chem 66:549–555. https://doi.org/10.1093/clinchem/hvaa029.
157. Cohen J, Kupferschmidt K. 2020. Labs scramble to produce new coronavirus diagnostics. Science 367:727. https://doi.org/10.1126/science.367.6479.727.
158. Zhang J, Zhou L, Yang Y, Peng W, Wang W, Chen X. 2020. Therapeutic and triage strategies for 2019 novel coronavirus disease in fever clinics. Lancet Respir Med 8:e11–e12. https://doi.org/10.1016/S2213-2600(20)30071-0.
159. Shi H, Han X, Jiang N, Cao Y, Alwalid O, Gu J, Fan Y, Zheng C. 2020. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. Lancet Infect Dis 20:425–434. https://doi.org/10.1016/S1473-3099(20)30086-4.
160. Kim H. 2020. Outbreak of novel coronavirus (COVID-19): what is the role of radiologists? Eur Radiol 30:3266–3267. https://doi.org/10.1007/s00330-020-06748-2.
161. Munjal A, Khandia R, Dhama K, Sachan S, Karthik K, Tiwari R, Malik YS, Kumar D, Singh RK, Iqbal HMN, Joshi SK. 2017. Advances in developing therapies to combat Zika virus: current knowledge and future perspectives. Front Microbiol 8:1469. https://doi.org/10.3389/fmicb.2017.01469.
162. Dhama K, Karthik K, Khandia R, Chakraborty S, Munjal A, Latheef SK, Kumar D, Ramakrishnan MA, Malik YS, Singh R, Malik SVS, Singh RK, Chaicumpa W. 2018. Advances in designing and developing vaccines, drugs, and therapies to counter Ebola virus. Front Immunol 9:1803. https://doi.org/10.3389/fimmu.2018.01803.
163. Singh RK, Dhama K, Chakraborty S, Tiwari R, Natesan S, Khandia R, Munjal A, Vora KS, Latheef SK, Karthik K, Singh Malik Y, Singh R, Chaicumpa W, Mourya DT. 2019. Nipah virus: epidemiology, pathology, immunobiology and advances in diagnosis, vaccine designing and control strategies—a comprehensive review. Vet Q 39:26–55. https://doi.org/10.1080/01652176.2019.1580827.
164. Yang ZY, Kong WP, Huang Y, Roberts A, Murphy BR, Subbarao K, Nabel GJ. 2004. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. Nature 428:561–564. https://doi.org/10.1038/nature02463.
165. Chu CM, Cheng VC, Hung IF, Wong MM, Chan KH, Chan KS, Kao RY, Poon LL, Wong CL, Guan Y, Peiris JS, Yuen KY, HKU/UCH SARS Study Group. 2004. Role of lopinavir/ritonavir in the treatment of SARS: initial

virological and clinical findings. Thorax 59:252–256. https://doi.org/10.1136/thorax.2003.012658.
166. Kumar V, Jung Y-S, Liang P-H. 2013. Anti-SARS coronavirus agents: a patent review (2008–present). Expert Opin Ther Pat 23:1337–1348. https://doi.org/10.1517/13543776.2013.823159.
167. Li E, Yan F, Huang P, Chi H, Xu S, Li G, Liu C, Feng N, Wang H, Zhao Y, Yang S, Xia X. 2020. Characterization of the immune response of MERS-CoV vaccine candidates derived from two different vectors in mice. Viruses 12:125. https://doi.org/10.3390/v12010125.
168. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. 2009. The spike protein of SARS-CoV—a target for vaccine and therapeutic development. Nat Rev Microbiol 7:226–236. https://doi.org/10.1038/nrmicro2090.
169. Wirblich C, Coleman CM, Kurup D, Abraham TS, Bernbaum JG, Jahrling PB, Hensley LE, Johnson RF, Frieman MB, Schnell MJ. 2017. One-health: a safe, efficient, dual-use vaccine for humans and animals against Middle East Respiratory syndrome coronavirus and rabies virus. J Virol 91:e02040-16. https://doi.org/10.1128/JVI.02040-16.
170. Buchholz UJ, Bukreyev A, Yang L, Lamirande EW, Murphy BR, Subbarao K, Collins PL. 2004. Contributions of the structural proteins of severe acute respiratory syndrome coronavirus to protective immunity. Proc Natl Acad Sci U S A 101:9804–9809. https://doi.org/10.1073/pnas.0403492101.
171. Chen R, Fu J, Hu J, Li C, Zhao Y, Qu H, Wen X, Cao S, Wen Y, Wu R, Zhao Q, Yan Q, Huang Y, Ma X, Han X, Huang X. 2020. Identification of the immunodominant neutralizing regions in the spike glycoprotein of porcine deltacoronavirus. Virus Res 276:197834. https://doi.org/10.1016/j.virusres.2019.197834.
172. Liu WJ, Zhao M, Liu K, Xu K, Wong G, Tan W, Gao GF. 2017. T-cell immunity of SARS-CoV: implications for vaccine development against MERS-CoV. Antiviral Res 137:82–92. https://doi.org/10.1016/j.antiviral.2016.11.006.
173. Jiang S, Du L, Shi Z. 2020. An emerging coronavirus causing pneumonia outbreak in Wuhan, China: calling for developing therapeutic and prophylactic strategies. Emerg Microbes Infect 9:275–277. https://doi.org/10.1080/22221751.2020.1723441.
174. Veljkovic V, Vergara-Alert J, Segalés J, Paessler S. 2020. Use of the informational spectrum methodology for rapid biological analysis of the novel coronavirus 2019-nCoV: prediction of potential receptor, natural reservoir, tropism and therapeutic/vaccine target. F1000Res 9:52. https://doi.org/10.12688/f1000research.22149.3.
175. Jiang S, He Y, Liu S. 2005. SARS vaccine development. Emerg Infect Dis 11:1016–1020. https://doi.org/10.3201/1107.050219.
176. Baruah V, Bose S. 2020. Immunoinformatics-aided identification of T cell and B cell epitopes in the surface glycoprotein of 2019-nCoV. J Med Virol 92:495–500. https://doi.org/10.1002/jmv.25698.
177. Kim MH, Kim HJ, Chang J. 2019. Superior immune responses induced by intranasal immunization with recombinant adenovirus-based vaccine expressing full-length spike protein of Middle East respiratory syndrome coronavirus. PLoS One 14:e0220196. https://doi.org/10.1371/journal.pone.0220196.
178. Shi J, Zhang J, Li S, Sun J, Teng Y, Wu M, Li J, Li Y, Hu N, Wang H, Hu Y. 2015. Epitope-based vaccine target screening against highly pathogenic MERS-CoV: an in silico approach applied to emerging infectious diseases. PLoS One 10:e0144475. https://doi.org/10.1371/journal.pone.0144475.
179. Xie Q, He X, Yang F, Liu X, Li Y, Liu Y, Yang Z, Yu J, Zhang B, Zhao W. 2018. Analysis of the genome sequence and prediction of B-cell epitopes of the envelope protein of Middle East respiratory syndrome-coronavirus. IEEE/ACM Trans Comput Biol Bioinform 15:1344–1350. https://doi.org/10.1109/TCBB.2017.2702588.
180. NIAID. 2020. Developing therapeutics and vaccines for coronaviruses. https://www.niaid.nih.gov/diseases-conditions/coronaviruses-therapeutics-vaccines. Accessed 15 February 2020.
181. CEPI. 2020. CEPI to fund three programmes to develop vaccines against the novel coronavirus, nCoV-2019. https://cepi.net/news_cepi/cepi-to-fund-three-programmes-to-develop-vaccines-against-the-novel-coronavirus-ncov-2019/. Accessed 15 February 2020.
182. Moderna. 2020. Moderna announces funding award from CEPI to accelerate development of messenger RNA (mRNA) vaccine against novel coronavirus. https://investors.modernatx.com/news-releases/news-release-details/moderna-announces-funding-award-cepi-accelerate-development. Accessed 15 February 2020.
183. Adedeji AO, Severson W, Jonsson C, Singh K, Weiss SR, Sarafianos SG. 2013. Novel inhibitors of severe acute respiratory syndrome coronavirus entry that act by three distinct mechanisms. J Virol 87:8017–8028. https://doi.org/10.1128/JVI.00998-13.
184. Falzarano D, de Wit E, Rasmussen AL, Feldmann F, Okumura A, Scott DP, Brining D, Bushmaker T, Martellaro C, Baseler L, Benecke AG, Katze MG, Munster VJ, Feldmann H. 2013. Treatment with interferon-α2b and ribavirin improves outcome in MERS-CoV-infected rhesus macaques. Nat Med 19:1313–1317. https://doi.org/10.1038/nm.3362.
185. Lu L, Liu Q, Du L, Jiang S. 2013. Middle East respiratory syndrome coronavirus (MERS-CoV): challenges in identifying its source and controlling its spread. Microbes Infect 15:625–629. https://doi.org/10.1016/j.micinf.2013.06.003.
186. Dhama K, Sharun K, Tiwari R, Dadar M, Malik YS, Singh KP, Chaicumpa W. 18 March 2020. COVID-19, an emerging coronavirus infection: advances and prospects in designing and developing vaccines, immunotherapeutics, and therapeutics. Hum Vaccin Immunother https://doi.org/10.1080/21645515.2020.1735227.
187. Li H, Wang YM, Xu JY, Cao B. 2020. Potential antiviral therapeutics for 2019 novel coronavirus. Zhonghua Jie He He Hu Xi Za Zhi 43:E002. https://doi.org/10.3760/cma.j.issn.1001-0939.2020.0002.
188. Wang C, Horby PW, Hayden FG, Gao GF. 2020. A novel coronavirus outbreak of global health concern. Lancet 395:470–473. https://doi.org/10.1016/S0140-6736(20)30185-9.
189. Zumla A, Chan JF, Azhar EI, Hui DS, Yuen KY. 2016. Coronaviruses—drug discovery and therapeutic options. Nat Rev Drug Discov 15:327–347. https://doi.org/10.1038/nrd.2015.37.
190. Chan JF, Yao Y, Yeung ML, Deng W, Bao L, Jia L, Li F, Xiao C, Gao H, Yu P, Cai JP, Chu H, Zhou J, Chen H, Qin C, Yuen KY. 2015. Treatment with lopinavir/ritonavir or interferon-β1b improves outcome of MERS-CoV infection in a nonhuman primate model of common Marmoset. J Infect Dis 212:1904–1913. https://doi.org/10.1093/infdis/jiv392.
191. Arabi YM, Alothman A, Balkhy HH, Al-Dawood A, AlJohani S, Al Harbi S, Kojan S, Al Jeraisy M, Deeb AM, Assiri AM, Al-Hameed F, AlSaedi A, Mandourah Y, Almekhlafi GA, Sherbeeni NM, Elzein FE, Memon J, Taha Y, Almotairi A, Maghrabi KA, Qushmaq I, Al Bshabshe A, Kharaba A, Shalhoub S, Jose J, Fowler RA, Hayden FG, Hussein MA, MIRACLE Trial Group. 2018. Treatment of Middle East respiratory syndrome with a combination of lopinavir-ritonavir and interferon-β1b (MIRACLE trial): study protocol for a randomized controlled trial. Trials 19:81. https://doi.org/10.1186/s13063-017-2427-0.
192. WHO. 2020. Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected. Available on https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected. Accessed on 7 February 2020.
193. Wang Z, Chen X, Lu Y, Chen F, Zhang W. 2020. Clinical characteristics and therapeutic procedure for four cases with 2019 novel coronavirus pneumonia receiving combined Chinese and Western medicine treatment. Biosci Trends 14:64–68. https://doi.org/10.5582/bst.2020.01030.
194. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, Shi Z, Hu Z, Zhong W, Xiao G. 2020. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 30:269–271. https://doi.org/10.1038/s41422-020-0282-0.
195. Sheahan TP, Sims AC, Graham RL, Menachery VD, Gralinski LE, Case JB, Leist SR, Pyrc K, Feng JY, Trantcheva I, Bannister R, Park Y, Babusis D, Clarke MO, Mackman RL, Spahn JE, Palmiotti CA, Siegel D, Ray AS, Cihlar T, Jordan R, Denison MR, Baric RS. 2017. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med 9:eaal3653. https://doi.org/10.1126/scitranslmed.aal3653.
196. Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, Seidah NG, Nichol ST. 2005. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J 2:69. https://doi.org/10.1186/1743-422X-2-69.
197. Gao J, Tian Z, Yang X. 2020. Breakthrough: chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends 14:72–73. https://doi.org/10.5582/bst.2020.01047.
198. Widjaja I, Wang C, van Haperen R, Gutiérrez-Álvarez J, van Dieren B, Okba NMA, Raj VS, Li W, Fernandez-Delgado R, Grosveld F, van Kuppeveld FJM, Haagmans BL, Enjuanes L, Drabek D, Bosch BJ. 2019. Towards a solution to MERS: protective human monoclonal antibodies targeting different domains and functions of the MERS-coronavirus spike glycoprotein. Emerg Microbes Infect 8:516–530. https://doi.org/10.1080/22221751.2019.1597644.
199. Goo J, Jeong Y, Park Y-S, Yang E, Jung D-I, Rho S, Park U, Sung H, Park

P-G, Choi J-A, Seo SH, Cho NH, Lee H, Lee JM, Kim J-O, Song M. 2020. Characterization of novel monoclonal antibodies against MERS-coronavirus spike protein. Virus Res 278:197863. https://doi.org/10.1016/j.virusres.2020.197863.
200. Zeng LP, Ge XY, Peng C, Tai W, Jiang S, Du L, Shi ZL. 2017. Cross-neutralization of SARS coronavirus-specific antibodies against bat SARS-like coronaviruses. Sci China Life Sci 60:1399–1402. https://doi.org/10.1007/s11427-017-9189-3.
201. Cohen J. 2020. New coronavirus threat galvanizes scientists. Science 367:492–493. https://doi.org/10.1126/science.367.6477.492.
202. Tian X, Li C, Huang A, Xia S, Lu S, Shi Z, Lu L, Jiang S, Yang Z, Wu Y, Ying T. 2020. Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. Emerg Microbes Infect 9:382–385. https://doi.org/10.1080/22221751.2020.1729069.
203. Kilianski A, Baker SC. 2014. Cell-based antiviral screening against coronaviruses: developing virus-specific and broad-spectrum inhibitors. Antiviral Res 101:105–112. https://doi.org/10.1016/j.antiviral.2013.11.004.
204. Zhao J, Perera RA, Kayali G, Meyerholz D, Perlman S, Peiris M. 2015. Passive immunotherapy with dromedary immune serum in an experimental animal model for Middle East respiratory syndrome coronavirus infection. J Virol 89:6117–6120. https://doi.org/10.1128/JVI.00446-15.
205. Zhang L, Liu Y. 2020. Potential interventions for novel coronavirus in China: a systemic review. J Med Virol 92:479–490. https://doi.org/10.1002/jmv.25707.
206. Seesuay W, Jittavisutthikul S, Sae-Lim N, Sookrung N, Sakolvaree Y, Chaicumpa W. 2018. Human transbodies that interfere with the functions of Ebola virus VP35 protein in genome replication and transcription and innate immune antagonism. Emerg Microbes Infect 7:41. https://doi.org/10.1038/s41426-018-0031-3.
207. Sun ML, Yang JM, Sun YP, Su GH. 2020. Inhibitors of RAS might be a good choice for the therapy of COVID-19 pneumonia. Zhonghua Jie He He Hu Xi Za Zhi 43:E014. https://doi.org/10.3760/cma.j.issn.1001-0939.2020.0014.
208. de Wilde AH, Jochmans D, Posthuma CC, Zevenhoven-Dobbe JC, van Nieuwkoop S, Bestebroer TM, van den Hoogen BG, Neyts J, Snijder EJ. 2014. Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. Antimicrob Agents Chemother 58:4875–4884. https://doi.org/10.1128/AAC.03011-14.
209. Liu J, Zheng X, Tong Q, Li W, Wang B, Sutter K, Trilling M, Lu M, Dittmer U, Yang D. 2020. Overlapping and discrete aspects of the pathology and pathogenesis of the emerging human pathogenic coronaviruses SARS-CoV, MERS-CoV, and 2019-nCoV. J Med Virol 92:491–494. https://doi.org/10.1002/jmv.25709.
210. Xia S, Zhu Y, Liu M, Lan Q, Xu W, Wu Y, Ying T, Liu S, Shi Z, Jiang S, Lu L. 11 February 2020. Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein. Cell Mol Immunol https://doi.org/10.1038/s41423-020-0374-2.
211. Gretebeck LM, Subbarao K. 2015. Animal models for SARS and MERS coronaviruses. Curr Opin Virol 13:123–129. https://doi.org/10.1016/j.coviro.2015.06.009.
212. Yong CY, Ong HK, Yeap SK, Ho KL, Tan WS. 2019. Recent advances in the vaccine development against Middle East respiratory syndrome-coronavirus. Front Microbiol 10:1781. https://doi.org/10.3389/fmicb.2019.01781.
213. Martina BE, Haagmans BL, Kuiken T, Fouchier RA, Rimmelzwaan GF, Van Amerongen G, Peiris JS, Lim W, Osterhaus AD. 2003. Virology: SARS virus infection of cats and ferrets. Nature 425:915. https://doi.org/10.1038/425915a.
214. Lamirande EW, DeDiego ML, Roberts A, Jackson JP, Alvarez E, Sheahan T, Shieh WJ, Zaki SR, Baric R, Enjuanes L, Subbarao K. 2008. A live attenuated severe acute respiratory syndrome coronavirus is immunogenic and efficacious in golden Syrian hamsters. J Virol 82:7721–7724. https://doi.org/10.1128/JVI.00304-08.
215. Roberts A, Lamirande EW, Vogel L, Jackson JP, Paddock CD, Guarner J, Zaki SR, Sheahan T, Baric R, Subbarao K. 2008. Animal models and vaccines for SARS-CoV infection. Virus Res 133:20–32. https://doi.org/10.1016/j.virusres.2007.03.025.
216. Falzarano D, de Wit E, Feldmann F, Rasmussen AL, Okumura A, Peng X, Thomas MJ, van Doremalen N, Haddock E, Nagy L, LaCasse R, Liu T, Zhu J, McLellan JS, Scott DP, Katze MG, Feldmann H, Munster VJ. 2014. Infection with MERS-CoV causes lethal pneumonia in the common marmoset. PLoS Pathog 10:e1004250. https://doi.org/10.1371/journal.ppat.1004250.
217. Du L, Tai W, Zhou Y, Jiang S. 2016. Vaccines for the prevention against the threat of MERS-CoV. Expert Rev Vaccines 15:1123–1134. https://doi.org/10.1586/14760584.2016.1167603.
218. Enjuanes L, Zuñiga S, Castaño-Rodriguez C, Gutierrez-Alvarez J, Canton J, Sola I. 2016. Molecular basis of coronavirus virulence and vaccine development. Adv Virus Res 96:245–286. https://doi.org/10.1016/bs.aivir.2016.08.003.
219. Yang XH, Deng W, Tong Z, Liu YX, Zhang LF, Zhu H, Gao H, Huang L, Liu YL, Ma CM, Xu YF, Ding MX, Deng HK, Qin C. 2007. Mice transgenic for human angiotensin-converting enzyme 2 provide a model for SARS coronavirus infection. Comp Med 57:450–459.
220. Leist SR, Cockrell AS. 2020. Genetically engineering a susceptible mouse model for MERS-CoV-induced acute respiratory distress syndrome. Methods Mol Biol 2099:137–159. https://doi.org/10.1007/978-1-0716-0211-9_12.
221. Zhou Y, Jiang S, Du L. 2018. Prospects for a MERS-CoV spike vaccine. Expert Rev Vaccines 17:677–686. https://doi.org/10.1080/14760584.2018.1506702.
222. Cockrell AS, Yount BL, Scobey T, Jensen K, Douglas M, Beall A, Tang XC, Marasco WA, Heise MT, Baric RS. 2016. A mouse model for MERS coronavirus-induced acute respiratory distress syndrome. Nat Microbiol 2:16226. https://doi.org/10.1038/nmicrobiol.2016.226.
223. Eckerle I, Corman VM, Müller MA, Lenk M, Ulrich RG, Drosten C. 2014. Replicative capacity of MERS coronavirus in livestock cell lines. Emerg Infect Dis 20:276–279. https://doi.org/10.3201/eid2002.131182.
224. Milewska A, Nowak P, Owczarek K, Szczepanski A, Zarebski M, Hoang A, Berniak K, Wojarski J, Zeglen S, Baster Z, Rajfur Z, Pyrc K. 2017. Entry of human coronavirus NL63 into the cell. J Virol 92:e01933-17. https://doi.org/10.1128/JVI.01933-17.
225. Nkengasong J. 2020. China's response to a novel coronavirus stands in stark contrast to the 2002 SARS outbreak response. Nat Med 26:310–311. https://doi.org/10.1038/s41591-020-0771-1.
226. Khan S, Ali A, Siddique R, Nabi G. 2020. Novel coronavirus is putting the whole world on alert. J Hosp Infect 104:252–253. https://doi.org/10.1016/j.jhin.2020.01.019.
227. Sun K, Chen J, Viboud C. 2020. Early epidemiological analysis of the coronavirus disease 2019 outbreak based on crowdsourced data: a population-level observational study. Lancet Digital Health 2:e201–e208. https://doi.org/10.1016/S2589-7500(20)30026-1.
228. Tang B, Wang X, Li Q, Bragazzi NL, Tang S, Xiao Y, Wu J. 2020. Estimation of the transmission risk of the 2019-nCoV and its implication for public health interventions. J Clin Med 9:462. https://doi.org/10.3390/jcm9020462.
229. Kang L, Li Y, Hu S, Chen M, Yang C, Yang BX, Wang Y, Hu J, Lai J, Ma X, Chen J, Guan L, Wang G, Ma H, Liu Z. 2020. The mental health of medical workers in Wuhan, China dealing with the 2019 novel coronavirus. Lancet Psychiatry 7:e14. https://doi.org/10.1016/S2215-0366p(20)30047-X.
230. Guan W, Xian J. 2020. The progress of 2019 novel coronavirus (2019-nCoV) event in China. J Med Virol 92:468–472. https://doi.org/10.1002/jmv.25705.
231. Wu JT, Leung K, Leung GM. 2020. Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study. Lancet 395:689–697. https://doi.org/10.1016/S0140-6736(20)30260-9.
232. Kickbusch I, Leung G. 2020. Response to the emerging novel coronavirus outbreak. BMJ 368:m406. https://doi.org/10.1136/bmj.m406.
233. Bogoch II, Watts A, Thomas-Bachli A, Huber C, Kraemer MUG, Khan K. 2020. Potential for global spread of a novel coronavirus from China. J Travel Med 27:taaa011. https://doi.org/10.1093/jtm/taaa011.
234. Khan S, Siddique R, Ali A, Xue M, Nabi G. 2020. Novel coronavirus, poor quarantine, and the risk of pandemic. J Hosp Infect 104:449–450. https://doi.org/10.1016/j.jhin.2020.02.002.
235. Thompson RN. 2020. Novel Coronavirus outbreak in Wuhan, China, 2020: intense surveillance is vital for preventing sustained transmission in new locations. J Clin Med 9:498. https://doi.org/10.3390/jcm9020498.
236. Du Z, Wang L, Cauchemez S, Xu X, Wang X, Cowling BJ, Meyers LA. 2020. Risk for transportation of 2019 novel coronavirus disease from Wuhan to other cities in China. Emerg Infect Dis 26:1049–1052. https://doi.org/10.3201/eid2605.200146.
237. Wood C. 2020. Infections without borders: a new coronavirus in Wuhan,

China. Br J Nurs 29:166–167. https://doi.org/10.12968/bjon.2020.29.3.166.
238. Dong E, Du H, Gardner L. 2020. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 20:533–534. https://doi.org/10.1016/S1473-3099(20)30120-1.
239. Sawano T, Ozaki A, Rodriguez-Morales AJ, Tanimoto T, Sah R. 2020. Limiting spread of COVID-19 from cruise ships—lessons to be learnt from Japan. QJM 113:309–310. https://doi.org/10.1093/qjmed/hcaa092.
240. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, Li J, Zhao D, Xu D, Gong Q, Liao J, Yang H, Hou W, Zhang Y. 2020. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet 395:809–815. https://doi.org/10.1016/S0140-6736(20)30360-3.
241. Wang X, Zhou Z, Zhang J, Zhu F, Tang Y, Shen X. 28 February 2020. A case of 2019 novel coronavirus in a pregnant woman with preterm delivery. Clin Infect Dis https://doi.org/10.1093/cid/ciaa200.
242. Yu N, Li W, Kang Q, Xiong Z, Wang S, Lin X, Liu Y, Xiao J, Liu H, Deng D, Chen S, Zeng W, Feng L, Wu J. 2020. Clinical features and obstetric and neonatal outcomes of pregnant patients with COVID-19 in Wuhan, China: a retrospective, single-centre, descriptive study. Lancet Infect Dis 20:559–564. https://doi.org/10.1016/S1473-3099(20)30176-6.
243. Zambrano LI, Fuentes-Barahona IC, Bejarano-Torres DA, Bustillo C, Gonzales G, Vallecillo-Chinchilla G, Sanchez-Martínez FE, Valle-Reconco JA, Sierra M, Bonilla-Aldana DK, Cardona-Ospina JA, Rodríguez-Morales AJ. 25 March 2020. A pregnant woman with COVID-19 in Central America. Travel Med Infect Dis https://doi.org/10.1016/j.tmaid.2020.101639.
244. Rodriguez-Morales AJ, Cardona-Ospina JA, Gutiérrez-Ocampo E, Villamizar-Peña R, Holguin-Rivera Y, Escalera-Antezana JP, Alvarado-Arnez LE, Bonilla-Aldana DK, Franco-Paredes C, Henao-Martinez AF, Paniz-Mondolfi A, Lagos-Grisales GJ, Ramírez-Vallejo E, Suárez JA, Zambrano LI, Villamil-Gómez WE, Balbin-Ramon GJ, Rabaan AA, Harapan H, Dhama K, Nishiura H, Kataoka H, Ahmad T, Sah R, Latin American Network of Coronavirus Disease 2019-COVID-19 Research (LANCOVID-19). 2020. Clinical, laboratory and imaging features of COVID-19: a systematic review and meta-analysis. Travel Med Infect Dis 34:101623. https://doi.org/10.1016/j.tmaid.2020.101623.
245. Xie C, Jiang L, Huang G, Pu H, Gong B, Lin H, Ma S, Chen X, Long B, Si G, Yu H, Jiang L, Yang X, Shi Y, Yang Z. 2020. Comparison of different samples for 2019 novel coronavirus detection by nucleic acid amplification tests. Int J Infect Dis 93:264–267. https://doi.org/10.1016/j.ijid.2020.02.050.
246. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. 11 March 2020. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA https://doi.org/10.1001/jama.2020.3786.
247. Kam K-Q, Yung CF, Cui L, Lin RTP, Mak TM, Maiwald M, Li J, Chong CY, Nadua K, Tan NWH, Thoon KC. 28 February 2020. A well infant with coronavirus disease 2019 with high viral load. Clin Infect Dis https://doi.org/10.1093/cid/ciaa201.
248. Woelfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Mueller MA, Niemeyer D, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Bruenink T, Schneider J, Ehmann R, Zwirglmaier K, Drosten C, Wendtner C. 2020. Clinical presentation and virological assessment of hospitalized cases of coronavirus disease 2019 in a travel-associated transmission cluster. medRxiv https://doi.org/10.1101/2020.03.05.20030502.
249. He Y, Wang Z, Li F, Shi Y. 2020. Public health might be endangered by possible prolonged discharge of SARS-CoV-2 in stool. J Infect 80:e18–e19. https://doi.org/10.1016/j.jinf.2020.02.031.
250. Ianiro G, Mullish BH, Kelly CR, Sokol H, Kassam Z, Ng S, Fischer M, Allegretti JR, Masucci L, Zhang F, Keller J, Sanguinetti M, Costello SP, Tilg H, Gasbarrini A, Cammarota G. 2020. Screening of faecal microbiota transplant donors during the COVID-19 outbreak: suggestions for urgent updates from an international expert panel. Lancet Gastroenterol Hepatol 5:430–432. https://doi.org/10.1016/S2468-1253(20)30082-0.
251. Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H. 2020. Evidence for gastrointestinal infection of SARS-CoV-2. Gastroenterology 158:1831–1833. https://doi.org/10.1053/j.gastro.2020.02.055.
252. Yeo C, Kaushal S, Yeo D. 2020. Enteric involvement of coronaviruses: is faecal-oral transmission of SARS-CoV-2 possible? Lancet Gastroenterol Hepatol 5:335–337. https://doi.org/10.1016/S2468-1253(20)30048-0.
253. Kim JY, Ko JH, Kim Y, Kim YJ, Kim JM, Chung YS, Kim HM, Han MG, Kim SY, Chin BS. 2020. Viral load kinetics of SARS-CoV-2 infection in first two patients in Korea. J Korean Med Sci 35:e86. https://doi.org/10.3346/jkms.2020.35.e86.
254. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu N-H, Nitsche A, Müller MA, Drosten C, Pöhlmann S. 2020. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181:271–280. https://doi.org/10.1016/j.cell.2020.02.052.
255. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. 2020. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 181:281–292. https://doi.org/10.1016/j.cell.2020.02.058.
256. Su YC, Anderson DE, Young BE, Zhu F, Linster M, Kalimuddin S, Low JGH, Yan Z, Jayakumar J, Sun L, Yan GZ, Mendenhall IH, Leo Y-S, Lye DC, Wang L-F, Smith G. 2020. Discovery of a 382-nt deletion during the early evolution of SARS-CoV-2. bioRxiv https://doi.org/10.1101/2020.04.17.20069641.
257. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A, Harcourt JL, Thornburg NJ, Gerber SI, Lloyd-Smith JO, de Wit E, Munster VJ. 2020. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med 382:1564–1567. https://doi.org/10.1056/NEJMc2004973.
258. Loeffelholz MJ, Tang YW. 2020. Laboratory diagnosis of emerging human coronavirus infections—the state of the art. Emerg Microbes Infect 9:1–26. https://doi.org/10.1080/22221751.2020.1745095.
259. US Food and Drug Administration. 2020. Coronavirus disease (COVID-19) Emergency Use Authorization (EUA) information—in vitro diagnostic EUAs. https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd.
260. Wang C, Liu Z, Chen Z, Huang X, Xu M, He T, Zhang Z. 2020. The establishment of reference sequence for SARS-CoV-2 and variation analysis. J Med Virol 92:667–674. https://doi.org/10.1002/jmv.25762.
261. Colson P, Rolain JM, Lagier JC, Brouqui P, Raoult D. 2020. Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. Int J Antimicrob Agents 55:105932. https://doi.org/10.1016/j.ijantimicag.2020.105932.
262. Cortegiani A, Ingoglia G, Ippolito M, Giarratano A, Einav S. 2020. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. Crit Care 57:279–283. https://doi.org/10.1016/j.jcrc.2020.03.005.
263. Devaux CA, Rolain JM, Colson P, Raoult D. 2020. New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? Int J Antimicrob Agents 55:105938. https://doi.org/10.1016/j.ijantimicag.2020.105938.
264. Sahraei Z, Shabani M, Shokouhi S, Saffaei A. 2020. Aminoquinolines against coronavirus disease 2019 (COVID-19): chloroquine or hydroxychloroquine. Int J Antimicrob Agents 55:105945. https://doi.org/10.1016/j.ijantimicag.2020.105945.
265. Touret F, de Lamballerie X. 2020. Of chloroquine and COVID-19. Antiviral Res 177:104762. https://doi.org/10.1016/j.antiviral.2020.104762.
266. Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, Liu X, Zhao L, Dong E, Song C, Zhan S, Lu R, Li H, Tan W, Liu D. 9 March 2020. In vitro antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Clin Infect Dis https://doi.org/10.1093/cid/ciaa237.
267. Zhou D, Dai SM, Tong Q. 20 March 2020. COVID-19: a recommendation to examine the effect of hydroxychloroquine in preventing infection and progression. J Antimicrob Chemother https://doi.org/10.1093/jac/dkaa114.
268. Srinivasa A, Tosounidou S, Gordon C. 2017. Increased incidence of gastrointestinal side effects in patients taking hydroxychloroquine: a brand-related issue? J Rheumatol 44:398. https://doi.org/10.3899/jrheum.161063.
269. U.S. National Library of Medicine. 2020. Clinical trials registry–chloroquine. COVID-19. https://www.clinicaltrials.gov/ct2/results?cond=COVID-19&term=Chloroquine&cntry=&state=&city=&dist=2020.
270. Multicenter Collaboration Group of Department of Science and Technology of Guangdong Province and Health Commission of Guangdong Province for Chloroquine in the Treatment of Novel Coronavirus. 2020. Expert consensus on chloroquine phosphate for the treatment of novel coronavirus pneumonia. Zhonghua Jie He He Hu Xi Za Zhi 43:185–188. https://doi.org/10.3760/cma.j.issn.1001-0939.2020.03.009.
271. Gautret P, Lagier J-C, Parola P, Hoang VT, Meddeb L, Mailhe M, Doudier B, Courjon J, Giordanengo V, Vieira VE, Dupont HT, Honoré S, Colson P, Chabrière E, La Scola B, Rolain JM, Brouqui P, Raoult D. 20 March 2020. Hydroxychloroquine and azithromycin as a treatment of COVID-19:

results of an open-label non-randomized clinical trial. Int J Antimicrob Agents https://doi.org/10.1016/j.ijantimicag.2020.105949.
272. Keyaerts E, Vijgen L, Maes P, Neyts J, Van Ranst M. 2004. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. Biochem Biophys Res Commun 323:264–268. https://doi.org/10.1016/j.bbrc.2004.08.085.
273. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, Li X, Xia J, Chen N, Xiang J, Yu T, Bai T, Xie X, Zhang L, Li C, Yuan Y, Chen H, Li H, Huang H, Tu S, Gong F, Liu Y, Wei Y, Dong C, Zhou F, Gu X, Xu J, Liu Z, Zhang Y, Li H, Shang L, Wang K, Li K, Zhou X, Dong X, Qu Z, Lu S, Hu X, Ruan S, Luo S, Wu J, Peng L, Cheng F, Pan L, Zou J, Jia C, Wang J, Liu X, Wang S, Wu X, Ge Q, He J, Zhan H, Qiu F, Guo L, Huang C, Jaki T, Hayden FG, Horby PW, Zhang D, Wang C. 2020. A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. N Engl J Med 382:1787–1799. https://doi.org/10.1056/NEJMoa2001282.
274. Bao L, Deng W, Gao H, Xiao C, Liu J, Xue J, Lv Q, Liu J, Yu P, Xu Y, Qi F, Qu Y, Li F, Xiang Z, Yu H, Gong S, Liu M, Wang G, Wang S, Song Z, Zhao W, Han Y, Zhao L, Liu X, Wei Q, Qin C. 2020. Reinfection could not occur in SARS-CoV-2 infected rhesus macaques. bioRxiv https://doi.org/10.1101/2020.03.13.990226.
275. Cheng VC, Lau SK, Woo PC, Yuen KY. 2007. Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. Clin Microbiol Rev 20:660–694. https://doi.org/10.1128/CMR.00023-07.
276. Ison MG, Hirsch HH. 2019. Community-acquired respiratory viruses in transplant patients: diversity, impact, unmet clinical needs. Clin Microbiol Rev 32:e00042-19. https://doi.org/10.1128/CMR.00042-19.
277. Sanchez JL, Cooper MJ, Myers CA, Cummings JF, Vest KG, Russell KL, Sanchez JL, Hiser MJ, Gaydos CA. 2015. Respiratory infections in the U.S. military: recent experience and control. Clin Microbiol Rev 28:743–800. https://doi.org/10.1128/CMR.00039-14.
278. Chan JF, Lau SK, To KK, Cheng VC, Woo PC, Yuen KY. 2015. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. Clin Microbiol Rev 28:465–522. https://doi.org/10.1128/CMR.00102-14.
279. Cheng VC, To KK, Tse H, Hung IF, Yuen KY. 2012. Two years after pandemic influenza A/2009/H1N1: what have we learned? Clin Microbiol Rev 25:223–263. https://doi.org/10.1128/CMR.05012-11.
280. Gillim-Ross L, Subbarao K. 2006. Emerging respiratory viruses: challenges and vaccine strategies. Clin Microbiol Rev 19:614–636. https://doi.org/10.1128/CMR.00005-06.
281. Norkin LC. 1995. Virus receptors: implications for pathogenesis and the design of antiviral agents. Clin Microbiol Rev 8:293–315. https://doi.org/10.1128/CMR.8.2.293.
282. Dhama K, Patel SK, Pathak M, Yatoo MI, Tiwari R, Malik YS, Singh R, Sah R, Rabaan AA, Bonilla-Aldana DK, Rodriguez-Morales AJ. 2020. An update on SARS-COV-2/COVID-19 with particular reference on its clinical pathology, pathogenesis, immunopathology and mitigation strategies–a review. Preprints https://www.preprints.org/manuscript/202003.0348/v1.
283. Kimberlin DW, Stagno S. 26 March 2020. Can SARS-CoV-2 infection be acquired in utero? More definitive evidence is needed. JAMA https://doi.org/10.1001/jama.2020.4868.
284. Akarsh HP. 2020. Wuhan virus: Chinese animal markets reopened with almost no precautions. https://metrosaga.com/wuhan-virus-chinese-animal-markets-reopened-with-almost-no-precautions/.
285. Blakeman BA. 2020. China must close down "wet markets" now. https://thehill.com/opinion/international/490528-china-must-close-down-wet-markets-now.
286. Knowles G. 2020. Will they ever learn? Chinese markets are still selling bats and slaughtering rabbits on blood-soaked floors as Beijing celebrates "victory" over the coronavirus. https://www.dailymail.co.uk/news/article-8163761/Chinese-markets-selling-bats.html.
287. Dhama K, Patel SK, Sharun K, Pathak M, Tiwari R, Yatoo MI, Malik YS, Sah R, Rabaan Aa, Panwar Pk, Singh KP, Michalak I, Chaicumpa W, Bonilla-Aldana DK, Rodriguez-Morales AJ. 2020. SARS-CoV-2: jumping the species barrier, lessons from SARS and MERS, its zoonotic spillover, transmission to humans, preventive and control measures and recent developments to counter this pandemic virus. Preprints https://www.preprints.org/manuscript/202004.0011/v1.
288. Young BE, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J, Ng OT, Marimuthu K, Ang LW, Mak TM, Lau SK, Anderson DE, Chan KS, Tan TY, Ng TY, Cui L, Said Z, Kurupatham L, Chen MI, Chan M, Vasoo S, Wang LF, Tan BH, Lin RTP, Lee VJM, Leo YS, Lye DC, Singapore 2019 Novel Coronavirus Outbreak Research Team. 2020. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. JAMA 323:1488. https://doi.org/10.1001/jama.2020.3204.
289. Ong SWX, Tan YK, Chia PY, Lee TH, Ng OT, Wong MSY, Marimuthu K. 2020. Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a symptomatic patient. JAMA 323:1610. https://doi.org/10.1001/jama.2020.3227.
290. Zhang T, Cui X, Zhao X, Wang J, Zheng J, Zheng G, Guo W, Cai C, He S, Xu Y. 29 March 2020. Detectable SARS-CoV-2 viral RNA in feces of three children during recovery period of COVID-19 pneumonia. J Med Virol https://doi.org/10.1002/jmv.25795.
291. Yu F, Yan L, Wang N, Yang S, Wang L, Tang Y, Gao G, Wang S, Ma C, Xie R, Wang F, Tan C, Zhu L, Guo Y, Zhang F. 28 March 2020. Quantitative detection and viral load analysis of SARS-CoV-2 in infected patients. Clin Infect Dis https://doi.org/10.1093/cid/ciaa345.
292. Huang Y, Cheng W, Zhao N, Qu H, Tian J. 26 March 2020. CT screening for early diagnosis of SARS-CoV-2 infection. Lancet Infect Dis https://doi.org/10.1016/S1473-3099(20)30241-3.
293. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, Guo L, Guo R, Chen T, Hu J, Xiang Z, Mu Z, Chen X, Chen J, Hu K, Jin Q, Wang J, Qian Z. 2020. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun 11:1620. https://doi.org/10.1038/s41467-020-15562-9.
294. Ahmed SF, Quadeer AA, McKay MR. 2020. Preliminary identification of potential vaccine targets for the COVID-19 coronavirus (SARS-CoV-2) based on SARS-CoV immunological studies. Viruses 12:254. https://doi.org/10.3390/v12030254.
295. Ramaiah A, Arumugaswami V. 2020. Insights into cross-species evolution of novel human coronavirus 2019-nCoV and defining immune determinants for vaccine development. bioRxiv https://doi.org/10.1101/2020.01.29.925867.
296. Cohen J. 2020. Vaccine designers take first shots at COVID-19. Science 368:14–16. https://doi.org/10.1126/science.368.6486.14.
297. Precision Vaccinations. 2020. Coronavirus vaccines. https://www.precisionvaccinations.com/vaccines/coronavirus-vaccines.
298. WHO. 2020. Draft landscape of COVID-19 candidate vaccines–20 March 2020. https://www.who.int/blueprint/priority-diseases/key-action/novel-coronavirus-landscape-ncov.pdf?ua=1.
299. Cascella M, Rajnik M, Cuomo A, Dulebohn SC, Di Napoli R. 2020. Features, evaluation and treatment coronavirus (COVID-19). *In* StatPearls [Internet]. StatPearls Publishing, Treasure Island, FL.
300. Shetty R, Ghosh A, Honavar SG, Khamar P, Sethu S. 2020. Therapeutic opportunities to manage COVID-19/SARS-CoV-2 infection: present and future. Indian J Ophthalmol 68:693. https://doi.org/10.4103/ijo.IJO_639_20.
301. Zhang W, Zhao Y, Zhang F, Wang Q, Li T, Liu Z, Wang J, Qin Y, Zhang X, Yan X, Zeng X, Zhang S. 2020. The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): the experience of clinical immunologists from China. Clin Immunol 214:108393. https://doi.org/10.1016/j.clim.2020.108393.
302. Little P. 2020. Non-steroidal anti-inflammatory drugs and Covid-19. BMJ 368:m1185. https://doi.org/10.1136/bmj.m1185.
303. Alhazzani W, Møller MH, Arabi YM, Loeb M, Gong MN, Fan E, Oczkowski S, Levy MM, Derde L, Dzierba A, Du B, Aboodi M, Wunsch H, Cecconi M, Koh Y, Chertow DS, Maitland K, Alshamsi F, Belley-Cote E, Greco M, Laundy M, Morgan JS, Kesecioglu J, McGeer A, Mermel L, Mammen MJ, Alexander PE, Arrington A, Centofanti JE, Citerio G, Baw B, Memish ZA, Hammond N, Hayden FG, Evans L, Rhodes A. 2020. Surviving sepsis campaign: guidelines on the management of critically ill adults with coronavirus disease 2019 (COVID-19). Intensive Care Med 46:854–887. https://doi.org/10.1007/s00134-020-06022-5.
304. Atluri S, Manchikanti L, Hirsch JA. 2020. Expanded umbilical cord mesenchymal stem cells (UC-MSCs) as a therapeutic strategy in managing critically ill COVID-19 patients: the case for compassionate use. Pain Physician 23:E71–E83.
305. Elfiky AA. 2020. Ribavirin, remdesivir, sofosbuvir, galidesivir, and tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): a molecular docking study. Life Sci 253:117592. https://doi.org/10.1016/j.lfs.2020.117592.
306. Ekins S, Lane TR, Madrid PB. 2020. Tilorone: a broad-spectrum antiviral invented in the USA and commercialized in Russia and beyond. Pharm Res 37:71. https://doi.org/10.1007/s11095-020-02799-8.

307. Molina JM, Delaugerre C, Goff JL, Mela-Lima B, Ponscarme D, Goldwirt L, de Castro N. 2020. No evidence of rapid antiviral clearance or clinical benefit with the combination of hydroxychloroquine and azithromycin in patients with severe COVID-19 infection. Med Mal Infect 50:384. https://doi.org/10.1016/j.medmal.2020.03.006.
308. Caly L, Druce JD, Catton MG, Jans DA, Wagstaff KM. 2020. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. Antiviral Res 178:104787. https://doi.org/10.1016/j.antiviral.2020.104787.
309. Ong J, Cross GB, Dan YY. 2020. Prevention of nosocomial SARS-CoV-2 transmission in endoscopy: international recommendations and the need for a gold standard. Gut 69:1145–1148. https://doi.org/10.1136/gutjnl-2020-321154.
310. Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, Wang F, Li D, Yang M, Xing L, Wei J, Xiao H, Yang Y, Qu J, Qing L, Chen L, Xu Z, Peng L, Li Y, Zheng H, Chen F, Huang K, Jiang Y, Liu D, Zhang Z, Liu Y, Liu L. 2020. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. JAMA 323:1582. https://doi.org/10.1001/jama.2020.4783.
311. Keith P, Day M, Perkins L, Moyer L, Hewitt K, Wells A. 2020. A novel treatment approach to the novel coronavirus: an argument for the use of therapeutic plasma exchange for fulminant COVID-19. Crit Care 24:128. https://doi.org/10.1186/s13054-020-2836-4.
312. Repici A, Maselli R, Colombo M, Gabbiadini R, Spadaccini M, Anderloni A, Carrara S, Fugazza A, Di Leo M, Galtieri PA, Pellegatta G, Ferrara EC, Azzolini E, Lagioia M. 14 March 2020. Coronavirus (COVID-19) outbreak: what the department of endoscopy should know. Gastrointest Endosc https://doi.org/10.1016/j.gie.2020.03.019.
313. Liu X, Zhang S. 29 March 2020. COVID-19: face masks and human-to-human transmission. Influenza Other Respir Viruses https://doi.org/10.1111/irv.12740.
314. Yang C. 2020. Does hand hygiene reduce SARS-CoV-2 transmission? Graefes Arch Clin Exp Ophthalmol 258:1133–1134. https://doi.org/10.1007/s00417-020-04652-5.
315. Lai THT, Tang EWH, Fung KSC, Li K. 2020. Reply to "Does hand hygiene reduce SARS-CoV-2 transmission?" Graefes Arch Clin Exp Ophthalmol 258:1135–1135. https://doi.org/10.1007/s00417-020-04653-4.
316. Chu J, Yang N, Wei Y, Yue H, Zhang F, Zhao J, He L, Sheng G, Chen P, Li G, Wu S, Zhang B, Zhang S, Wang C, Miao X, Li J, Liu W, Zhang H. 29 March 2020. Clinical characteristics of 54 medical staff with COVID-19: a retrospective study in a single center in Wuhan, China. J Med Virol https://doi.org/10.1002/jmv.25793.
317. Bonilla-Aldana DK, Dhama K, Rodriguez-Morales AJ. 2020. Revisiting the One Health approach in the context of COVID-19: a look into the ecology of this emerging disease. Adv Anim Vet Sci 8:234–237. https://doi.org/10.17582/journal.aavs/2020/8.3.234.237.
318. Malik YS, Sircar S, Bhat S, Vinodhkumar OR, Tiwari R, Sah R, Rabaan AA, Rodriguez-Morales AJ, Dhama K. 2020. Emerging coronavirus disease (COVID-19), a pandemic public health emergency with animal linkages: current status update. Indian J Anim Sci 90:156–173.
319. Rodriguez-Morales AJ, Tiwari R, Sah R, Dhama K. 2020. COVID-19, an emerging coronavirus infection: current scenario and recent developments—an overview. J Pure Appl Microbiol 14:5–12. https://doi.org/10.22207/JPAM.14.1.02.
320. Moriarty LF, Plucinski MM, Marston BJ, Kurbatova EV, Knust B, Murray EL, Pesik N, Rose D, Fitter D, Kobayashi M, Toda M, Cantey PT, Scheuer T, Halsey ES, Cohen NJ, Stockman L, Wadford DA, Medley AM, Green G, Regan JJ, Tardivel K, White S, Brown C, Morales C, Yen C, Wittry B, Freeland A, Naramore S, Novak RT, Daigle D, Weinberg M, Acosta A, Herzig C, Kapella BK, Jacobson KR, Lamba K, Ishizumi A, Sarisky J, Svendsen E, Blocher T, Wu C, Charles J, Wagner R, Stewart A, Mead PS, Kurylo E, Campbell S, Murray R, Weidle P, Cetron M, Friedman CR, CDC Cruise Ship Response Team, California Department of Public Health COVID-19 Team, Solano County COVID-19 Team. 2020. Public health responses to COVID-19 outbreaks on cruise ships—worldwide, February–March 2020. MMWR Morb Mortal Wkly Rep 69:347–352. https://doi.org/10.15585/mmwr.mm6912e3.
321. Hasan Z, Narasimhan M. 25 March 2020. Preparing for the COVID-19 pandemic: our experience in New York. Chest https://doi.org/10.1016/j.chest.2020.03.027.
322. Colbourn T. 2020. COVID-19: extending or relaxing distancing control measures. Lancet Public Health 5:e236–e237. https://doi.org/10.1016/S2468-2667(20)30072-4.
323. Zheng J. 2020. SARS-CoV-2: an emerging coronavirus that causes a global threat. Int J Biol Sci 16:1678–1685. https://doi.org/10.7150/ijbs.45053.
324. Chatterjee P, Nagi N, Agarwal A, Das B, Banerjee S, Sarkar S, Gupta N, Gangakhedkar RR. 2020. The 2019 novel coronavirus disease (COVID-19) pandemic: a review of the current evidence. Indian J Med Res https://doi.org/10.4103/ijmr.IJMR_519_20.
325. Xiao DAT, Gao DC, Zhang DS. 21 March 2020. Profile of specific antibodies to SARS-CoV-2: the first report. J Infect https://doi.org/10.1016/j.jinf.2020.03.012.
326. Ali M, Zaid M, Saqib MAN, Ahmed H, Afzal MS. 8 April 2020. SARS-CoV-2 and the hidden carriers—sewage, feline, and blood transfusion. J Med Virol https://doi.org/10.1002/jmv.25956.
327. Ahmed W, Angel N, Edson J, Bibby K, Bivins A, O'Brien JW, Choi PM, Kitajima M, Simpson SL, Li J, Tscharke B, Verhagen R, Smith WJM, Zaugg J, Dierens L, Hugenholtz P, Thomas KV, Mueller JF. 2020. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. Sci Total Environ 728:138764. https://doi.org/10.1016/j.scitotenv.2020.138764.
328. Núñez-Delgado A. 2020. What do we know about the SARS-CoV-2 coronavirus in the environment? Sci Total Environ 727:138647. https://doi.org/10.1016/j.scitotenv.2020.138647.
329. Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, Liu R, He X, Shuai L, Sun Z, Zhao Y, Liu P, Liang L, Cui P, Wang J, Zhang X, Guan Y, Tan W, Wu G, Chen H, Bu Z. 8 April 2020. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. Science https://doi.org/10.1126/science.abb7015.
330. McNamara T, Richt JA, Glickman L. 2020. A critical needs assessment for research in companion animals and livestock following the pandemic of COVID-19 in humans. Vector Borne Zoonotic Dis 20:393–405. https://doi.org/10.1089/vbz.2020.2650.
331. Shanthikumar SR. 2020. We should err on side of caution with Covid-19 advice. Vet Rec 186:458. https://doi.org/10.1136/vr.m1488.
332. Li X. 2020. Can cats become infected with Covid-19? Vet Rec 186:457–458. https://doi.org/10.1136/vr.m1455.
333. Almendros A, Gascoigne E. 2020. Can companion animals become infected with Covid-19? Vet Rec 186:419–420. https://doi.org/10.1136/vr.m1322.
334. IDEXX. 2020. Leading veterinary diagnostic company sees no COVID-19 cases in pets. www.idexx.com/en/about-idexx/news/no-covid-19-cases-pets. Accessed 11 May 2020.
335. AVMA. 2020. SARS-CoV-2 in animals. https://www.avma.org/resources-tools/animal-health-and-welfare/covid-19/sars-cov-2-animals-including-pets. Accessed 11 May 2020.
336. Opriessnig T, Huang YW. 2020. Coronavirus disease 2019 (COVID-19) outbreak: could pigs be vectors for human infections? Xenotransplantation 27:e12591. https://doi.org/10.1111/xen.12591.
337. Kim YI, Kim SG, Kim SM, Kim EH, Park SJ, Yu KM, Chang JH, Kim EJ, Lee S, Casel MAB, Um J, Song MS, Jeong HW, Lai VD, Kim Y, Chin BS, Park JS, Chung KH, Foo SS, Poo H, Mo IP, Lee OJ, Webby RJ, Jung JU, Choi YK. 2020. Infection and rapid transmission of SARS-CoV-2 in ferrets. Cell Host Microbe 27:704–709. https://doi.org/10.1016/j.chom.2020.03.023.
338. Sharun K, Shyamkumar TS, Aneesha VA, Dhama K, Pawde AM, Pal A. 2019. Current therapeutic applications and pharmacokinetic modulations of ivermectin. Vet World 12:1204–1211. https://doi.org/10.14202/vetworld.2019.1204-1211.
339. Patrì A, Fabbrocini G. 2020. Hydroxychloroquine and ivermectin: a synergistic combination for COVID-19 chemoprophylaxis and/or treatment? J Am Acad Dermatol 82:e221. https://doi.org/10.1016/j.jaad.2020.04.017.
340. Schmith VD, Zhou JJ, Lohmer LR. 2020. The approved dose of ivermectin alone is not the ideal dose for the treatment of COVID-19. Clin Pharmacol Ther 18:630. https://doi.org/10.1002/cpt.1889.
341. Bray M, Rayner C, Noël F, Jans D, Wagstaff K. 2020. Ivermectin and COVID-19: a report in antiviral research, widespread interest, an FDA warning, two letters to the editor and the authors' responses. Antiviral Res 178:104805. https://doi.org/10.1016/j.antiviral.2020.104805.
342. Graham BS. 8 May 2020. Rapid COVID-19 vaccine development. Science https://doi.org/10.1126/science.abb8923.
343. Mukherjee R. 2020. Global efforts on vaccines for COVID-19: since, sooner or later, we all will catch the coronavirus. J Biosci 45:68. https://doi.org/10.1007/s12038-020-00040-7.

344. Abbott. 2020. Abbott launches molecular point-of-care test to detect novel coronavirus in as little as five minutes. https://abbott.mediaroom.com/2020-03-27-Abbott-Launches-Molecular-Point-of-Care-Test-to-Detect-Novel-Coronavirus-in-as-Little-as-Five-Minutes. Accessed 2 April 2020.
345. Ai JW, Zhang Y, Zhang HC, Xu T, Zhang WH. 2020. Era of molecular diagnosis for pathogen identification of unexplained pneumonia, lessons to be learned. Emerg Microbes Infect 9:597–600. https://doi.org/10.1080/22221751.2020.1738905.
346. Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, Miao X, Streithorst JA, Granados A, Sotomayor-Gonzalez A, Zorn K, Gopez A, Hsu E, Gu W, Miller S, Pan CY, Guevara H, Wadford DA, Chen JS, Chiu CY. 16 Apr 2020. CRISPR-Cas12-based detection of SARS-CoV-2. Nat Biotechnol https://doi.org/10.1038/s41587-020-0513-4.
347. Cheng MP, Papenburg J, Desjardins M, Kanjilal S, Quach C, Libman M, Dittrich S, Yansouni CP. 13 May 2020. Diagnostic testing for severe acute respiratory syndrome-related coronavirus-2: a narrative review. Ann Intern Med 13:M20-1301. https://doi.org/10.7326/M20-1301.
348. Li L, Qin L, Xu Z, Yin Y, Wang X, Kong B, Bai J, Lu Y, Fang Z, Song Q, Cao K, Liu D, Wang G, Xu Q, Fang X, Zhang S, Xia J, Xia J. 19 March 2020. Artificial intelligence distinguishes COVID-19 from community acquired pneumonia on chest CT. Radiology https://doi.org/10.1148/radiol.2020200905.
349. Lu R, Wu X, Wan Z, Li Y, Zuo L, Qin J, Jin X, Zhang C. 1 April 2020. Development of a novel reverse transcription loop-mediated isothermal amplification method for rapid detection of SARS-CoV-2. Virol Sin https://doi.org/10.1007/s12250-020-00218-1.
350. Pathak M, Patel SK, Jigyasa R, Tiwari R, Dhama K, Sah R, Rabaan AA, Bonilla-Aldana DK, Rodriguez-Morales AJ. 15 April 2020. Global threat of SARS-CoV-2/COVID-19 and the need for more and better diagnostic tools. Arch Med Res https://doi.org/10.1016/j.arcmed.2020.04.003.
351. Udugama B, Kadhiresan P, Kozlowski HN, Malekjahani A, Osborne M, Li VYC, Chen H, Mubareka S, Gubbay JB, Chan W. 2020. Diagnosing COVID-19: the disease and tools for detection. ACS Nano 14:3822–3835. https://doi.org/10.1021/acsnano.0c02624.
352. Vashist SK. 2020. In vitro diagnostic assays for COVID-19: recent advances and emerging trends. Diagnostics 10:202. https://doi.org/10.3390/diagnostics10040202.
353. Yang T, Wang YC, Shen CF, Cheng CM. 2020. Point-of-care RNA-based diagnostic device for COVID-19. Diagnostics 10:165. https://doi.org/10.3390/diagnostics10030165.
354. Yu L, Wu S, Hao X, Li X, Liu X, Ye S, Han H, Dong X, Li X, Li J. 2020. Rapid colorimetric detection of COVID-19 coronavirus using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic plat-form: iLACO. medRxiv https://10.1101/2020.02.20.20025874.
355. Zhang F, Abudayyeh OO, Gootenberg JS. 2020. A protocol for detection of COVID-19 using CRISPR diagnostics. Broad Institute, Cambridge, MA.
356. Arab-Mazar Z, Sah R, Rabaan AA, Dhama K, Rodriguez-Morales AJ. 2020. Mapping the incidence of the COVID-19 hotspot in Iran—implications for travellers. Travel Med Infect Dis 34:101630. https://doi.org/10.1016/j.tmaid.2020.101630.
357. Ebrahim SH, Memish ZA. 2020. COVID-19: preparing for superspreader potential among Umrah pilgrims to Saudi Arabia. Lancet 395:e48. https://doi.org/10.1016/S0140-6736(20)30466-9.
358. Kamel Boulos MN, Geraghty EM. 2020. Geographical tracking and mapping of coronavirus disease COVID-19/severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epidemic and associated events around the world: how 21st century GIS technologies are supporting the global fight against outbreaks and epidemics. Int J Health Geogr 19:8. https://doi.org/10.1186/s12942-020-00202-8.
359. Lin C, Braund WE, Auerbach J. 2020. Policy decisions and use of information technology to fight 2019 novel coronavirus disease, Taiwan. Emerg Infect Dis https://doi.org/10.3201/eid2607.200574.
360. Wynants L, Calster BV, Bonten MMJ, Collins GS, Debray TPA, De Vos M, Haller MC, Heinze G, Moons KGM, Riley RD, Schuit E, Smits LJM, Snell KIE, Steyerberg EW, Wallisch C, van Smeden M. 2020. Prediction models for diagnosis and prognosis of Covid-19 infection: systematic review and critical appraisal. BMJ 369:m1328. https://doi.org/10.1136/bmj.m1328.
361. Whitehead CL, Walker SP. 13 May 2020. Consider pregnancy in COVID-19 therapeutic drug and vaccine trials. Lancet https://doi.org/10.1016/S0140-6736(20)31029-1.
362. Burton DR, Walker LM. 2020. Rational vaccine design in the time of COVID-19. Cell Host Microbe 27:695–698. https://doi.org/10.1016/j.chom.2020.04.022.
363. O'Connor E, Teh J, Kamat AM, Lawrentschuk N. 14 May 2020. Bacillus Calmette Guérin (BCG) vaccination use in the fight against COVID-19–what's old is new again? Future Oncol https://doi.org/10.2217/fon-2020-0381.
364. Hamiel U, Kozer E, Youngster I. 13 May 2020. SARS-CoV-2 rates in BCG-vaccinated and unvaccinated young adults. JAMA https://doi.org/10.1001/jama.2020.8189.
365. Ojha R, Gupta N, Naik B, Singh S, Verma VK, Prusty D, Prajapati VK. 14 May 2020. High throughput and comprehensive approach to develop multiepitope vaccine against minacious COVID-19. Eur J Pharm Sci https://doi.org/10.1016/j.ejps.2020.105375.
366. Tang X, Wu C, Li X, Song Y, Yao X, Wu X, Duan Y, Zhang H, Wang Y, Qian Z, Cui J, Lu J. 3 March 2020. On the origin and continuing evolution of SARS-CoV-2. Natl Sci Rev https://doi.org/10.1093/nsr/nwaa036.
367. Lockey E. 2020. COVID-19: the race for a vaccine. J Renin Angiotensin Aldosterone Syst 21:1470320320926902. https://doi.org/10.1177/1470320320926902.

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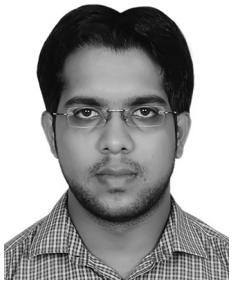
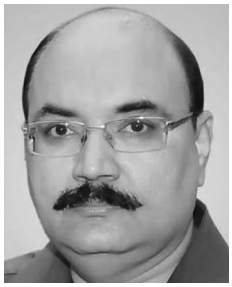
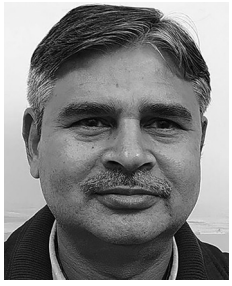


Shubhankar Sircar, a Ph.D. scholar, received his master's degree from Integral University Lucknow, India, in 2012 and is presently serving as a Senior Research Fellow in an ICAR-National Fellow Scheme in the Division of Biological Standardization at the ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, India. His area of interest is molecular epidemiology and genotype distribution of major enteric viruses, with a focus on developing different molecular as well as serological diagnostic testing assays. Apart from his expertise in viral diagnosis, he has particular interest and knowledge in the fields of infectious diseases of farms, animals, and wildlife. With good academic records, he has received a few awards and recognitions (Best Poster and Young Scientist) and participated in several national and international workshops, training programs, and conferences. He has published 30 papers in journals of repute. He is highly enthusiastic about gaining knowledge of advancements in educational and scientific research areas.

Sudipta Bhat, a Ph.D. scholar, received his bachelor's (B.V.Sc.) from West Bengal University of Animal and Fishery Sciences, Kolkata, India, and masters (M.V.Sc.) from ICAR-Indian Veterinary Research Institute (IVRI), Bareilly, India. He has been pursuing a Ph.D. in Veterinary Virology, ICAR-IVRI, since 2016. He has worked on the highly pathogenic H5N1 avian influenza virus and now is working on emerging enteric viruses of zoonotic importance from different animal species. He has published his research findings in international journals. His area of interest is infectious diseases with a focus on understanding the antigenic and genetic diversity of viruses causing disease of several livestock species. With brilliant academic records, he has also been awarded several fellowships and scholarships and participated in several national and international workshops, training programs, and courses.



Yashpal Singh Malik, M.V.Sc., Ph.D., serving as an ICAR-National Fellow and Professor, is an expert on enteric viral infections, zoonosis, and emerging viral diseases of animals and humans. He has contributed immensely to viral disease epidemiology, virus-host interactions, microbial biodiversity, characterization, and diagnosis of pathogens. He performed his postdoctoral work at the University of Minnesota. He acquired advanced training in molecular virology from the Division of Virology, University of Ottawa, Canada, and Wuhan Institute of Virology, China. He has represented India in the scientific arena in more than 12 countries. He is the Secretary-General of the Indian Virological Society and Secretary for the World Society for Virology (USA). He is a study group member of the ICTV on *Birnaviridae* and *Picobirnaviridae*. He has authored five books from reputed publishers, including Elsevier and Springer Nature, and has published 225 scientific research articles and reviews in journals of high impact factor. His h-index is 29, and his RG score is 39.



Karam Pal Singh, Ph.D., obtained his B.V.Sc. and A.H. degrees from CSA University of Agriculture & Technology, Kanpur, India, and M.V.Sc. (1987) and Ph.D. (1990) degrees in veterinary pathology from the ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, India. He worked as Scientist, Senior Scientist, and Principal Scientist before taking charge as the Acting Head, Division of Pathology, on 1 January 2019. He worked at the Institute of Animal Health, Pirbright, United Kingdom, as a visiting fellow from April to December 1996. He worked at the Institute of Animal Health, Pirbright, United Kingdom, as a Postdoctoral Fellow on a Wellcome Trust Fellowship from September 2002 to August 2004. Further, he worked at the Veterinary Research Centre, Muscat, Sultanate of Oman, as an Expert Pathologist from June 2008 to May 2009. Dr. Singh is a veterinary pathologist. His area of interest is infectious diseases with a focus on understanding the pathogenesis and molecular diagnosis of viral diseases, with particular reference to rabies and bluetongue viruses.

Wanpen Chaicumpa, D.V.M. (Hons.), Ph.D. (microbiology), is Emeritus Professor, Research Consultant, and Head of the Center of Research Excellence on Therapeutic Proteins and Antibody Engineering at the Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, and consultant of the Faculty of Allied Health Sciences, Thammasat University, Thailand. Her research interests are intestinal immunity against enteric infections, vaccine development, immuno- and molecular diagnoses of tropical infections, allergy, immunotherapy, and antibody engineering. She is an executive member of the Thailand Academy of Sciences. She has served as Editor in Chief of the Asian Pacific Journal of Allergy and Immunology. She served as a consultant to the WHO Southeast Asian Regional Office, India. She has published over 250 publications, owns more than 30 patents/patent applications, and has published three textbooks (*Animal Viruses*, *Immunology for Diagnosis of Diseases*, and *Practical Immunology for Students of Diploma of Tropical Medicines*).

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D. Katterine Bonilla-Aldana, D.V.M., M.Sc., graduated from Universidad de la Amazonia, School of Veterinary Medicine and Zootechnics, in Florencia, Colombia, in 2015. She completed a master of sciences in microbiology, Universidad Metropolitana, Barranquilla, Colombia, in 2019. She served as a Young Researcher, Public Health and Infection Research Group, Faculty of Health Sciences, Universidad Tecnológica de Pereira (UTP), in Pereira, Risaralda, Colombia. She is a member of the Colombian Infectious Diseases Association (ACIN) and the International Society for Infectious Diseases. She is a member of the Committee on Tropical Medicine, Zoonoses, and Travel Medicine of the ACIN. She has been recognized as Junior Researcher, Ministry of Science in Colombia, MinCiencias. She is a Professor of veterinary medicine and zootechnics, Fundación Universitaria Autónoma de las Americas (FUAM), in Pereira, Risaralda, Colombia. She is a Lead Professor of the Zoonoses Research Incubator (SIZOO), FUAM. Her main research interest is the study of zoonotic tick-borne and vector-borne diseases.

Alfonso J. Rodriguez-Morales, M.D., M.Sc., D.T.M. and H., F.R.S.T.M.H. (Lon), F.F.T.M-.R.C.P.S. (Glasg), F.A.C.E., Ph.D.(c), Hon.D.Sc., is an expert in tropical and emerging diseases, particularly in zoonotic and vector-borne diseases. He is President of the Travel Medicine Committee, Pan-American Infectious Diseases Association (API), as well as the Vice President, Colombian Infectious Diseases Association (ACIN). He is a member of the Committee on Tropical Medicine, Zoonoses, and Travel Medicine, ACIN. He is part of the Executive Board of the Latin American Society for Travel Medicine (SLAMVI) and of the Council of the International Society for Infectious Diseases. Since 2014, he has been recognized as Senior Researcher, Ministry of Science (MinCiencias), Colombia. He is Professor, Faculty of Health Sciences, Universidad Tecnológica de Pereira, and of the Fundacion Universitaria Autonoma de las Americas, in Pereira, Risaralda, Colombia. He is Codirector of the Public Health and Infection Research Group, UTP, classified A1 by Colciencias. His Scopus H index is 30 (Google scholar H index, 46).