To assess the genetic variation of different SARS-CoV-2 strains, the 2019 Novel Coronavirus Resource of China National Center for Bio information aligned77,801 genome sequences of SARS-CoV-2 detected glob-ally and identified a total of 15,018 mutations, including14,824 single-nucleotide polymorphisms (BIGD)".In the S protein, four amino acid alterations, V483A,14551, F456V and G4765, are located near the binding interface in the RBD, but their effects on binding to the host receptor are unknown. The alteration D614G in the S1 subunit was found far more frequently than other S variant sites, and it is the marker of a major subclade ofSARS-CoV-2 (clade G). Since March 2020, SARS-CoV-2variants with G614 in the S protein have replaced the original D614 variants and become the dominant form circulating globally. Compared with the D614 variant, higher viral loads were found in patients infected with the G614 variant, but clinical data suggested no significant link between the D614G alteration and disease severity”. Pseudo typed viruses carrying the S protein with G614 generated higher infectious titers than viruses carrying the S protein with D614, suggesting the alteration may have increased the infectivity of SARS-CoV-2(REF.”). However, the results of in vitro experiments based on pseudo virus models may not exactly reflect natural infection. This preliminary finding should be validated by more studies using wild-type SARS-CoV-2 variants to infect different target cells and animal models. Whether this amino acid change enhanced virus transmissibility is also to be determined. Another marker mutation for SARS-CoV-2 evolution is the single-nucleotide To assess the genetic variation of different SARS-CoV-2 strains, the 2019 Novel Coronavirus Resource of China National Center for Bio information aligned77,801 genome sequences of SARS-CoV-2 detected glob-ally and identified a total of 15,018 mutations, including14,824 single-nucleotide polymorphisms (BIGD)".In the S protein, four amino acid alterations, V483A,14551, F456V and G4765, are located near the binding interface in the RBD, but their effects on binding to the host receptor are unknown. The alteration D614G in the S1 subunit was found far more frequently than others variant sites, and it is the marker oaf major subclade ofSARS-CoV-2 (clade G). Since March 2020, SARS-CoV-2variants with G614 in the S protein have replaced the original D614 variants and become the dominant form circulating globally. Compared with the D614 variant, higher viral loads were found in patients infected with the G614 variant, but clinical data suggested no significant link between the D614G alteration and disease severity”. Pseudo typed viruses carrying the S protein with G614 generated higher infectious titers than viruses carrying the S protein with D614, suggesting the alteration may have increased the infectivity of SARS-CoV-2(REF.”). However, the results of in vitro experiments based on pseudo virus models may not exactly reflect natural infection. This preliminary finding should be validated by more studies using wild-type SARS-CoV-2 variants to infect different target cells and animal models. Whether this amino acid change enhanced virus transmissibility is also to be determined. Another marker mutation for SARS-CoV-2 evolution is the single-nucleotide appeared asymptomatic’’. Another serological study detected SARS-CoV-2 neutralizing antibodies in cat serum samples collected in Wuhan after the COVID-19outbreak, providing evidence for SARS-CoV-2 infection in cat populations in Wuhan, although the potential of SARS-CoV-2 transmission from cats to humans is currently uncertain.

**Receptor use and pathogenesis**

SARS-CoV-2 uses the same receptor as SARS-CoV, angiotensin-converting enzyme 2 (ACE2)''’. Besides human ACE2 (hACE2), SARS-CoV-2 also recognizesACE2 from pig, ferret, rhesus monkey, civet, cat, pan-golin, rabbit and dog''\*\*\*\*”. The broad receptor usage of SARS-CoV-2 implies that it may have a wide host range, and the varied efficiency of ACE2 usage in different animals may indicate their different susceptibilities to SARS-CoV-2 infection. The $1 subunit of a corona-virus is further divided into two functional domains, an N-terminal domain and a C-terminal domain. Structural and biochemical analyses identified a211 amino acid region (amino acids 319-529) at the S1C-terminal domain of SARS-CoV-2 as the RBD, which has a key role in virus entry and is the target of neutralizing antibodies™”' (FIG. 5a). The RBM mediates con-tact with the ACE2 receptor (amino acids 437-507 ofSARS-CoV-2 S protein), and this region in SARS-CoV-2differs from that in SARS-CoV in the five residues crit-

**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, novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19), emerged inflate 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 byte novel Cove (originally named 2019-nCoV), which was first identified in Wuhan City, Hubei Province, China, on 12 December 2019. On 11February 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). CoVsbelong to the family Coronaviridae (subfamilyCoronavirinae), 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 viruses 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-19is the third CoV outbreak in humans over the past 2decades (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 a 21 January N00: a heennently 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 ads to the complexity of disease transmission dynamics inCOVID-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 CoVrevealed 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-“OVZCAS | bat-SL-CoVZXC2 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-19showed 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 earlierSARS and MERS CoVs, the veterinary perspective of CoVs and this emerging novel pathogen, and an evaluation of the zoonotic potential of similar Cost 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 ‘nation into Iwi late lead 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 WNidovirales, family Coronaviridae, subfamily Or the corona virus, which is subdivided into four genera, viz., Alpha corona virus, Beta corona virus, Gamma corona virus, and Delta corona virus (3, 27): The generaAlphacoronavirus and Beta coronavirus originate from bats, while =Gamma coronavirus andDeltacoronavirus have evolved from bird and swine gene pools (24, 28, 29, 275).

Coronaviruses possess an unsegmented, single-stranded, positive-sense RNA genome of around 30kb, enclosed by a 5'-cap and 3’-poly(A) tail (30). The genome of SARS-CoV-2 is 29,891 bp long, with aG+C content of 38% (31). These viruses are encircled with an envelope containing — viralnucleocapsid. 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 diverging spherical outline with some degree ofpleomorphism, vision diameters varying from 60 to140 nm, and distinct spikes of 9 to 12 nm, giving the virus the appearance of a solar corona (3). The Cognomen 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/band 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 beta coronaviruses (31). The positive-sense genome of CoVs serves as the mRNA and is translated to polyprotein 1a/lab (pela/lab) (33). 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 setoff sub genomic RNAs (sgRNAs) via discontinuous transcription (35).

Based on molecular characterization, SARS-CoV-2 is considered a new \_ Betacoronavirusbelonging to the subgenus Sarbecovirus (3). A few other critical zoonotic viruses (MERS-related CoVand SARS-related CoV) belong to the same genus. However, SARS-CoV-2 was identified as a distinct virus based on the percent identity with otherBetacoronavirus; conserved open reading frame la/b (ORF la/b) is below 90% identity (3). An overall80% nucleotide identity was observed betweenSARS-CoV-2 and the original SARS-CoV, along with 89% identity with ZC45 and ZXC21 SARS-related CoVs of bats (2, 31, and 36). In addition, 82%identity has been observed between SARS-CoV-2and human SARS-CoV Tor2 and human SARS-CoVBJO1 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 thatSARS-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 arose in disease transmission to humans (31). Of note, the other two zoonotic CoVs (MERS-related Coven 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 sub genomes encode six ORFs (31). The majority of the 5’ end is occupied by Offlay/b, which produces 16 nsps. The two polyproteins, pela and pplab, are initially produced from ORFla/b by a —1 frameshift between ORFlaand ORF 1b (32). The virus-encoded proteases cleavepolyproteins into individual nsps (main protease[Mpro], chymotrypsin-like protease [3CLpro], and papain-like proteases [PLPs]) (42). SARS-CoV-2also encodes these nsps, and their functions have been elucidated recently (31). Remarkably, adifference between SARS-CoV-2 and other CoVs is the identification of a novel short putative proteinwithin the ORF3 band, a secreted protein with an alpha helix and beta-sheet with six strands encodedby 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 class I viral transmembrane protein. The size of thisabundant S protein varies from 1,160 amino acids(IBV, infectious bronchitis virus, in poultry) to 1,400amino acids (FCoV, feline coronavirus) (43). It lies in a trimer on the virion surface, giving the virion acorona or crown-like appearance. Functionally it is required for the entry of the infectious virionparticles into the cell through interaction with various host cellular receptors (44).

Furthermore, it acts as a critical factor for tissuetropism and the determination of host range (45).Notably, S protein is one of the vital immunodominant proteins of CoVs capable ofinducing host immune responses (45). Theectodomains in all CoVs S proteins have similardomain organizations, divided into two subunits, S1and 82 (43). The first one, $1, helps in host receptorbinding, while the second one, $2, accounts forfusion. The former (S1) is further divided into twosubdomains, namely, the N-terminal domain (NTD)and C-terminal domain (CTD). Both of thesesubdomains act as\_ receptor-binding domains,interacting efficiently with various host receptors(45). The Sl CTD contains the receptor-bindingmotif (RBM). In each coronavirus spike protein, thetrimeric S1 locates itself on top of the trimeric S2stalk (45). Recently, structural analyses of the Sproteins of COVID-19 have revealed 27 amino acidsubstitutions within a 1,273-amino-acid stretch (16).Six substitutions are located in the RBD (aminoacids 357 to 528), while four substitutions are in theRBM at the CTD of the S1 domain (16). Of note, noamino acid change is seen in the RBM, which bindsdirectly to the angiotensin-converting enzyme-2(ACE2) receptor in SARS-CoV (16, 46). At present,the main emphasis is knowing how many differenceswould be required to change the host tropism.Sequence comparison revealed 17 nonsynonymouschanges between the early sequence of SARS-CoV-2and the later isolates of SARS-CoV. The changeswere found scattered over the genome of the virus,with nine substitutions in ORFlab, ORF8 (4substitutions), the spike gene (3 substitutions), andORF7a (single substitution) (4). Notably, the samenonsynonymous changes were found in a familialcluster, indicating that the viral evolution happenedduring person-to-person transmission (4, 47). Suchadaptive evolution events are frequent and constitutea constantly ongoing process once the virus spreadsamong new hosts (47). Even though no functionalchanges occur in the virus associated with thisadaptive evolution, close monitoring of the viralmutations that occur during subsequent human-to-human transmission is warranted.

**M Protein**

The M protein is the most abundant viral proteinpresent in the virion particle, giving a definite shapeto the viral envelope (48). It binds to thenucleocapsid and acts as a central organizer ofcoronavirus assembly (49). Coronavirus M proteinsare highly diverse in amino acid contents butmaintain overall structural similarity within differentgenera (50). The M protein has three transmembranedomains, flanked by a short amino terminus outsidethe virion and a long carboxy terminus inside thevirion (50). Overall, the viral scaffold is maintainedby M-M interaction. Of note, the M protein ofSARS-CoV-2. does not have an amino acidsubstitution compared to that of SARS-CoV (16).

**E Protein**

The coronavirus E protein is the most enigmaticand 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 aviroporin (ion channel) (53). The inactivation or 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 multi purpose. Among several functions, it plays a role in complex formation with the viral genome, facilitates Mprotein interaction needed during virion assembly,and enhances the transcription efficiency of the virus(55, 56). It contains three highly conserved anddistinct domains, namely, an NTD, an RNA-bindingdomain 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). Thecharged LKR is serine and arginine rich and is also known as the SR (serine and arginine) domain (59).The LKR is capable of direct 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 interfere on N Protein The N protein of coronavirus is multipurpose.Among several functions, it plays a role in complexformation with the viral genome, facilitates Mprotein interaction needed during virion assembly,and enhances the transcription efficiency of the virus(55, 56). It contains three highly conserved anddistinct domains, namely, an NTD, an RNA-bindingdomain 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 highlydiverged both in length and sequence (58). Thecharged LKR is serine and arginine rich and is alsoknown as the SR (serine and arginine) domain (59).The LKR is capable of direct interaction with in vitroRNA interaction and is responsible for cell signaling(60, 61). It also modulates the antiviral response ofthe host by working as an antagonist for interferon(IFN) and RNA interference (62). Compared to thatof SARS-CoV, the N protein of SARS-CoV-2possess five amino acid mutations, where two are inthe intrinsically dispersed region (IDR; positions 25and 26), one each in the NTD (position 103), LKR(position 217), and CTD (position 334) (16).

**nsps and Accessory Proteins**

~ 4 re | .nsps and Accessory ProteinsBesides the important structural proteins, theSARS-CoV-2 genome contains 15 nsps, nspl tonsp10 and nsp12 to nsp16, and 8 accessory proteins(3a, 3b, p6, 7a, 7b, 8b, 9b, and ORF 14) (16). Allthese 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 alonger 8b and shorter 3b protein (16). The nsp7,nsp13, envelope, matrix, and p6 and 8b accessoryproteins have not been detected with any amino acidsubstitutions compared to the sequences of othercoronaviruses (16).

The virus structure of SARS-CoV-2 is depicted inFig.. 2.Spike glycoprotein (S)(required for the entry of theinfectious virion particle)Membrane protein (M)(most abundant viral protein) Major structural proteinsEnvelope glycoprotein (E)(smallest among the majorstructural proteins)Nucleocapsid protein (N)+ single-stranded positivesense RNA genomeLipid bilayerFIG 2 SARS-CoV-2 virus structure.VO ssGsslU UL ik ICULIGE CLIUCLIL & Lilyia Bb Pe,using the MegAlign otis program, — thesimilarity between the novel SARS-CoV-2 isolateswas in the range of 99.4% to 100%. Among the otherSerbecovirus CoV sequences, the novel SARS-CoV-2 sequences revealed the highest similarity to bat-SL-CoV, with nucleotide percent identity rangesbetween 88.12 and 89.65%. Meanwhile, earlierreported 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% to46.3% to the other four subgenera, namely,Hibecovirus, Nobecovirus, Merbecovirus, andEmbecovirus, respectively. The percent similarityindex of current outbreak isolates indicates a closerelationship 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 arenecessary to draw any conclusions, although it was ascertained that the current novel SARS-CoV-2isolates belong to the subgenus Sarbecovirus in the diverse range of betacoronaviruses. Their possibleancestor was hypothesized to be from bat CoVstrains, wherein bats might have played a crucial rolein harboring this class of viruses.

**Splits Tree phylogeny analysis.**

In the unrooted phylogenetic tree of different betacoronaviruses based on the S protein, virussequences from different subgenera grouped intoseparate clusters. SARS-CoV-2 sequences fromWuhan and other countries exhibited a closerelationship and appeared in a single cluster (Fig. 1).The CoVs from the subgenus Sarbecovirus appearedjointly in SplitsTree and divided into threesubclusters, namely, SARS-CoV-2, bat-SARS-like-CoV (bat-SL-CoV), and SARS-CoV (Fig. 1). In thecase of other subgenera, like Merbecovirus, all of thesequences grouped in a single cluster, whereas inEmbecovirus, different species, comprised of caninerespiratory CoVs, bovine CoVs, equine CoVs, andhuman CoV strain (OC43), grouped in a commoncluster. Isolates in the subgenera Nobecovorus andHibecovirus were found to be placed separatelyaway from other reported SARS-CoVs but shared abat origin.

**CURRENT WORLDWIDE SCENARIO OFSARS-CoV-2**

This novel virus, SARS-CoV-2, comes under thesubgenus Sarbecovirus of the Orthocoronavirinaesubfamily and is entirely different from the virusesresponsible for MERS-CoV and SARS-CoV (3). Thenewly emerged SARS-CoV-2 is a group 2Bcoronavirus (2). The genome sequences of SARS-CoV-2 obtained from patients share 79.5% sequencesimilarity to the sequence of SARS-CoV (63).As of 13 May 2020, a total of 4,170,424confirmed cases of COVID-19 (with 287,399 deaths)have been reported in more than 210 affectedcountries worldwide (WHO Situation Report 114Initially, the epicenter of the SARS-CoV-2pandemic was China, which reported a significantnumber of deaths associated with COVID-19, with84,458 laboratory-confirmed cases and 4,644 deathsas of 13 May 2020 (Fig. 4). As of 13 May 2020,SARS-CoV-2 confirmed cases have been reported inmore than 210 countries apart from China (Fig. 3and 4) (WHO Situation Report 114) (25, 64).COVID-19 has been reported on all continents except Antarctica. For many weeks, Italy was thefocus of concerns regarding the large number of cases, with 221,216 cases and 30,911 deaths, butnow, 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) than Italy. A JohnHopkins University web platform has provided daily updates on the basic epidemiology of the COVID-19outbreak

Covid-19 has been confirmed on a cruiseship, named Diamond Princess, quarantined in Japanese waters (Port of Yokohama), as well as onother cruise ships around the world (239) (Fig. 3).The significant events of the SARS-CoV-2/COVID-19 virus outbreak occurring since 8 December 2019are presented as a timeline in Fig. 5.Major events of current coronavirus COVID-19 disease outbreak(03 Feb, 20208 Jan, 2020 + First complete genome from Japan\_| [711 Feb, 20205 Jan, 2020 CCDC announces a + 3,711 passengersand crew were | |. WHO names the31 Dec, 2019 Wuhan health committee novel coronavirus 24 Jan, 2020 quarantined by the Japanese disease COVID-19Wuhan notes 27, exclude SARS and MERS as isolated from Wuhan | | First complete Ministry of Health on Diamond + Virus was namedpneumonia cases | | possible cause of pneumonia | | pneumonia patient | | genomefrom USA | | Princess cruise ship SARS-CoV-2 by ICTV8 Dec, 2019 ‘Jan, 2020 7 Jan, 2020 10,Jan, 2020 30 Jan, 2020 02 Feb, 2020Symptom onset of China closes Wuhan | | First cell cultureisolation | | First genome released by | |WHOdeclaresitaPublic| | First death reportedthe 41 confirmed seafood market d idan University China Health Emergency of outside of China, in2019-nCoV cases in GenBank International Concern Philippines(05 Apr, 202013May,2020 | | The UniTotal confirmed s 27 Mar, 2020 (06 Mar, 2020cases reach Laboratories confirms Belgium health +3533 people (including 21 20 Feb, 2020 14 Feb, 20204170424 with | | SARS-CoV-2intigerand | | authorities report positive) on board cruise ship |\_\_| First death (n=02) Second death outside China287399 destte possible transmission of | | Grand Princess quarantined off | | reportedon Diamond | | was reported in Japan havingworldwide COVID-19 to domestic cat | | the coast of California (US) Princess cruise ship no travel history to China22 Apr, 2020 (02 Apr, 2020 11 Mar, 2020 28 Feb, 2020 16 Feb, 2020 13 Feb, 2020The US.CDC and the USDA National | | Total confirmed cases WHO declares covio-19 | | Hong Kong authorities Fitst death reported First complete genomeVeterinary Services Laboratories reaches 896,450 with as global pandemic report possible transmission from Europe in France from Taiwan(NVSL) confirms cases of SARS-CoV-2 | | 45,526 deaths worldwide of COVID-19 to pet dogsintwo pet catsFIG 5 Timeline depicting the significant events thatoccurred during the SARS-CoV-2/COVID-19 virusoutbreak. The timeline describes the significant eventsduring the current SARS-CoV-2 outbreak, from 8December 2019 to 13 May 2020.At the beginning, China experienced the majorityof the burden associated with COVID-19 in the formof disease morbidity and mortality (65), but overtime the COVID-19 menace moved to Europe,particularly Italy and Spain, and now the UnitedStates has the hichest number of confirmed casesassociated with severe economic impacts globallydue to the sudden interruption of global trade andsupply chains that forced multinational companies tomake decisions that led to significant economiclosses (66). The recent increase in the number ofconfirmed critically ill patients with COVID-19 hasalready surpassed the intensive care supplies,limiting intensive care services to only a smallportion of critically ill patients (67). This might alsohave contributed to the increased case fatality rateobserved in the COVID-19 outbreak.

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

The novel coronavirus was identified within 1month (28 days) of the outbreak. This is impressivelyfast compared to the time taken to identify SARS-CoV reported in Foshan, Guangdong Province,China (125days) (68). Immediately after theconfirmation of viral etiology, the Chinesevirologists rapidly released the genomic sequence ofSARS-CoV-2, which played a crucial role incontrolling the spread of this newly emerged novelcoronavirus to other parts of the world (69). Thepossible origin of SARS-CoV-2 and the first mode ofdisease transmission are not yet identified (70).Analysis of the initial cluster of infections suggeststhat the infected individuals had a common exposurepoint, a seafood market in Wuhan, Hubei Province,China (Fig. 6). The restaurants of this market arewell-known for providing different types of wildanimals for human consumption (71). The HuananSouth China Seafood Market also sells live animals,such as poultry, bats, snakes, and marmots (72). Thismight be the point where zoonotic (animal-to-human) transmission occurred (71). AlthoughSARS-CoV-2 is alleged to have originated from ananimal host (zoonotic origin) with further human-to-human transmission (Fig. 6), the likelihood offoodborne transmission should be ruled out withfurther investigations, since it is a latent possibility(1). Additionally, other potential and expected routeswould be associated with transmission, as in otherrespiratory viruses, by direct contact, such as shakingcontaminated hands, or by direct contact withcontaminated surfaces (Fig. 6). Still, whether bloodtransfusion and organ transplantation (276), as wellas transplacental and perinatal routes, are possibleroutes for SARS-CoV-2 transmission needs to bedetermined (Fig. 6).associated with known emerging viruses, higherpathogenicity of a virus is often associated withlower transmissibility. Compared to emergingviruses like Ebola virus, avian H7N9, SARS-CoV,and MERS-CoV, SARS-CoV-2 has relatively lowerpathogenicity and moderate transmissibility (15).The risk of death among individuals infected withCOVID-19 was calculated using the infectionfatality risk (IFR). The IFR was found to be in therange of 0.3% to 0.6%, which is comparable to thatof a previous Asian influenza pandemic (1957 to1958) (73, 277).

Notably, the reanalysis of the COVID-19pandemic curve from the initial cluster of casespointed to considerable human-to-humantransmission. It is opined that the exposure history ofSARS-CoV-2 at the Wuhan seafood marketoriginated from human-to-human transmission ratherthan animal-to-human transmission (74); however, inlight of the zoonotic spillover in COVID-19, is tooearly to fully endorse this idea (1). Following theinitial infection, human-to-human transmission hasbeen observed with a preliminary reproductionnumber (Ro) estimate of 1.4 to 2.5 (70, 75), andrecently it is estimated to be 2.24 to 3.58 (76). Inanother study, the average reproductive number ofCOVID-19 was found to be 3.28, which issignificantly higher than the initial WHO estimate of1.4 to 2.5 (77). It is too early to obtain the exact Rovalue, since there is a possibility of bias due toinsufficient data. The higher Ro value is indicative ofthe more significant potential of SARS-CoV-2transmission in a susceptible population. This is notthe first time where the culinary practices of Chinahave been blamed for the origin of novel coronavirusinfection in humans. Previously, the animals presentin the live-animal market were identified to be theintermediate hosts of the SARS outbreak in China(78). Several wildlife species were found to harborpotentially evolving coronavirus strains that canovercome the species barrier (79). One of the mainprinciples of Chinese food culture is that live-slaughtered animals are considered more nutritious(5).

After 4 months of struggle that lasted fromDecember 2019 to March 2020, the COVID-19situation now seems under control in China. The wetanimal markets have reopened, and people havestarted buying bats, dogs, cats, birds, scorpions,badgers, rabbits, pangolins (scaly anteaters), minks,soup from palm civet, ostriches, hamsters, snappingturtles, ducks, fish, Siamese crocodiles, and otheranimal meats without any fear of COVID-19. TheChinese government is encouraging people to feelthey can return to normalcy. However, this could bea risk, as it has been mentioned in advisories thatpeople should avoid contact with live-dead animalsas much as possible, as SARS-CoV-2 has shownzoonotic spillover. Additionally, we cannot rule outthe possibility of new mutations in the same virusbeing closely related to contact with both animalsand humans at the market (284). In January 2020,China imposed a temporary ban on the sale of live-dead animals in wet markets. However, nowhundreds of such wet markets have been reopenedwithout optimizing standard food safety andsanitation practices (286).

With China being the most populated country inthe world and due to its domestic and internationalfood exportation policies, the whole world is nowfacing the menace of COVID-19, including Chinaitself. Wet markets of live-dead animals do notmaintain strict food hygienic practices. Fresh bloodsplashes are present everywhere, on the floor andtabletops, and such food customs could encouragemany pathogens to adapt, mutate, and jump thespecies barrier. As a result, the whole world issuffering from novel SARS-CoV-2, with more than4,170,424 cases and 287,399 deaths across the globe.There is an urgent need for a rational internationalcampaign against the unhealthy food practices ofChina to encourage the sellers to increase hygienicfood practices or close the crude live-dead animalwet markets. There is a need to modify food policiesat national and international levels to avoid furtherlife threats and economic consequences from anyemerging or reemerging pandemic due to closeanimal-human interaction (285).

Even though individuals of all ages and sexes aresusceptible to COVID-19, older people with anunderlying chronic disease are more likely tobecome severely infected (80). Recently, individualswith asymptomatic infection were also found to actas a source of infection to susceptible individuals(81). Both the asymptomatic and symptomaticpatients secrete similar viral loads, which indicatesthat the transmission capacity of asymptomatic orminimally symptomatic patients is very high. Thus,SARS-CoV-2 transmission can happen early in thecourse of infection (82). Atypical clinicalmanifestations have also been reported in COVID-19in which the only reporting symptom was fatigue.Such patients may lack respiratory signs, such asfever, cough, and sputum (83). Hence, the cliniciansmust be on the look-out for the possible occurrenceof atypical clinical manifestations to avoid thepossibility of missed diagnosis. The earlytransmission ability of SARS-CoV-2 was found to besimilar to or slightly higher than that of SARS-CoV,reflecting that it could be controlled despitemoderate to high transmissibility (84).Increasing reports of SARS-CoV-2 in sewage andwastewater warrants the need for furtherinvestigation due to the possibility of fecal-oraltransmission. SARS-CoV-2 present in environmentalcompartments such as soil and water will finally endup in the wastewater and sewage sludge of treatmentplants (328). Therefore, we have to reevaluate thecurrent wastewater and sewage sludge treatmentprocedures and introduce advanced techniques thatare specific and effective against SARS-CoV-2.Since there is active shedding of SARS-CoV-2 in thestool, the prevalence of infections in a\_ largepopulation can be studied using wastewater-basedepidemiology. Recently, reverse transcription-quantitative PCR (RT-qPCR) was used to enumeratethe copies of SARS-CoV-2 RNA concentrated fromwastewater collected from a wastewater treatmentplant (327). The calculated viral RNA copy numbersdetermine the number of infected individuals. Therf e rfnucleic acid test results as one of the additionaldischarge criteria in laboratory-confirmed cases ofCOVID-19 (326).

The COVID-19 pandemic does not have anynovel factors, other than the genetically uniquepathogen and a further possible reservoir. The causeand the likely future outcome are just repetitions ofour previous interactions with fatal coronaviruses.The only difference is the time of occurrence and thegenetic distinctness of the pathogen involved.Mutations on the RBD of CoVs facilitated theircapability of infecting newer hosts, therebyexpanding their reach to all corners of the world(85). This is a potential threat to the health of bothanimals and humans. Advanced studies usingBayesian phylogeographic reconstruction identifiedthe most probable origin of SARS-CoV-2 as the batSARS-like coronavirus, circulating in theRhinolophus bat family (86).

Phylogenetic analysis of 10 whole-genomesequences of SARS-CoV-2 showed that they arerelated to two CoVs of bat origin, namely, bat-SL-CoVZC45 and bat-SL-CoVZXC21, which werereported during 2018 in China (17). It was reportedthat SARS-CoV-2 had been confirmed to use ACE2as an entry receptor while exhibiting an RBD similarto that of SARS-CoV (17, 87, 254, 255). Severalcountries have provided recommendations to theirpeople traveling to China (88, 89). Compared to theprevious coronavirus outbreaks caused by SARS-CoV and MERS-CoV, the efficiency of SARS-CoV-2 human-to-human transmission was thought to beless. This assumption was based on the finding thathealth workers were affected less than they were inprevious outbreaks of fatal coronaviruses (2).Superspreading events are considered the mainculprit for the extensive transmission of SARS andMERS (90, 91). Almost half of the MERS-CoVcases reported in Saudi Arabia are of secondaryorigin that occurred through contact with infectedasymptomatic or symptomatic individuals throughhuman-to-human transmission (92). The occurrenceof superspreading events in the COVID-19 outbreakcannot be ruled out until its possibility is evaluated.Like SARS and MERS, COVID-19 can also infectthe lower respiratory tract, with milder symptoms(27). The basic reproduction number of COVID-19has been found to be in the range of 2.8 to 3.3 basedon real-time reports and 3.2 to 3.9 based on predictedinfected cases (84).

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

Coronavirus infection in humans is commonlyassociated with mild to severe respiratory diseases,with high fever, severe inflammation, cough, andinternal organ dysfunction that can even lead todeath (92). Most of the identified coronavirusescause the common cold in humans. However, thischanged when SARS-CoV was identified, paving theway for severe forms of the disease in humans (22).Our previous experience with the outbreaks of othercoronaviruses, like SARS and MERS, suggests thatthe mode of transmission in COVID-19 as mainlyhuman-to-human transmission via direct contact,droplets, and fomites (25). Recent studies havedemonstrated that the virus could remain viable forhours in aerosols and up to days on surfaces; thus,aerosol and fomite contamination could play potentroles in the transmission of SARS-CoV-2 (257).

The immune response against coronavirus is vitalto control and get rid of the infection. However,maladjusted immune responses may contribute to theimmunopathology of the disease, resulting inimpairment of pulmonary gas \_\_ exchange.Understanding the interaction between CoVs andhost innate immune systems could enlighten ourwith this infection (24).SARS is a viral respiratory disease caused by aformerly unrecognized animal CoV that originatedfrom the wet markets in southern China afteradapting to the human host, thereby enablingtransmission between humans (90). The SARSoutbreak reported in 2002 to 2003 had 8,098confirmed cases with 774 total deaths (9.6%) (93).The outbreak severely affected the Asia Pacificregion, especially mainland China (94). Even thoughthe case fatality rate (CFR) of SARS-CoV-2(COVID-19) is lower than that of SARS-CoV, thereexists a severe concern linked to this outbreak due toits 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 wasfirst 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 thatthe incubation period of SARS-CoV-2, SARS-CoV,and MERS-CoV is in almost the same range. Thelongest predicted incubation time of SARS-CoV-2 is14 days. Hence, suspected individuals are isolatedfor 14 days to avoid the risk of further spread (98).Even though a high similarity has been reportedbetween the genome sequence of the newcoronavirus (SARS-CoV-2) and SARS-like CoVs,the comparative analysis recognized a furin-likecleavage site in the SARS-CoV-2 S protein that ismissing from other SARS-like CoVs (99). The furin-like cleavage site is expected to play a role in the lifecycle of the virus and disease pathogenicity andmight even act as a therapeutic target for furininhibitors. The highly contagious nature of SARS-CoV-2 compared to that of its predecessors might bethe result of a stabilizing mutation that occurred inthe endosome-associated-protein-like domain ofnsp2 protein.

Similarly, the destabilizing mutation near thephosphatase domain of nsp3 proteins in SARS-CoV-2 could indicate a potential mechanism thatdifferentiates it from other CoVs (100). Even thoughthe CFR reported for COVID-19 is meagercompared to those of the previous SARS and MERSoutbreaks, it has caused more deaths than SARS andMERS combined (101). Possibly related to the viralpathogenesis is the recent finding of an 832-nucleotide (nt) deletion in ORF8, which appears toreduce the replicative fitness of the virus and leads toattenuated phenotypes of SARS-CoV-2 (256).Coronavirus is the most prominent example of avirus that has crossed the species barrier twice fromwild animals to humans during SARS and MERSoutbreaks (79, 102). The possibility of crossing thespecies barrier for the third time has also beensuspected in the case of SARS-CoV-2 (COVID-19).Bats are recognized as a possible natural reservoirhost of both SARS-CoV and MERS-CoV infection.In contrast, the possible intermediary host is thepalm civet for SARS-CoV and the dromedary camelfor MERS-CoV infection (102). Bats are consideredthe ancestral hosts for both SARS and MERS (103).Bats are also considered the reservoir host of humancoronaviruses like HCoV-229E and HCoV-NL63(104). In the case of COVID-19, there are twopossibilities for primary transmission: it can betransmitted either through intermediate hosts, similarto that of SARS and MERS, or directly from bats(103). The emergence paradigm put forward in theSARS outbreak suggests that SARS-CoV originatedfrom bats (reservoir host) and later jumped to civets(intermediate host) and incorporated changes withinthe receptor-binding domain (RBD) to improvebinding to civet ACE2. This civet-adapted virus,during their subsequent exposure to humans at livemarkets, promoted further adaptations that resultedin the epidemic strain (104). Transmission can alsoin the epidemic strain (1U4). 1ransmission can alsooccur directly from the reservoir host to humanswithout RBD adaptations. The bat coronavirus that iscurrently in circulation maintains specific “poised”spike proteins that facilitate human infection withoutthe requirement of any mutations or adaptations(105). Altogether, different species of bats carry amassive number of coronaviruses around the world(106).

The high plasticity in receptor usage, along withthe feasibility of adaptive mutation andrecombination, may result in frequent interspeciestransmission of coronavirus from bats to animals andhumans (106). The pathogenesis of most batcoronaviruses is unknown, as most of these virusesare not isolated and studied (4). Hedgehogcoronavirus HKU31, a Betacoronavirus, has beenidentified from amur hedgehogs in China. Studiesshow that hedgehogs are the reservoir ofBetacoronavirus, and there is evidence ofrecombination (107).

The current scientific evidence available onMERS infection suggests that the significantreservoir host, as well as the animal source of MERSinfection in humans, is the dromedary camels (97).The infected dromedary camels may not show any‘sible © infact king it challengiidentify animals actively excreting MERS-CoV thathas the potential to infect humans. However, theymay shed MERS-CoV through milk, urine, feces,and nasal and eye discharge and can also be found inthe raw organs (108). In a study conducted toevaluate the susceptibility of animal species toMERS-CoV infection, llamas and pigs were found tobe susceptible, indicating the possibility of MERS-CoV circulation in animal species other thandromedary camels (109).

Following the outbreak of SARS in China,SARS-CoV-like viruses were isolated fromHimalayan palm civets (Paguma larvata) andraccoon dogs (Nyctereutes procyonoides) found in alive-animal market in Guangdong, China. Theanimal isolates obtained from the live-animal marketretained a 29-nucleotide sequence that was notpresent in most of the human isolates (78). Thesefindings were critical in identifying the possibility ofinterspecies transmission in SARS-CoV. The higherdiversity and prevalence of bat coronaviruses in thisregion compared to those in previous reports indicatea host/pathogen coevolution. SARS -likecoronaviruses also have been found circulating in theChinese horseshoe bat (Rhinolophus \_ sinicus)populations. The in vitro and in vivo studies carriedout on the isolated virus confirmed that there is apotential risk for the reemergence of SARS-CoVinfection from the viruses that are currentlycirculating in the bat population (105).

**CLINICAL PATHOLOGY OF SARS-CoV-2**

(COVID-19)The disease caused by SARS-CoV-2 is alsonamed severe specific contagious pneumonia(SSCP), Wuhan pneumonia, and, recently, COVID-19 (110). Compared to SARS-CoV, SARS-CoV-2has less severe pathogenesis but has superiortransmission capability, as evidenced by the rapidlyincreasing number of COVID-19 cases (111). Theincubation period of SARS-CoV-2 in familialclusters was found to be 3 to 6 days (112). The meanincubation period of COVID-19 was found to be 6.4days, ranging from 2.1 to 11.1 days (113). Among anearly affected group of 425 patients, 59 years was themedian age, of which more males were affected(114). Similar to SARS and MERS, the severity ofthis 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 tothe SARS-CoV-2-infected patients in Wuhan duringsymptoms were noticed in those patients that areinfected by human-to-human transmission (14).

The initial trends suggested that the mortalityassociated with COVID-19 was less than that ofprevious outbreaks of SARS (101). The updatesobtained from countries like China, Japan, Thailand,and South Korea indicated that the COVID-19patients had relatively mild manifestations comparedto those with SARS and MERS (4). Regardless ofthe coronavirus type, immune cells, like mast cells,that are present in the submucosa of the respiratorytract and nasal cavity are considered the primarybarrier against this virus (92). Advanced in-depthanalysis of the genome has identified 380 amino acidsubstitutions between the amino acid sequences ofSARS-CoV-2 and the SARS/SARS-likecoronaviruses. These differences in the amino acidsequences might have contributed to the differencein the pathogenic divergence of SARS-CoV-2 (16).Further research is required to evaluate the possibledifferences in tropism, pathogenesis, andtransmission of this novel agent associated with thischange in the amino acid sequence. With the currentoutbreak of COVID-19, there is an expectancy of asignificant increase in the number of publishedstudies about this emerging coronavirus, as occurredSARS-CoV-2 invades the lung parenchyma,resulting in severe interstitial inflammation of thelungs. This is evident on computed tomography (CT)images as ground-glass opacity in the lungs. Thislesion initially involves a single lobe but laterexpands to multiple lung lobes (118). Thehistological assessment of lung biopsy samplesobtained from COVID-19-infected patients revealeddiffuse alveolar damage, cellular fibromyxoidexudates, hyaline membrane formation, anddesquamation of pneumocytes, indicative of acuterespiratory distress syndrome (119). It was alsofound that the SARS-CoV-2-infected patients oftenhave lymphocytopenia with or without leukocyteabnormalities. The degree of lymphocytopenia givesan idea about disease prognosis, as it is found to bepositively correlated with disease severity (118).Pregnant women are considered to have a higher riskof getting infected by COVID-19. The coronavirusescan cause adverse outcomes for the fetus, such asintrauterine growth restriction, spontaneous abortion,preterm delivery, and perinatal death.

Nevertheless, the possibility of intrauterinematernal-fetal transmission (vertical transmission) ofCoVs is low and was not seen during either theSARS- or MERS-CoV outbreak (120). However,there has been concern regarding the impact ofSARS-CoV-2/COVID-19 on pregnancy. Researchershave mentioned the probability of in uterotransmission of novel SARS-CoV-2 from COVID-19-infected mothers to their neonates in China basedupon the rise in IgM and IgG antibody levels andcytokine values in the blood obtained from newborninfants immediately postbirth; however, RI-PCRfailed to confirm the presence of SARS-CoV-2genetic material in the infants (283). Recent studiesshow that at least in some cases, preterm deliveryand its consequences are associated with the virus.Nonetheless, some cases have raised doubts for thelikelihood of vertical transmission (240—243).

COVID-19 infection was associated withpneumonia, and some developed acute respiratorydistress syndrome (ARDS). The blood biochemistryindexes, such as albumin, lactate dehydrogenase, C-reactive protein, lymphocytes (percent), andneutrophils (percent) give an idea about the diseaseseverity in COVID-19 infection (121). DuringCOVID-19, patients may present leukocytosis,leukopenia with lymphopenia (244),hypoalbuminemia, and an increase of lactatedehydrogenase, aspartate transaminase, alanineaminotransferase, bilirubin, and, expecially, D-dimerSPR AAN We VGW 4 +17 171 1 "114(244). Middle-aged and elderly patients with primarychronic diseases, especially high blood pressure anddiabetes, were found to be more susceptible torespiratory failure and, therefore, had poorerprognoses. Providing respiratory support at earlystages improved the disease prognosis and facilitatedrecovery (18). The ARDS in COVID-19 is due to theoccurrence of cytokine storms that results inexaggerated immune response, immune regulatorynetwork imbalance, and, finally, miultiple-organfailure (122). In addition to the exaggeratedinflammatory response seen in patients withCOVID-19 pneumonia, the bile duct epithelial cell-derived hepatocytes upregulate ACE2 expression inliver tissue by compensatory proliferation that mightresult in hepatic tissue injury (123).

**CORONAVIRUSES IN ANIMALS ANDZOONOTIC LINKS—A BRIEF VIEWPOINT**

Coronavirus can cause disease in several speciesof domestic and wild animals, as well as humans(23). The different animal species that are infectedwith 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 todifferent kinds of clinical manifestations, varyingfrom enteritis in cows and pigs, upper respiratorydisease in chickens, and fatal respiratory infectionsin humans (30).

Among the CoV genera, Alphacoronavirus andBetacoronavirus infect mammals, whileGammacoronavirus and Deltacoronavirus mainlyinfect birds, fishes, and, sometimes, mammals (27,29, 106). Several novel coronaviruses that comeunder the genus Deltacoronavirus have beendiscovered in the past from birds, like Wigeoncoronavirus HKU20, Bulbul coronavirus HKU11,Munia coronavirus HKU13, white-eye coronavirusHKU16, night-heron coronavirus HKU19, andcommon moorhen coronavirus HKU21, as well asfrom pigs (porcine coronavirus HKU15) (6, 29).Transmissible gastroenteritis virus (TGEV), porcineepidemic diarrhea virus (PEDV), and \_ porcinehemagglutinating encephalomyelitis virus (PHEV)are some of the coronaviruses of swine. Amongthem, TGEV and PEDV are responsible for causingsevere gastroenteritis in young piglets withnoteworthy morbidity and mortality. Infection withPHEV also causes enteric infection but can causeencephalitis due to its ability to infect the nervousBovine coronaviruses (BoCoVs) are known toinfect several domestic and wild ruminants (126).BoCoV inflicts neonatal calf diarrhea in adult cattle,leading to bloody diarrhea (winter dysentery) andrespiratory disease complex (shipping fever) in cattleof all age groups (126). BoCoV-like viruses havebeen noted in humans, suggesting its zoonoticpotential as well (127). Feline enteric and felineinfectious peritonitis (FIP) viruses are the two majorfeline CoVs (128), where feline CoVs can affect thegastrointestinal tract, abdominal cavity (peritonitis),respiratory tract, and central nervous system (128).Canines are also affected by CoVs that fall underdifferent genera, namely, canine enteric coronavirusin Alphacoronavirus and canine \_\_ respiratorycoronavirus in Betacoronavirus, affecting the entericand respiratory tract, respectively (129, 130). IBV,under Gammacoronavirus, causes diseases ofrespiratory, urinary, and reproductive systems, withsubstantial economic losses in chickens (131, 132).In small laboratory animals, mouse hepatitis virus,rat sialodacryoadenitis coronavirus, and guinea pigand rabbit coronaviruses are the major CoVsassociated with disease manifestations like enteritis,hepatitis, and respiratory infections (10, 133).

Swine acute diarrhea syndrome coronavirus(SADS-CoV) was first identified in suckling pigletshaving severe enteritis and belongs to the genusAlphacoronavirus (106). The outbreak wasassociated with considerable scale mortality ofpiglets (24,693 deaths) across four farms in China(134). The virus isolated from the piglets was almostidentical to and had 95% genomic similarity withhorseshoe bat (Rhinolophus species) coronavirusHKU2, suggesting a bat origin of the pig virus (106,134, 135). It is also imperative to note that theSADS-CoV outbreak started in Guangdong province,near the location of the SARS pandemic origin(134). Before this outbreak, pigs were not known tobe infected with bat-origin coronaviruses. Thisindicates that the bat-origin coronavirus jumped topig by breaking the species barrier. The next step ofthis jump might not end well, since pigs areconsidered the mixing vessel for influenza A virusesdue to their ability to be infected by both human andavian influenza A viruses (136).

Similarly, they may act as the mixing vessel forcoronaviruses, since they are in frequent contact withboth humans and multiple wildlife species.Additionally, pigs are also found to be susceptible toinfection with human SARS-CoV and MERS-CoV,making this scenario a nightmare (109, 137). It iscoronavirus results in an epidemic by jumping theso-called species barrier (287).

The host spectrum of coronavirus increased whena novel coronavirus, namely, SW1, was recognizedin the liver tissue of a captive beluga whale(Delphinapterus leucas) (138). In recent decades,several novel coronaviruses were identified fromdifferent animal species. Bats can harbor theseviruses without manifesting any clinical disease butare persistently infected (30). They are the onlymammals with the capacity for self-powered flight,which enables them to migrate long distances, unlikeland mammals. Bats are distributed worldwide andalso account for about a fifth of all mammalianspecies (6). This makes them the ideal reservoir hostfor many viral agents and also the source of novelcoronaviruses that have yet to be identified. It hasbecome a necessity to study the diversity ofcoronavirus in the bat population to prevent futureoutbreaks that could jeopardize livestock and publichealth. The repeated outbreaks caused by bat-origincoronaviruses calls for the development of efficientmolecular surveillance strategies for studyingBetacoronavirus among animals (12), especially inthe Rhinolophus bat family (86). Chinese bats havehigh commercial value, since they are used intraditional Chinese medicine (TCM). Therefore, thehandling of bats for trading purposes poses aconsiderable risk of transmitting zoonotic CoVepidemics (139).Due to the possible role played by farm and wildanimals in SARS-CoV-2 infection, the WHO, intheir novel coronavirus (COVID-19) situation report,recommended the avoidance of unprotected contactwith both farm and wild animals (25). The live-animal markets, like the one in Guangdong, China,provides a setting for animal coronaviruses toamplify and to be transmitted to new hosts, likehumans (78). Such markets can be considered acritical place for the origin of novel zoonoticdiseases and have enormous public healthsignificance in the event of an outbreak. Bats are thereservoirs for several viruses; hence, the role of batsin the present outbreak cannot be ruled out (140). Ina qualitative study conducted for evaluating thezoonotic risk factors among rural communities ofsouthern China, the frequent human-animalinteractions along with the low levels ofenvironmental biosecurity were identified assignificant risks for the emergence of zoonoticdisease in local communities (141, 142).

The comprehensive sequence analysis of theSARS-CoV-2 RNA genome identified that the CoVfrom Wuhan is a recombinant virus of the batcoronavirus and another coronavirus of unknownorigin. The recombination was found to havehappened within the viral spike glycoprotein, whichrecognizes the cell surface receptor. Further analysisof the genome based on codon usage identified thesnake as the most probable animal reservoir ofSARS-CoV-2 (143). Contrary to these findings,another genome analysis proposed that the genomeof SARS-CoV-2 is 96% identical to bat coronavirus,reflecting its origin from bats (63). The involvementof bat-derived materials in causing the currentoutbreak cannot be ruled out. High risk is involvedin the production of bat-derived materials for TCMpractices involving the handling of wild bats. Theuse of bats for TCM practices will remain a severerisk for the occurrence of zoonotic coronavirusepidemics in the future (139).

Furthermore, the pangolins are an endangeredspecies of animals that harbor a wide variety ofviruses, including coronaviruses (144). Thecoronavirus isolated from Malayan pangolins (Manisjavanica) showed a very high amino acid identitywith COVID-19 at E (100%), M (98.2%), N(96.7%), and S genes (90.4%). The RBD of S proteinin CoV isolated from pangolin was almost identical(one amino acid difference) to that of SARS-CoV-2.A comparison of the genomes \_ suggestsrecombination between pangolin-CoV-like viruseswith the bat-CoV-RaTG13-like virus. All thissuggests the potential of pangolins to act as theintermediate host of SARS-CoV-2 (145).Human-wildlife interactions, which § areincreasing in the context of climate change (142), arefurther considered high risk and responsible for theemergence of SARS-CoV. COVID-19 is alsosuspected of having a similar mode of origin. Hence,to prevent the occurrence of another zoonoticspillover (1), exhaustive coordinated efforts areneeded to identify the high-risk pathogens harboredby wild animal populations, conducting surveillanceamong the people who are susceptible to zoonoticspillover events (12), and to improve the biosecuritymeasures associated with the wildlife trade (146).The serological surveillance studies conducted inpeople living in proximity to bat caves had earlieridentified the serological confirmation of SARS-related CoVs in humans. People living at thewildlife-human interface, mainly in rural China, areregularly exposed to SARS-related CoVs (147).These findings will not have any significance until asignificant outbreak occurs due to a virus-likeSARS-CoV-2.

There is a steady increase in the reports ofCOVID-19 in companion and wild animals aroundthe world. Further studies are required to evaluatethe potential of animals (especially companionanimals) to serve as an efficient reservoir host thatcan further alter the dynamics of human-to-humantransmission (330). To date, two pet dogs (HongKong) and four pet cats (one each from Belgium andHong Kong, two from the United States) have testedpositive for SARS-CoV-2 (335). The WorldOrganization for Animal Health (OIE) has confirmedthe diagnosis of COVID-19 in both dogs and catsdue to human-to-animal transmission (331). Thesimilarity observed in the gene sequence of SARS-CoV-2 from an infected pet owner and his dogfurther confirms the occurrence of human-to-animaltransmission (333). Even though asymptomatic,feline species should be considered a\_ potentialtransmission 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 thatcats are susceptible to SARS-CoV-2 and can getinfected by human beings. However, evidence of cat-to-human transmission is lacking and requiresfurther studies (332). Rather than waiting for firmerevidence on animal-to-human transmission,necessary preventive measures are advised, as wellas following social distancing practices amongcompanion animals of different households (331).One of the leading veterinary diagnostic companies,IDEXX, has conducted large-scale testing forCOVID-19 in specimens collected from dogs andcats. However, none of the tests turned out to bepositive (334).

In a study conducted to investigate the potentialof different animal species to act as the intermediatehost of SARS-CoV-2, it was found that both ferretsand cats can be infected via experimental inoculationof the virus. In addition, infected cats efficientlytransmitted the disease to naive cats (329). SARS-CoV-2 infection and subsequent transmission inferrets were found to recapitulate the clinical aspectsof COVID-19 in humans. The infected ferrets alsoshed virus via multiple routes, such as saliva, nasalwashes, feces, and urine, postinfection, making theman ideal animal model for studying diseasetransmission (337). Experimental inoculation wasalso done in other animal species and found that thedogs have low susceptibility, while the chickens,ducks, and pigs are not at all susceptible to SARS-CoV-2 (329).Similarly, the National Veterinary ServicesLaboratories of the USDA have reported COVID-19in tigers and lions that exhibited respiratory signslike dry cough and wheezing. The zoo animals aresuspected to have been infected by an asymptomaticzookeeper (335). The total number of COVID-19-positive cases in human beings is increasing at a highrate, thereby creating ideal conditions for viralspillover to other species, such as pigs. The evidenceobtained from SARS-CoV suggests that pigs can getinfected with SARS-CoV-2 (336). However,experimental inoculation with SARS-CoV-2 failed toinfect pigs (329).

Further studies are required to identify thepossible animal reservoirs of SARS-CoV-2 and theseasonal variation in the circulation of these virusesin the animal population. Research collaborationbetween human and animal health sectors isbecoming a necessity to evaluate and identify thepossible risk factors of transmission between animalsand humans. Such cooperation will help to deviseefficient strategies for the management of emergingzoonotic 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 ornext-generation sequencing (148, 149, 245, 246). Atpresent, nucleic acid detection techniques, like RT-PCR, are considered an effective method forconfirming the diagnosis in clinical cases of COVID-19 (148). Several companies across the world arecurrently focusing on developing and marketingSARS-CoV-2-specific nucleic acid detection kits.Multiple laboratories are also developing their ownin-house RT-PCR. One of them is the SARS-CoV-2nucleic acid detection kit produced by ShuoshiBiotechnology (double fluorescence PCR method)(150). Up to 30 March 2020, the U.S. Food and DrugAdministration (FDA) had granted 22 in vitrodiagnostics Emergency Use Authorizations (EUAs),including for the RT-PCR diagnostic panel for theuniversal detection of SARS-like betacoronavirusesand specific detection of SARS-CoV-2, developedby the U.S. CDC (Table 1) (258, 259).SARAS-CoV-2. strains available in the NationalCenter for Biotechnology Information and GISAIDdatabases were subjected to multiple-sequencealignment and phylogenetic analyses for studyingvariations in the viral genome (260). All the viralstrains 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. Overallvariation was found to be low in ORF regions, with13 variation sites recognized in la, 1b, S, 3a, M, 8,and N regions. Mutation rates of 30.53% (29/95) and29.47% (28/95) were observed at nt 28144 (ORF8)and nt 8782 (ORF 1a) positions, respectively. Owingto such selective mutations, a few specific regions ofSARS-CoV-2 should not be considered for designingprimers and probes. The SARS-CoV-2 referencesequence could pave the way to study molecularbiology and pathobiology, along with developingdiagnostics and appropriate prevention and controlstrategies for countering SARS-CoV-2 (260).

Nucleic acids of SARS-CoV-2 can be detectedfrom samples (64) such as bronchoalveolar lavagefluid, sputum, nasal swabs, fiber bronchoscope brushbiopsy specimen, pharyngeal swabs, feces, blood,and urine, with different levels of diagnosticperformance (Table 2) (80, 245, 246). The viral loadsof SARS-CoV-2 were measured using N-gene-specific quantitative RT-PCR in throat swab andsputum samples collected from COVID-19-infectedindividuals. The results indicated that the viral loadpeaked at around 5 to 6 days following the onset ofsymptoms, and it ranged from 10\* to 107 copies/mlduring this time (151). In another study, the viralload was found to be higher in the nasal swabs thanthe throat swabs obtained from COVID-19symptomatic patients (82). Although initially it wasthought that viral load would be associated with pooroutcomes, some case reports have shownasymptomatic individuals with high viral loads(247). Recently, the viral load in nasal and throatswabs of 17 symptomatic patients was determined,and higher viral loads were recorded soon after theonset of symptoms, particularly in the nosecompared to the throat. The pattern of viral nucleicacid shedding of SARS-CoV-2-infected patients wassimilar to that of influenza patients but seemed to bedifferent from that of SARS-CoV patients. The viralload detected in asymptomatic patients resembledthat of symptomatic patients as studied in China,which reflects the transmission perspective ofasymptomatic or symptomatic patients havingminimum signs and symptoms (82). Another study,Bas YUP ly atl UL Sy Up ryiliatle Paelielils did Vilisminimum signs and symptoms (82). Another study,conducted in South Korea, related to SARS-CoV-2viral load, opined that SARS-CoV-2 kinetics weresignificantly different from those of earlier reportedCoV infections, including SARS-CoV (253). SARS-CoV-2 transmission can occur early in the viralinfection phase; thus, diagnosing cases and isolationattempts for this virus warrant different strategiesthan those needed to counter SARS-CoV. Studies arerequired to establish any correlation between SARS-CoV-2 viral load and cultivable virus. Recognizingpatients with fewer or no symptoms, along withhaving modest detectable viral RNA in\_ theoropharynx for 5 days, indicates the requirement ofdata for assessing SARS-CoV-2 transmissiondynamics and updating the screening procedures inthe clinics (82).

The results of the studies related to SARS-CoV-2viral loads reflect active replication of this virus inthe upper respiratory tract and prolonged viralshedding after symptoms disappear, including viastool. Thus, the current case definition needs to beupdated along with a reassessment of the strategiesto be adopted for restraining the SARS-CoV-2outbreak spread (248). In some cases, the viral loadstudies of SARS-CoV-2 have also been useful torecommend precautionary measures when handlingspecific samples, e.g., feces. In a recent survey from17 confirmed cases of SARS-CoV-2 infection withavailable data (representing days 0 to 13 after onset),stool samples from nine cases (53%; days 0 to 11after onset) were positive on RT-PCR analysis.Although the viral loads were lower than those ofrespiratory samples (range, 550 copies per ml to1.21 x 10° copies per ml), this has essential biosafetyimplications (151).The samples from 18 SARS-CoV-2-positivepatients in Singapore who had traveled from Wuhanto Singapore showed the presence of viral RNA instool and whole blood but not in urine by real-timeRT-PCR (288). Further, novel SARS-CoV-2infections have been detected in a variety of clinicalspecimens, like bronchoalveolar lavage fluid,sputum, nasal swabs, fibrobronchoscope brushbiopsy specimens, pharyngeal swabs, feces, andblood (246).

The presence of SARS-CoV-2 in fecal sampleshas posed grave public health concerns. In additionto the direct transmission mainly occurring viadroplets of sneezing and coughing, other routes, suchas fecal excretion and environmental and fomitecontamination, are contributing to SARS-CoV-2transmission and spread (249-252). Fecal excretionhas also been documented for SARS-CoV andMERS-CoV, along with the potential to stay viablein situations aiding fecal-oral transmission. Thus,SARS-CoV-2 has every possibility to be transmittedthrough this mode. Fecal-oral transmission of SARS-CoV-2, particularly in regions having low standardsof hygiene and poor sanitation, may have graveconsequences with regard to the high spread of thisvirus. Ethanol and disinfectants containing chlorineor bleach are effective against coronaviruses(249-252). Appropriate precautions need to befollowed strictly while handling the stools of patientsinfected with SARS-CoV-2. Biowaste materials andsewage from hospitals must be adequatelydisinfected, treated, and disposed of properly. Thesignificance of frequent and good hand hygiene andsanitation practices needs to be given due emphasis(249-252). Future explorative research needs to beconducted with regard to the fecal-oral transmissionof SARS-CoV-2, along with focusing onenvironmental investigations to find out if this viruscould stay viable in situations and atmospheresfacilitating such potent routes of transmission. Thecorrelation of fecal concentrations of viral RNA withdisease severity needs to be determined, along withassessing the gastrointestinal symptoms and thepossibility of fecal SARS-CoV-2 RNA detectionduring the COVID-19 incubation period orconvalescence phases of the disease (249-252).

The lower respiratory tract sampling techniques,like bronchoalveolar lavage fluid aspirate, areconsidered the ideal clinical materials, rather thanthe throat swab, due to their higher positive rate onthe nucleic acid test (148). The diagnosis of COVID-19 can be made by using upper-respiratory-tractspecimens collected using nasopharyngeal andoropharyngeal swabs. However, these techniques areassociated with unnecessary risks to health careworkers due to close contact with patients (152).Similarly, a single patient with a high viral load wasreported to contaminate an entire endoscopy room byshedding the virus, which may remain viable for atleast 3 days and is considered a great risk foruninfected patients and health care workers (289).Recently, it was found that the anal swabs gave morepositive results than oral swabs in the later stages ofinfection (153). Hence, clinicians have to be cautiouswhile discharging any COVID-19-infected patientbased on negative oral swab test results due to thepossibility of fecal-oral transmission. Even thoughthe viral loads in stool samples were found to be lessthan those of respiratory samples, \_ strictprecautionary measures have to be followed whilehandling stool samples of COVID-19 suspected orinfected patients (151). Children infected withSARS-CoV-2 experience only a mild form of illnessand recover immediately after treatment. It wasrecently found that stool samples of SARS-CoV-2-infected children that gave negative throat swabresults were positive within ten days of negativeresults. This could result in the fecal-oraltransmission of SARS-CoV-2 infections, especiallyin children (290). Hence, to prevent the fecal-oraltransmission of SARS-CoV-2, infected COVID-19patients should only be considered negative whenthey test negative for SARS-CoV-2 in the stoolsample.to be confirmed if the respiratory tract aspirate orblood samples test positive for SARS-CoV-2 nucleicacid using RT-PCR or by the identification of SARS-CoV-2 genetic sequence in respiratory tract aspirateor blood samples (80). The patient will be confirmedas cured when two subsequent oral swab results arenegative (153). Recently, the live virus was detectedin the self-collected saliva of patients infected withCOVID-19. These findings were confirmative ofusing saliva as a noninvasive specimen for thediagnosis of COVID-19 infection in suspectedindividuals (152). It has also been observed that theinitial screening of COVID-19 patients infected withRT-PCR may give negative results even if they havechest CT findings that are suggestive of infection.Hence, for the accurate diagnosis of COVID-19, acombination of repeated swab tests using RT-PCRand CT scanning is required to prevent thepossibility of false-negative results during diseasescreening (154). RT-PCR is the most widely used testfor diagnosing COVID-19. However, it has somesignificant limitations from the clinical perspective,since it will not give any clarity regarding diseaseprogression. Droplet digital PCR (ddPCR) can beused for the quantification of viral load in thesamples obtained from lower respiratory tracts.Hence, based on the viral load, we can quicklyevaluate the progression of infection (291). Inaddition to all of the above findings, sequencing andphylogenetics are critical in the correct identificationand confirmation of the causative viral agent anduseful to establish relationships with previousisolates and sequences, as well as to know, especiallyduring an epidemic, the nucleotide and amino acidmutations and the molecular divergence. The rapiddevelopment and implementation of diagnostic testsagainst emerging novel diseases like COVID-19pose significant challenges due to the lack ofresources and logistical limitations associated withan outbreak (155).

SARS-CoV-2 infection can also be confirmed byisolation and culturing. The human airway epithelialcell culture was found to be useful in isolatingSARS-CoV-2 (3). The efficient control of anoutbreak depends on the rapid diagnosis of thedisease. Recently, in response to the COVID-19outbreak, 1-step quantitative real-time reversetranscription-PCR assays were developed that detectthe ORF1b and N regions of the SARS-CoV-2genome (156). That assay was found to achieve therapid detection of SARS-CoV-2. Nucleic acid-basedassays offer high accuracy in the diagnosis of SARS-CoV-2, but the current rate of spread limits its usedue to the lack of diagnostic assay kits. This willfurther result in the extensive transmission ofCOVID-19, since only a portion of suspected casescan be diagnosed. In such situations, conventionalserological assays, like enzyme-linkedimmunosorbent assay (ELISA), that are specific toCOVID-19 IgM and IgG antibodies can be used as ahigh-throughput alternative (149). At present, thereis no diagnostic kit available for detecting the SARS-CoV-2 antibody (150). The specific antibody profilesof COVID-19 patients were analyzed, and it wasfound that the IgM level lasted more than 1 month,indicating a prolonged stage of virus replication inSARS-CoV-2-infected patients. The IgG levels werefound to increase only in the later stages of thedisease. These findings indicate that the specificantibody profiles of SARS-CoV-2 and SARS-CoVwere similar (325). These findings can be utilized forthe development of specific diagnostic tests againstCOVID-19 and can be used for rapid screening.Even though diagnostic test kits are already availablethat can detect the genetic sequences of SARS-CoV-2 (95), their availability is a concern, as the numberof COVID-19 cases is skyrocketing (155, 157). Amajor problem associated with this diagnostic kit isthat it works only when the test subject has an activeinfection, limiting its use to the earlier stages ofinfection. Several laboratories around the world arecurrently developing antibody-based diagnostic testsagainst SARS-CoV-2 (157).

Chest CT is an ideal diagnostic tool foridentifying viral pneumonia. The sensitivity of chestCT is far superior to that of X-ray screening. Thechest CT findings associated with COVID-19-infected patients include characteristic patchyinfiltration that later progresses to ground-glassopacities (158). Early manifestations of COVID-19pneumonia might not be evident in X-ray chestradiography. In such situations, a chest CTexamination can be performed, as it is consideredhighly specific for COVID-19 pneumonia (118).Those patients having COVID-19 pneumonia willexhibit the typical ground-glass opacity in their chestCT images (154). The patients infected withCOVID-19 had elevated plasma angiotensin 2 levels.The level of angiotensin 2 was found to be linearlyassociated with viral load and lung injury, indicatingits potential as a diagnostic biomarker (121). Thechest CT imaging abnormalities associated withCOVID-19 pneumonia have also been observed evenin asymptomatic patients. These abnormalitiesprogress from the initial focal unilateral to diffusebilateral ground-glass opacities and will furtherprogress to or coexist with lung consolidationchanges within 1 to 3 weeks (159). The role playedby radiologists in the current scenario is veryimportant. Radiologists can help in the earlydiagnosis of lung abnormalities associated withCOVID-19 pneumonia. They can also help in theevaluation of disease severity, identifying itsprogression to acute respiratory distress syndromeand the presence of secondary bacterial infections(160). Even though chest CT is considered anessential diagnostic tool for COVID-19, theextensive use of CT for screening purposes in thesuspected individuals might be associated with adisproportionate risk-benefit ratio due to increasedradiation exposure as well as increased risk of cross-infection. Hence, the use of CT for early diagnosis ofSARS-CoV-2 infection in high-risk groups should bedone with great caution (292).

More recently, other advanced diagnostics havebeen designed and developed for the detection ofSARS-CoV-2 (345, 347, 350-352). A reversetranscriptional loop-mediated isothermalamplification (RT-LAMP), namely, iLACO, has beendeveloped for rapid and colorimetric detection of thisvirus (354). RT-LAMP serves as a simple, rapid, andsensitive diagnostic method that does not requiresophisticated equipment or skilled personnel (349).An interactive web-based dashboard for trackingSARS-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 POCTcombined with LAMP, is a useful point-of-carediagnostic (353). An Abbott ID Now COVID-19molecular POCT-based test, using isothermal nucleicacid amplification technology, has been designed asa point-of-care test for very rapid detection ofSARS-CoV-2 in just 5 min (344). A CRISPR-basedSHERLOCK (specific high-sensitivity enzymaticreporter unlocking) diagnostic for rapid detection ofSARS-CoV-2 without the requirement of specializedinstrumentation has been reported to be very usefulin the clinical diagnosis of COVID-19 (360). ACRISPR-Cas12-based lateral flow assay also hasbeen developed for rapid detection of SARS-CoV-2(346). Artificial intelligence, by means of a three-dimensional deep-learning model, has \_ beendeveloped for sensitive and specific diagnosis ofCOVID-19 via CT images (332).

Tracking and mapping of the rising incidencerates, disease outbreaks, community \_ spread,clustered transmission events, hot spots, andsuperspreader potential of SARS-CoV-2/COVIDwarrant full exploitation of real-time diseasemapping by employing geographical informationsystems (GIS), such as the GIS software Kosmo 3.1,web-based real-time tools and dashboards, apps, andadvances in information technology (356-359).Researchers have also developed a few predictiontools/models, such as the prediction model risk ofbias assessment tool (PROBAST) and criticalappraisal and data extraction for systematic reviewsof prediction modeling studies (CHARMS), whichcould aid in assessing the possibility of gettinginfection and estimating the prognosis in patients;however, such models may suffer from bias issuesand, hence, cannot be considered completelytrustworthy, which necessitates the development ofnew and reliable predictors (360).

**VACCINES, THERAPEUTICS, AND DRUGS**

Recently emerged viruses, such as Zika, Ebola,and Nipah viruses, and their grave threats to humanshave begun a race in exploring the designing anddeveloping of advanced vaccines, prophylactics,therapeutics, and drug regimens to counter emergingviruses (161-163, 280). Several attempts are beingmade to design and develop vaccines for CoVinfection, mostly by targeting the spike glycoprotein.Nevertheless, owing to extensive diversity inantigenic variants, cross-protection rendered by thevaccines is significantly limited, even within thestrains of a phylogenetic subcluster (104). Due to thelack of effective antiviral therapy and vaccines in thepresent scenario, we need to depend solely onimplementing effective infection control measures tolessen the risk of possible nosocomial transmission(68). Recently, the receptor for SARS-CoV-2 wasestablished as the human angiotensin-convertingenzyme 2 (hACE2), and the virus was found to enterthe host cell mainly through endocytosis. It was alsofound that the major components that have a criticalrole in viral entry include PIKfyve, TPC2, andcathepsin L. These findings are critical, since thecomponents described above might act as candidatesfor vaccines or therapeutic drugs against SARS-CoV-2 (293).

The majority of the treatment options andstrategies that are being evaluated for SARS-CoV-2(COVID-19) have been taken from our previousexperiences in treating SARS-CoV, MERS-CoV, andother emerging viral diseases. Several therapeuticand preventive strategies, including vaccines,immunotherapeutics, and antiviral drugs, have beenexploited against the previous CoV \_ outbreaks(SARS-CoV and MERS-CoV) (8, 104, 164-167).These valuable options have already been evaluatedfor their potency, efficacy, and safety, along withseveral other types of current research that will fuelour search for ideal therapeutic agents againstCOVID-19 (7, 9, 19, 21, 36). The primary cause ofthe unavailability of approved and commercialvaccines, drugs, and therapeutics to counter theearlier SARS-CoV and MERS-CoV seems to owe tothe lesser attention of the biomedicine andpharmaceutical companies, as these two CoVs didnot cause much havoc, global threat, and panic likethose posed by the SARS-CoV-2 pandemic (19).Moreover, for such outbreak situations, therequirement for vaccines and\_ therapeutics/drugsexists only for a limited period, until the outbreak iscontrolled. The proportion of the human populationinfected with SARS-CoV and MERS-CoV was alsomuch lower across the globe, failing to attract drugand vaccine manufacturers and producers. Therefore,by the time an effective drug or vaccine is designedagainst such disease outbreaks, the virus would havebeen controlled by adopting appropriate and strictprevention and control measures, and patients forclinical trials will not be available. The newlydeveloped drugs cannot be marketed due to the lackof end users.

**Vaccines**

The S protein plays a significant role in theinduction of protective immunity against SARS-CoVby mediating T-cell responses and neutralizingantibody production (168). In the past few decades,we have seen several attempts to develop a vaccineagainst human coronaviruses by using S protein asthe target (168, 169). However, the developedvaccines have minimal application, even amongclosely related strains of the virus, due to a lack ofcross-protection. That is mainly because of theextensive diversity existing among the differentantigenic variants of the virus (104). Thecontributions of the structural proteins, like spike(S), matrix (M), small envelope (E), andnucleocapsid (N) proteins, of SARS-CoV to induceprotective immunity has been evaluated byexpressing them in a recombinant parainfluenzavirus type 3 vector (BHPIV3). Of note, the resultwas conclusive that the expression of M, E, or Nproteins without the presence of S protein would notconfer any noticeable protection, with the absence ofdetectable serum SARS-CoV-neutralizing antibodies(170). Antigenic determinant sites present over S andN\_ structural proteins of SARS-CoV-2 can beexplored as suitable vaccine candidates (294). In theAsian population, S, E, M, and N proteins of SARS-CoV-2 are being targeted for developing subunitvaccines against COVID-19 (295).

The identification of the immunodominant regionamong the subunits and domains of S protein iscritical for developing an effective vaccine againstthe coronavirus. The C-terminal domain of the S1subunit is considered the immunodominant region ofthe porcine deltacoronavirus S\_ protein (171).Similarly, further investigations are needed todetermine the immunodominant regions of SARS-CoV-2 for facilitating vaccine development.

However, our previous attempts to develop auniversal vaccine that is effective for both SARS-CoV and MERS-CoV based on T-cell epitopesimilarity pointed out the possibility of cross-reactivity among coronaviruses (172). That can bemade possible by selected potential vaccine targetsthat are common to both viruses. SARS-CoV-2 hasbeen reported to be closely related to SARS-CoV(173, 174). Hence, knowledge and understanding ofS protein-based vaccine development in SARS-CoVwill help to identify potential S protein vaccinecandidates in SARS-CoV-2. Therefore, vaccinestrategies based on the whole S protein, S proteinsubunits, or specific potential epitopes of S proteinappear to be the most promising vaccine candidatesagainst coronaviruses. The RBD of the S1 subunit ofS protein has a superior capacity to induceneutralizing antibodies. This property of the RBDcan be utilized for designing potential SARS-CoVvaccines either by using RBD-containingrecombinant proteins or recombinant vectors thatencode RBD (175). Hence, the superior geneticsimilarity existing between SARS-CoV-2 and SARS-CoV can be utilized to repurpose vaccines that haveproven in vitro efficacy against SARS-CoV to beutilized for SARS-CoV-2. The possibility of cross-protection in COVID-19 was evaluated bycomparing the S protein sequences of SARS-CoV-2with that of SARS-CoV. The comparative analysisconfirmed that the variable residues were foundconcentrated on the S1 subunit of S protein, animportant vaccine target of the virus (150). Hence,the possibility of SARS-CoV-specific neutralizingantibodies providing cross-protection to COVID-19might be lower. Further genetic analysis is requiredbetween SARS-CoV-2 and different strains ofSARS-CoV and SARS-like (SL) CoVs to evaluatethe possibility of repurposed vaccines againstCOVID-19. This strategy will be helpful in thescenario of an outbreak, since much time can besaved, because preliminary evaluation, including invitro studies, already would be completed for suchvaccine candidates.

Multiepitope subunit vaccines can be considereda promising preventive strategy against the ongoingCOVID-19 pandemic. Jn silico and advancedimmunoinformatic tools can be used to developmultiepitope subunit vaccines. The vaccines that areengineered by this technique can be further evaluatedusing docking studies and, if found effective, thencan be further evaluated in animal models (365).Identifying epitopes that have the potential tobecome a vaccine candidate is critical to developingan effective vaccine against COVID-19. Theimmunoinformatics approach has been used forrecognizing essential epitopes of cytotoxic Tlymphocytes and B\_ cells from the surfaceglycoprotein of SARS-CoV-2. Recently, a fewepitopes have been recognized from the SARS-CoV-2 surface glycoprotein. The selected epitopesexplored targeting molecular dynamic simulations,evaluating their interaction with corresponding majorhistocompatibility complex class I molecules. Theypotentially induce immune responses (176). Therecombinant vaccine can be designed by using rabiesvirus (RV) as a viral vector. RV can be made toexpress 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 fromchallenge with MERS-CoV (169). The intranasalvaccine in BALB/c mice was found to induce long-lasting neutralizing immunity against MERS spikepseudotyped virus, characterized by the induction ofsystemic IgG, secretory IgA, and lung-residentmemory T-cell responses (177). Immunoinformaticsmethods have been employed for the genome-widescreening of potential vaccine targets among thedifferent immunogens of MERS-CoV (178). The Nprotein and the potential B-cell epitopes of MERS-CoV E\_ protein have been’ suggested asimmunoprotective targets inducing both T-cell andneutralizing antibody responses (178, 179).

The collaborative effort of the researchers ofRocky Mountain Laboratories and Oxford Universityis designing a chimpanzee adenovirus-vectoredvaccine to counter COVID-19 (180). The Coalitionfor Epidemic Preparedness Innovations (CEPI) hasinitiated three programs to design SARS-CoV-2vaccines (181). CEPI has a collaborative project withInovio for designing a MERS-CoV DNA vaccinethat could potentiate effective immunity. CEPI andthe University of Queensland are designing amolecular clamp vaccine platform for MERS-CoVand other pathogens, which could assist in the easieridentification of antigens by the immune system(181). CEPT has also funded Moderna to develon avaccine for COVID-19 in partnership with theVaccine Research Center (VRC) of the NationalInstitute of Allergy and Infectious Diseases (NIAID),part of the National Institutes of Health (NIH) (182).By employing mRNA vaccine platform technology, avaccine candidate expressing SARS-CoV-2 spikeprotein is likely to go through clinical testing in thecoming months (180). On 16 March 2020, JenniferHaller became the first person outside China toreceive an experimental vaccine, developed byModerna, against this pandemic virus. Moderna,along with China’s CanSino Biologics, became thefirst research group to launch small clinical trials ofvaccines against COVID-19. Their study isevaluating the vaccine’s safety and ability to triggerimmune responses (296).

Scientists from all over the world are trying hardto develop working vaccines with robust protectiveimmunity against COVID-19. Vaccine candidates,like mRNA-1273 SARS-CoV-2 vaccine, INO-4800DNA coronavirus vaccine, and adenovirus type 5vector vaccine candidate (Ad5-nCoV), are a fewexamples under phase I clinical trials, while self-amplifying RNA vaccine, oral recombinant COVID-19 vaccine, BNT162, plant-based COVID-19vaccine, and li-Key peptide COVID-19 vaccine areunder preclinical trials (297). Similarly, the WHO,on its official website, has mentioned a detailed listof COVID-19 vaccine agents that are underconsideration. Different phases of trials are ongoingfor live attenuated virus vaccines, formaldehydealum inactivated vaccine, adenovirus type 5 vectorvaccine, LNP-encapsulated mRNA vaccine, DNAplasmid vaccine, and S protein, S-trimer, and li-Keypeptide as a subunit protein vaccine, among others(298). The process of vaccine development usuallytakes approximately ten years, in the case ofinactivated or live attenuated vaccines, since itinvolves the generation of long-term efficacy data.However, this was brought down to 5 years duringthe Ebola emergency for viral vector vaccines. In theurgency associated with the COVID-19 outbreaks,we expect a vaccine by the end of this year (343).The development of an effective vaccine againstCOVID-19 with high speed and precision is thecombined result of advancements in computationalbiology, gene synthesis, protein engineering, and theinvention of advanced manufacturing platforms(342).

The recurring nature of the coronavirus outbreakscalls for the development of a pan-coronavirusvaccine that can produce cross-reactive antibodies.However, the success of such a vaccine relies greatlyon its ability to provide protection not only againstpresent versions of the virus but also the ones thatare likely to emerge in the future. This can beachieved by identifying antibodies that can recognizerelatively conserved epitopes that are maintained assuch even after the occurrence of considerablevariations (362). Even though several vaccineclinical trials are being conducted around the world,pregnant women have been completely excludedfrom these studies. Pregnant women are highlyvulnerable to emerging diseases such as COVID-19due to alterations in the immune system and otherphysiological systems that are associated withpregnancy. Therefore, in the event of successfulvaccine development, pregnant women will not getaccess to the vaccines (361). Hence, it isrecommended that pregnant women be included inthe ongoing vaccine trials, since successfulvaccination in pregnancy will protect the mother,fetus, and newborn.

The heterologous immune effects induced byBacillus Calmette Guérin (BCG) vaccination is apromising strategy for controlling the COVID-19pandemic and requires further investigations. BCG isa widely used vaccine against tuberculosis in high-strain of Mycobacterium bovis. At present, three newclinical trials have been registered to evaluate theprotective role of BCG vaccination against SARS-CoV-2 (363). Recently, a cohort study was conductedto evaluate the impact of childhood BCG vaccinationin COVID-19 PCR \_ positivity rates. However,childhood BCG vaccination was found to beassociated with a rate of COVID-19-positive testresults similar to that of the nonvaccinated group(364). Further studies are required to analyzewhether BCG vaccination in childhood can induceprotective effects against COVID-19 in adulthood.Population genetic studies conducted on 103genomes identified that the SARS-CoV-2 virus hasevolved into two major types, L and S. Among thetwo types, L type is expected to be the mostprevalent (~70%), followed by the S type (~30%)(366). This finding has a significant impact on ourrace to develop an ideal vaccine, since the vaccinecandidate has to target both strains to be consideredeffective. At present, the genetic differences betweenthe L and S types are very small and may not affectthe immune response. However, we can expectfurther genetic variations in the coming days thatcould lead to the emergence of new strains (367).There is no currently licensed specific antiviraltreatment for MERS- and SARS-CoV infections, andthe main focus in clinical settings remains onlessening clinical signs and providing supportivecare (183-186). Effective drugs to manage COVID-19 patients include remdesivir, lopinavir/ritonaviralone or in a blend with interferon beta, convalescentplasma, and monoclonal antibodies (MAbs);however, efficacy and safety issues of these drugsrequire additional clinical trials (187, 281). Acontrolled trial of ritonavir-boosted lopinavir andinterferon alpha 2b treatment was performed onCOVID-19 hospitalized patients(ChiCTR2000029308) (188). In addition, the use ofhydroxychloroquine and \_ tocilizumab for theirpotential role in modulating inflammatory responsesin the lungs and antiviral effect has been proposedand discussed in many research articles. Still, nofool-proof clinical trials have been published (194,196, 197, 261-272). Recently, a clinical trialconducted on adult patients suffering from severeCOVID-19 revealed no benefit of lopinavir-ritonavirtreatment over standard care (273).

The efforts to control SARS-CoV-2 infectionutilize defined strategies as followed against MERSand SARS, along with adopting and strengthening afew precautionary measures owing to the unknownnature of this novel virus (36, 189). Presently, themain course of treatment for severely affectedSARS-CoV-2 patients admitted to hospitals includesmechanical ventilation, intensive care unit (ICU)admittance, and symptomatic and \_ supportivetherapies. Additionally, RNA synthesis inhibitors(lamivudine and tenofovir disoproxil fumarate),remdesivir, neuraminidase inhibitors, peptide (EK 1),anti-inflammatory drugs, abidol, and Chinesetraditional medicine (Lianhuagingwen — andShuFengJieDu capsules) could aid in COVID-19treatment. However, further clinical trials are beingcarried out concerning their safety and efficacy (7).It might require months to a year(s) to design anddevelop effective drugs, therapeutics, and vaccinesagainst COVID-19, with adequate evaluation andapproval from regulatory bodies and moving to thebulk production of many millions of doses atcommercial levels to meet the timely demand ofmass populations across the globe (9). Continuousefforts are also warranted to identify and assessviable drugs and immunotherapeutic regimens thatrevealed proven potency in combating other viralagents similar to SARS-CoV-2.

COVID-19 patients showing severe signs aretreated symptomatically along with oxygen therapy.In such cases where the patients progress towardrespiratory failure and become refractory to oxygentherapy, mechanical ventilation is necessitated. TheCOVID-19-induced septic shock can be managed byproviding adequate hemodynamic support (299).Several classes of drugs are currently beingevaluated for their potential therapeutic actionagainst SARS-CoV-2. Therapeutic agents that haveanti-SARS-CoV-2 activity can be broadly classifiedinto three categories: drugs that block virus entryinto the host cell, drugs that block viral replication aswell as its survival within the host cell, and drugsthat attenuate the exaggerated host immune response(300). An inflammatory cytokine storm is commonlyseen in critically ill COVID-19 patients. Hence, theymay benefit from the use of timely anti-inflammationtreatment. Anti-inflammatory therapy using drugslike glucocorticoids, cytokine inhibitors, JAKinhibitors, and chloroquine/hydroxychloroquineshould be done only after analyzing the risk/benefitratio in COVID-19 patients (301). There have notbeen any studies concerning the application ofnonsteroidal anti-inflammatory drugs (NSAID) toCOVID-19-infected patients. However, reasonablepieces of evidence are available that link NSAIDuses with the occurrence of respiratory andcardiovascular adverse effects. Hence, as acautionary approach, it is better to recommend theuse of NSAIDs as the first-line option for managingCOVID-19 symptoms (302). The use \_ ofcorticosteroids in COVID-19 patients is still a matterof controversy and requires further systematicclinical studies. The guidelines that were put forwardto manage critically ill adults suggest the use ofsystemic corticosteroids in mechanically ventilatedadults with ARDS (303). The generalized use ofcorticosteroids is not indicated in COVID-19, sincethere are some concerns associated with the use ofcorticosteroids in viral pneumonia. Stem cell therapyusing mesenchymal stem cells (MSCs) is anotherhopeful strategy that can be used in clinical cases ofCOVID-19 owing to its potentialimmunomodulatory capacity. It may have abeneficial role in attenuating the cytokine storm thatis observed in severe cases of SARS-CoV-2infection, thereby reducing mortality. Among thedifferent types of MSCs, expanded umbilical cordMSCs can be considered a potential therapeuticagent that requires further validation for managingcritically ill COVID-19 patients (304).

Repurposed broad-spectrum antiviral drugsnaving proven uses against other viral pathogens Canbe employed for SARS-CoV-2-infected patients.These possess benefits of easy accessibility andrecognized pharmacokinetic and pharmacodynamicactivities, stability, doses, and side effects (9).Repurposed drugs have been studied for treatingCoV infections, like lopinavir/ritonavir, andinterferon-1B revealed in vitro anti-MERS-CoVaction. The in vivo experiment carried out in thenonhuman primate model of common marmosetstreated with lopinavir/ritonavir and interferon betashowed superior protective results in treated animalsthan in the untreated ones (190). A combination ofthese drugs is being evaluated to treat MERS inhumans (MIRACLE trial) (191). These two proteaseinhibitors (lopinavir and ritonavir), in combinationwith ribavirin, gave encouraging clinical outcomes inSARS patients, suggesting their therapeutic values(165). However, in the current scenario, due to thelack of specific therapeutic agents against SARS-CoV-2, hospitalized patients confirmed for thedisease are given supportive care, like oxygen andfluid therapy, along with antibiotic therapy formanaging secondary bacterial infections (192).Patients with novel coronavirus or COVID-19pneumonia who are mechanically ventilated often; lati ;relaxation drugs to prevent ventilator-related lunginjury associated with human-machineincoordination (122). The result obtained from aclinical study of four patients infected with COVID-19 claimed that combination therapy usinglopinavir/ritonavir, arbidol, and Shufeng Jieducapsules (traditional Chinese medicine) was found tobe effective in managing COVID-19 pneumonia(193). It is difficult to evaluate the therapeuticpotential of a drug or a combination of drugs formanaging a disease based on such a limited samplesize. Before choosing the ideal therapeutic agent forthe management of COVID-19, randomized clinicalcontrol studies should be performed with a sufficientstudy population.

**Antiviral Drugs**

Several classes of routinely used antiviral drugs,like oseltamivir (neuraminidase inhibitor), acyclovir,ganciclovir, and ribavirin, do not have any effect onCOVID-19 and, hence, are not recommended (187).Oseltamivir, a neuraminidase inhibitor, has beenexplored in Chinese hospitals for treating suspectedCOVID-19 cases, although proven efficacy againstSARS-CoV-2 is still lacking for this drug (7). The invitro antiviral potential of FAD-approved drugs, viz.,ribavirin, penciclovir, nitazoxanide, nafamostat, andchloroquine, tested in comparison to remdesivir andfavipiravir (broad-spectrum antiviral drugs) revealedremdesivir and chloroquine to be highly effectiveagainst SARS-CoV-2 infection in vitro (194).Ribavirin, penciclovir, and favipiravir might notpossess noteworthy in vivo antiviral actions forSARS-CoV-2, since higher concentrations of thesenucleoside analogs are needed in vitro to lessen theviral infection. Both remdesivir and chloroquine arebeing used in humans to treat other diseases, andsuch safer drugs can be explored for assessing theireffectiveness in COVID-19 patients.

Several therapeutic agents, such aslopinavir/ritonavir, chloroquine, andhydroxychloroquine, have been proposed for theclinical management of COVID-19 (299). Amolecular docking study, conducted in the RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2using different commercially availableantipolymerase drugs, identified that drugs such asribavirin, remdesivir, galidesivir, tenofovir, andsofosbuvir bind RdRp tightly, indicating their vastpotential to be used against COVID-19 (305). Abroad-spectrum antiviral drug that was developed inthe United States, tilorone dihydrochloride (tilorone),was previously found to possess potent antiviralactivity against MERS, Marburg, Ebola, andChikungunya viruses (306). Even though it hadbroad-spectrum activity, it was neglected for anextended period. Tilorone is another antiviral drugthat 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 thereplication of SARS-CoV and MERS-CoV inprimary human airway epithelial cell culture systems(195). Recently, in vitro study has proven thatremdesivir has better antiviral activity than lopinavirand ritonavir. Further, in vivo studies conducted inmice also identified that treatment with remdesivirimproved pulmonary function and reduced viralloads and lung pathology both in prophylactic andtherapeutic regimens compared tolopinavir/ritonavir-IFN-y treatment in MERS-CoVinfection (8). Remdesivir also inhibits a diverserange of coronaviruses, including circulating humanCoV, zoonotic bat CoV, and prepandemic zoonoticCoV (195). Remdesivir is also considered the onlytherapeutic drug that significantly reducespulmonary pathology (8). All these findings indicatethat remdesivir has to be further evaluated for itsefficacy in the treatment of COVID-19 infection inhumans. The broad-spectrum activity exhibited byremdesivir will help control the spread of disease inthe event of a new coronavirus outbreak.

Chloroquine is an antimalarial drug known topossess antiviral activity due to its ability to blockvirus-cell fusion by raising the endosomal pHnecessary for fusion. It also interferes with virus-receptor binding by interfering with the terminalglycosylation of SARS-CoV cellular receptors, suchas ACE2 (196). In a recent multicenter clinical trialthat was conducted in China, chloroquine phosphatewas found to exhibit both efficacy and safety in thetherapeutic management of SARS-CoV-2-associatedpneumonia (197). This drug is already included inthe treatment guidelines issued by the NationalHealth Commission of the People’s Republic ofChina. The preliminary clinical trials usinghydroxychloroquine, another aminoquinoline drug,gave promising results. The COVID-19 patientsreceived 600 mg of hydroxychloroquine daily alongwith azithromycin as a single-arm protocol. Thisprotocol was found to be associated with anoteworthy reduction in viral load. Finally, itresulted in a complete cure (271); however, the studycomprised a small population and, hence, thepossibility of misinterpretation could arise. However,in another case study, the authors raised concernsover the efficacy of hydroxychloroquine-azithromycin in the treatment of COVID-19 patients,since no observable effect was seen when they wereused. In some cases, the treatment was discontinueddue to the prolongation of the QT interval (307).Hence, further randomized clinical trials are requiredbefore concluding this matter.

Recently, another FDA-approved drug,ivermectin, was reported to inhibit the in vitroreplication of SARS-CoV-2. The findings from thisstudy indicate that a single treatment of this drug wasable to induce an ~5,000-fold reduction in the viralRNA at 48 h in cell culture. (308). One of the maindisadvantages that limit the clinical utility ofivermectin is its potential to cause cytotoxicity.However, altering the vehicles used in \_ theformulations, the pharmacokinetic properties can bemodified, thereby having significant control over thesystemic concentration of ivermectin (338). Basedon the pharmacokinetic simulation, it was also foundthat ivermectin may have limited therapeutic utilityin managing COVID-19, since the inhibitoryconcentration that has to be achieved for effectiveanti-SARS-CoV-2 activity is far higher than themaximum plasma concentration achieved byadministering the approved dose (340). However,ivermectin, being a host-directed agent, exhibitsantiviral activity by targeting a critical cellularprocess of the mammalian cell. Therefore, theadministration of ivermectin, even at lower doses,will reduce the viral load at a minor level. This slightdecrease will provide a great advantage to theimmune system for mounting a large-scale antiviralresponse against SARS-CoV-2 (341). Further, acombination of ivermectin and hydroxychloroquinemight have a synergistic effect, since ivermectinreduces viral replication, while hydroxychloroquineinhibits the entry of the virus in the host cell (339).Further, in vivo studies and randomized clinicalcontrol trials are required to understand themechanism as well as the clinical utility of thispromising drug.

Nafamostat is a potent inhibitor of MERS-CoVthat acts by preventing membrane fusion.Nevertheless, it does not have any sort of inhibitoryaction against SARS-CoV-2 infection (194).Recently, several newly synthesized halogenatedtriazole compounds were evaluated, usingfluorescence resonance energy transfer (FRET)-based helicase assays, for their ability to inhibitAmong the evaluated compounds, 4-(cyclopent-1-en-3-ylamino)-5-[2-(4-iodophenyl)hydrazinyl]-4H-1,2,4-triazole-3-thiol and4-(cyclopent- 1-en-3-ylamino)-5-[2-(4-chlorophenyl)hydrazinyl]-4H-1,2,4-triazole-3-thiolwere found to be the most potent. These compoundswere used for in silico studies, and moleculardocking was accomplished into the active bindingsite of MERS-CoV helicase nsp13 (21). Furtherstudies are required for evaluating the therapeuticpotential of these newly identified compounds in themanagement of COVID-19 infection.

**Passive Immunization/Antibody Therapy/Mab**

Monoclonal antibodies (MAbs) may be helpful inthe intervention of disease in CoV-exposedindividuals. Patients recovering from SARS showedrobust neutralizing antibodies against this CoVinfection (164). A set of MAbs aimed at the MERS-CoV S protein-specific domains, comprising sixspecific epitope groups interacting with receptor-binding, membrane fusion, and sialic acid-bindingsites, make up crucial entry tasks of S protein (198,199). Passive immunization employing weaker andstrongly neutralizing antibodies providedconsiderable protection in mice against a MERS-CoV lethal challenge. Such antibodies may play acrucial role in enhancing protective humoralresponses against the emerging CoVs by aimingappropriate epitopes and functions of the S protein.The cross-neutralization ability of SARS-CoV RBD-specific neutralizing MAbs considerably relies onthe resemblance between their RBDs; therefore,SARS-CoV RBD-specific antibodies could cross-neutralized SL CoVs, 1.e., bat-SL-CoV strain WIV1(RBD with eight amino acid differences from SARS-CoV) but not bat-SL-CoV strain SHCO14 (24 aminoacid differences) (200).

Appropriate RBD-specific MAbs can berecognized by a relative analysis of RBD of SARS-CoV-2 to that of SARS-CoV, and cross-neutralizingSARS-CoV RBD-specific MAbs could be exploredfor their effectiveness against COVID-19 and furtherneed to be assessed clinically. The USS.biotechnology company Regeneron is attempting torecognize potent and specific MAbs to combatCOVID-19. An ideal therapeutic option suggestedfor SARS-CoV-2 (COVID-19) is the combinationtherapy comprised of MAbs and the drug remdesivir(COVID-19) (201). The SARS-CoV-specific humanMAb CR3022 is found to bind with SARS-CoV-2RBD, indicating its potential as a therapeutic agentin the management of COVID-19. It can be usedalone or in combination with other effectiveneutralizing antibodies for the treatment andprevention of COVID-19 (202). Furthermore, SARS-CoV-specific neutralizing antibodies, like m396 andCR3014, failed to bind the S protein of SARS-CoV-2, indicating that a particular level of similarity ismandatory between the RBDs of SARS-CoV andSARS-CoV-2 for the cross-reactivity to occur.

Further assessment is necessary beforeconfirming the effectiveness of such combinationtherapy. In addition, to prevent further communityand nosocomial spread of COVID-19, thepostprocedure risk management program should notbe neglected (309). Development of broad-spectruminhibitors against the human coronaviral pathogenswill help to facilitate clinical trials on theeffectiveness of such inhibitors against endemic andemerging coronaviruses (203). A promising animalstudy revealed the protective effect of passiveimmunotherapy with immune serum from MERS-immune camels on mice infected with MERS-CoV(204). Passive immunotherapy using convalescentplasma is another strategy that can be used fortreating COVID-19-infected, critically ill patients(205).(human single-chain antibodies; HuscFvs) orhumanized nanobodies (single-domain antibodies;sdAb, VH/VHH) could aid in blocking virusreplication, as these agents can traverse the virus-infected cell membranes (transbodies) and caninterfere with the biological characteristics of thereplicating virus proteins. Such examples includetransbodies to the influenza virus, hepatitis C virus,Ebola virus, and dengue virus (206). Producingsimilar transbodies against intracellular proteins ofcoronaviruses, such as papain-like proteases (PLpro),cysteine-like protease (3CLpro), or other nsps, whichare essential for replication and transcription of thevirus, might formulate a practical move forward for asafer and potent passive immunization approach forvirus-exposed persons and rendering therapy toinfected patients.

In a case study on five grimly sick patientshaving symptoms of severe pneumonia due toCOVID-19, convalescent plasma administration wasfound to be helpful in patients recoveringsuccessfully. The convalescent plasma containing aSARS-CoV-2-specific ELISA (serum) antibody titerhigher than 1:1,000 and neutralizing antibody titermore significant than 40 was collected from therecovered patients and used for plasma transfusiontwice in a volume of 200 to 250 ml on the day ofcollection (310). At present, treatment for sepsis andARDS mainly involves antimicrobial therapy, sourcecontrol, and supportive care. Hence, the use oftherapeutic plasma exchange can be considered anoption in managing such severe conditions. Furtherrandomized trials can be designed to investigate itsefficacy (311).

**Potential Therapeutic Agents**

Potent therapeutics to combat SARS-CoV-2infection include virus binding molecules, moleculesor inhibitors targeting particular enzymes implicatedin replication and transcription process of the virus,helicase inhibitors, vital viral proteases and proteins,protease inhibitors of host cells, endocytosisinhibitors, short interfering RNA \_ (siRNA),neutralizing antibodies, MAbs against the hostreceptor, MAbs interfering with the Sl RBD,antiviral peptide aimed at S2, and \_ naturaldrugs/medicines (7, 166, 186). The S protein acts asthe critical target for developing CoV antivirals, likeinhibitors of S protein and S cleavage, neutralizingantibodies, RBD-ACE2 blockers, siRNAs, blockersof the fusion core, and proteases (168).All of these therapeutic approaches have revealedboth in vitro and in vivo anti-CoV potential.Although in vitro research carried out with thesetherapeutics showed efficacy, most need appropriatesupport from randomized animal or human trials.Therefore, they might be of limited applicability andrequire trials against SARS-CoV-2 to gain practicalusefulness. The binding of SARS-CoV-2 with ACE2leads to the exacerbation of pneumonia as aconsequence of the imbalance in the renin-angiotensin system (RAS). The virus-inducedpulmonary inflammatory responses may be reducedby the administration of ACE inhibitors (ACEI) andangiotensin type-1 receptor (AT1R) (207).

Several investigations have suggested the use ofsmall-molecule inhibitors for the potential control ofSARS-CoV infections. Drugs of the FDA-approvedcompound library were screened to identify foursmall-molecule inhibitors of | MERS-CoV(chlorpromazine, chloroquine, loperamide, andlopinavir) that inhibited viral replication. Thesecompounds also hinder SARS-CoV and humanCoVs (208). Therapeutic strategies involving the useof specific antibodies or compounds that neutralizecytokines and their receptors will help to restrain thehost inflammatory responses. Such drugs actingspecifically in the respiratory tract will help toreduce virus-triggered immune pathologies inCOVID-19 (209). The later stages of coronavirus-induced inflammatory cascades are characterized bythe release of proinflammatory interleukin-1 (IL-1)family members, such as IL-1 and IL-33. Hence,there exists a possibility that the inflammationassociated with coronavirus can be inhibited byutilizing anti-inflammatory cytokines that belong tothe IL-1 family (92). It has also been suggested thatthe actin protein is the host factor that is involved incell entry and pathogenesis of SARS-CoV-2. Hence,those drugs that modulate the biological activity ofthis protein, like ibuprofen, might have sometherapeutic application in managing the disease(174). The plasma angiotensin 2 level was found tobe markedly elevated in COVID-19 infection andwas correlated with viral load and lung injury.Hence, drugs that block angiotensin receptors mayhave potential for treating COVID-19 infection(121). A scientist from Germany, named RolfHilgenfeld, has been working on the identification ofdrugs for the treatment of coronaviral infection sincethe time of the first SARS outbreak (19).

The SARS-CoV S2 subunit has a significantfunction in mediating virus fusion that provides entryinto the host cell. Heptad repeat 1 (HR1) and heptadrepeat 2 (HR2) can interact and form a six-helixbundle that brings the viral and cellular membranesin close proximity, facilitating its fusion. Thesequence alignment study conducted betweenCOVID-19 and SARS-CoV identified that the S2subunits are highly conserved in these CoVs. TheHR1 and HR2 domains showed 92.6% and 100%overall identity, respectively (210). From thesefindings, we can confirm the significance ofCOVID-19 HR1 and HR2 and their vital role in hostcell entry. Hence, fusion inhibitors target the HR1domain of S protein, thereby preventing viral fusionand entry into the host cell. This is another potentialtherapeutic strategy that can be used in themanagement of COVID-19. Other than the specifictherapy directed against COVID-19, generaltreatments play a vital role in the enhancement ofhost immune responses against the viral agent.Inadequate nutrition is linked to the weakening ofthe host immune response, making the individualmore susceptible. The role played by nutrition indisease susceptibility should be measured byevaluating the nutritional status of patients withCOVID-19 (205).

**Animal Models and Cell Cultures**

For evaluating the potential of vaccines andtherapeutics against CoVs, including SARS-CoV,MERS-CoVs, and the presently emerging SARS-CoV-2, suitable animal models that can mimic theclinical disease are needed (211, 212). Variousanimal models were assessed for SARS- and MERS-CoVs, such as mice, guinea pigs, golden Syrianhamsters, ferrets, rabbits, nonhuman primates likerhesus macaques and marmosets, and cats (185,213-218). The specificity of the virus to hACE2(receptor of SARS-CoV) was found to be asignificant barrier in developing animal models.Consequently, a SARS-CoV transgenic mouse modelhas been developed by inserting the hACE2 geneinto the mouse genome (219). The inability ofMERS-CoV to replicate in the respiratory tracts ofanimals (mice, hamsters, and ferrets) is anotherlimiting factor. However, with genetic engineering, a288-330'/\* MERS-CoV genetically modified mousemodel was developed and now is in use for theassessment of novel drugs and vaccines againstMERS-CoV (220). In the past, small animals (miceor hamsters) have been targeted for being closer to ahumanized structure, such as mouse DPP4 alteredwith human DPP4 (hDPP4), hDPP4-transducedmice. and hDPP4-Teg mice (transgenic for expressinghDPP4) for MERS-CoV infection (221). TheCRISPR-Cas9 gene-editing tool has been used forinserting genomic alterations in mice, making themsusceptible to MERS-CoV infection (222). Effortsare under way to recognize suitable animal modelsfor SARS-CoV2/COVID-19, identify the receptoraffinity of this virus, study pathology in experimentalanimal models, and explore virus-specific immuneresponses and protection studies, which togetherwould increase the pace of efforts being made fordeveloping potent vaccines and drugs to counter thisemerging virus. Cell lines, such as monkey epithelialcell lines (LLC-MK2 and Vero-B4), goat lung cells,alpaca kidney cells, dromedary umbilical cord cells,and advanced ex \_ vivo \_ three-dimensionaltracheobronchial tissue, have been explored to studyhuman CoVs (MERS-CoV) (223, 224). Vero andHuh-7 cells (human liver cancer cells) have beenused for isolating SARS-CoV-2 (194).

Recently, an experimental study with rhesusmonkeys as animal models revealed the absence ofany viral loads in nasopharyngeal and anal swabs,and no viral replication was recorded in the primarytissues at a time interval of 5 days post-reinfection inreexposed monkeys (274). The subsequentvirological, radiological, and \_ pathologicalobservations indicated that the monkeys withreexposure had no recurrence of COVID-19, like theSARS-CoV-2-infected monkeys without rechallenge.These findings suggest that primary infection withSARS-CoV-2 could protect from later exposures tothe virus, which could help in defining diseaseprognosis and crucial inferences for designing anddeveloping potent vaccines against COVID-19(274).

**PREVENTION, CONTROL, ANDMANAGEMENT**

In contrast to their response to the 2002 SARSoutbreak, China has shown immense politicalopenness in reporting the COVID-19 outbreakpromptly. They have also performed rapidsequencing of COVID-19 at multiple levels andshared the findings globally within days ofidentifying the novel virus (225). The move made byChina opened a new chapter in global health securityand diplomacy. Even though complete lockdown wasdeclared following the COVID-19 outbreak inWuhan, the large-scale movement of people hasresulted in a radiating spread of infections in thesurrounding provinces as well as to several othercountries. Large-scale screening programs mighthelp us to control the spread of this virus. However,this is both challenging as well as time-consumingdue to the present extent of infection (226). Thecurrent scenario demands effective implementationof vigorous prevention and control strategies owingto the prospect of COVID-19 for nosocomialinfections (68). Follow-ups of infected patients bytelephone on day 7 and day 14 are advised to avoidany further unintentional spread or nosocomialtransmission (312). The availability of public datasets provided by independent analytical teams willact as robust evidence that would guide us indesigning interventions against the COVID-19outbreak. Newspaper reports and social media can beused to analyze and reconstruct the progression of anoutbreak. They can help us to obtain detailed patient-level data in the early stages of an outbreak (227).Immediate travel restrictions imposed by severalcountries might have contributed significantly topreventing the spread of SARS-CoV-2 globally (89,228). Following the outbreak, a temporary ban wasimposed on the wildlife trade, keeping in mind thepossible role played by wild animal species in theorigin of SARS-CoV-2/COVID-19 (147). Making apermanent and bold decision on the trade of wildanimal species is necessary to prevent the possibilityof virus spread and initiation of an outbreak due tozoonotic spillover (1).

Personal protective equipment (PPE), like facemasks, will help to prevent the spread of respiratoryinfections like COVID-19. Face masks not onlyprotect from infectious aerosols but also prevent thetransmission of disease to other susceptibleindividuals while traveling through public transportsystems (313). Another critical practice that canreduce the transmission of respiratory diseases is themaintenance of hand hygiene. However, the efficacyof this practice in reducing the transmission ofrespiratory viruses like SARS-CoV-2 is muchdependent upon the size of droplets produced. Handhygiene will reduce disease transmission only if thevirus is transmitted through the formation of largedroplets (314). Hence, it is better not tooveremphasize that hand hygiene will prevent thetransmission of SARS-CoV-2, since it may produce afalse sense of safety among the general public thatfurther contributes to the spread of COVID-19. Eventhough airborne spread has not been reported inSARS-CoV-2 infection, transmission can occurthrough droplets and fomites, especially when thereis close, unprotected contact between infected andsusceptible individuals. Hence, hand hygiene isequally as important as the use of appropriate PPE,like face masks, to break the transmission cycle ofthe virus; both hand hygiene and face masks help tolessen the risk of COVID-19 transmission (315).

Medical staff are in the group of individuals mostat risk of getting COVID-19 infection. This isbecause they are exposed directly to infectedpatients. Hence, proper training must be given to allhospital staff on methods of prevention andprotection so that they become competent enough toprotect themselves and others from this deadlydisease (316). As a preventive measure, health careworkers caring for infected patients should takeextreme precautions against both contact andairborne transmission. They should use PPE such asface masks (N95 or FFP3), eye protection (goggles),gowns, and gloves to nullify the risk of infection(299).

The human-to-human transmission reported inSARS-CoV-2 infection occurs mainly throughdroplet or direct contact. Due to this finding,frontline health care workers should follow stringentinfection control and preventive measures, such asthe use of PPE, to prevent infection (110). Themental health of the medical/health workers who areinvolved in the COVID-19 outbreak is of greatimportance, because the strain on their mental well-being will affect their attention, concentration, anddecision-making capacity. Hence, for control of theCOVID-19 outbreak, rapid steps should be taken toprotect the mental health of medical workers (229).

Since the living mammals sold in the wet marketare suspected to be the intermediate host of SARS-CoV-2, there is a need for strengthening theregulatory mechanism for wild animal trade (13).The total number of COVID-19 confirmed cases ison a continuous rise and the cure rate is relativelylow, making disease control very difficult to achieve.The Chinese government is making continuousefforts to contain the disease by taking emergencycontrol and prevention measures. They have alreadybuilt a hospital for patients affected by this virus andare currently building several more foraccommodating the continuously increasing infectedpopulation (230). The effective control of SARS-CoV-2/COVID-19 requires high-level interventionslike intensive contact tracing, as well as\_ thequarantine of people with suspected infection and theisolation of infected individuals. The implementationof rigorous control and preventive measures togethermight control the Rg number and reduce thetransmission risk (228). Considering the zoonoticlinks associated with SARS-CoV-2, the One Healthapproach may play a vital role in the prevention andcontrol measures being followed to restrain thispandemic virus (317-319). The © substantialimportation of COVID-19 presymptomatic casesfrom Wuhan has resulted in independent, self-sustaining outbreaks across major cities both withinthe country and across the globe. The majority ofChinese cities are now facing localized outbreaks ofCOVID-19 (231). Hence, deploying efficient publichealth interventions might help to cut the spread ofthis virus globally.The occurrence of COVID-19 infection onseveral cruise ships gave us a preliminary idearegarding the transmission pattern of the disease.Cruise ships act as a closed environment and providean ideal setting for the occurrence of respiratorydisease outbreaks. Such a situation poses asignificant threat to travelers, since people fromdifferent countries are on board, which favors theintroduction of the pathogen (320). Although nearly30 cruise ships from different countries have beenfound harboring COVID-19 infection, the majorcruise ships that were involved in the COVID-19outbreaks are the Diamond Princess, GrandPrincess, Celebrity Apex, and Ruby Princess. Thenumber of confirmed COVID-19 cases around theworld is on the rise. The success of preventivemeasures put forward by every country is mainlydependent upon their ability to anticipate theapproaching waves of patients. This will help toproperly prepare the health care workers andincrease the intensive care unit (ICU) capacity (321).Instead of entirely relying on lockdown protocols,countries should focus mainly on \_ alternativeintervention strategies, such as large-scale testing,contract tracing, and localized quarantine ofsuspected cases for limiting the spread of thispandemic virus. Such intervention strategies will beuseful either at the beginning of the pandemic orafter lockdown relaxation (322). Lockdown shouldbe imposed only to slow down disease progressionamong the population so that the health care systemis not overloaded.

The reproduction number (Ro) of COVID-19infection was earlier estimated to be in the range of1.4 to 2.5 (70); recently, it was estimated to be 2.24to 3.58 (76). Compared to its coronaviruspredecessors, COVID-19 has an Rg value that isgreater than that of MERS (Ro < 1) (108) but lessthan that of SARS (Ro value of 2 to 5) (93). Still, toprevent further spread of disease at mass gatherings,functions remain canceled in the affected cities, andpersons are asked to work from home (232). Hence,it is a relief that the current outbreak of COVID-19infection can be brought under control with theadoption of strategic preventive and \_ controlmeasures along with the early isolation ofsubsequent cases in the coming days. Studies alsoreport that since air traffic between China andAfrican countries increased many times over in thedecade after the SARS outbreak, African countriesneed to be vigilant to prevent the spread of novelcoronavirus in Africa (225). Due to fear of virusspread, Wuhan City was completely shut down(233). The immediate control of the ongoingCOVID-19 outbreaks appears a mammoth task,especially for developing countries, due to theirinability to allocate quarantine stations that couldscreen infected individuals’ movements (234). Suchunderdeveloped countries should divert theirresources and energy to enforcing the primary levelof preventive measures, like controlling the entry ofindividuals from China or countries where thedisease has flared up, isolating the infectedindividuals, and quarantining individuals withsuspected infection. Most of the sub-Saharan Africancountries have a fragile health system that can becrippled in the event of an outbreak. Effectivemanagement of COVID-19 would be difficult forlow-income countries due to their inability torespond rapidly due to the lack of an efficient healthcare system (65). Controlling the imported cases iscritical in preventing the spread of COVID-19 toother countries that have not reported the diseaseuntil now. The possibility of an imported case ofCOVID-19 leading to sustained human-to-humantransmission was estimated to be 0.41. This can bereduced to a value of 0.012 by decreasing the meantime from the onset of symptoms to hospitalizationand can only be made possible by using intensedisease surveillance systems (235). The silentimportations of infected individuals (before themanifestation of clinical signs) also contributedsignificantly to the spread of disease across themajor cities of the world. Even though the travel banwas implemented in Wuhan (89), infected personswho traveled out of the city just before theimposition of the ban might have remainedundetected and resulted in local outbreaks (236).Emerging novel diseases like COVID-19 are difficultto contain within the country of origin, sinceglobalization has led to a world without borders.Hence, international collaboration plays a vital roleWe also predict the possibility of anotheroutbreak, as predicted by Fan et al. (6). Indeed, thepresent outbreak caused by SARS-CoV-2 (COVID-19) was expected. Similar to previous outbreaks, thecurrent outbreak also will be contained shortly.However, the real issue is how we are planning tocounter the next zoonotic CoV epidemic that is likelyto 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 hasserved as a reminder of how novel pathogens canrapidly emerge and spread through the humanpopulation and eventually cause severe public healthcrises. Further research should be conducted toestablish animal models for SARS-CoV-2\_ toinvestigate replication, transmission dynamics, andpathogenesis in humans. This may help develop andevaluate potential therapeutic strategies againstzoonotic CoV epidemics. Present trends suggest theoccurrence of future outbreaks of CoVs due tochanges in the climate, and ecological conditionsmay be associated with human-animal contact. Live-animal markets, such as the Huanan South ChinaSeafood Market, represent ideal conditions forinterspecies contact of wildlife with domestic birds,pigs, and mammals, which substantially increasesthe probability of interspecies transmission of CoVinfections and could result in high risks to humansdue to adaptive genetic recombination in theseviruses (323-325).

The COVID-19-associated symptoms are fever,cough, expectoration, headache, and myalgia orfatigue. Individuals with asymptomatic and atypicalclinical manifestations were also identified recently,further adding to the complexity of diseasetransmission dynamics. Atypical clinicalmanifestations may only express symptoms such asfatigue instead of respiratory signs such as fever,cough, and sputum. In such cases, the clinician mustbe vigilant for the possible occurrence ofasymptomatic and atypical clinical manifestations toavoid the possibility of missed diagnoses.The present outbreak caused by SARS-CoV-2was, indeed, expected. Similar to previous outbreaks,the current pandemic also will be contained shortly.However, the real question is, how are we planningto counter the next zoonotic CoV epidemic that islikely to occur within the next 5 to 10 years orperhaps sooner? Our knowledge of most of the batCoVs is scarce, as these viruses have not beenisolated and studied, and extensive studies on suchviruses are typically only conducted when they areassociated with specific disease outbreaks. The nextstep following the control of the COVID-19 outbreakin China should be focused on \_ screening,identification, isolation, and characterization ofCoVs\_ present in wildlife species of China,particularly in bats. Both in vitro and in vivo studies(using suitable animal models) should be conducted(using sultable animal modeis) snouid be Conauctedto 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 andvaccines against several other emerging diseases willhelp develop suitable therapeutic agents againstCOVID-19 in a short time. Until then, we must relyexclusively on various control and preventionmeasures to prevent this new disease from becominga pandemic.

**4 VIROLOGY**

Coronaviruses, a family of viruses within thenidoviruses superfamily, are further classifiedaccording to their genera, alpha-, beta-, gamma-and deltacoronaviruses (a-, B-, y- and 5-).Among those, alpha and beta species arecapable of contaminating only mammals,whereas the other two genera can infect birdsand could also infect mammals.'\* '\* Two ofthese genera belong to human coronaviruses(HCoVs): a-coronaviruses, which comprisehuman coronavirus 229E (hcov229E) andhuman coronavirus NL63 (hcovNL63), and B-coronaviruses, which are human coronavirusHKU1, human coronavirus OC43, MERS-COV(known as Middle East respiratory syndromecoronavirus) and SARS-CoV (referred to assevere acute respiratory syndromecoronavirus).

The severe acute respiratory syndrome CoV-2(SARS-CoV-2) is now named novel COVID-19(coronavirus disease 2019).'° Genomesequencing and phylogenetic research revealedthat the COVID-19-causing coronavirus is abeta-coronavirus that belongs to the samesubtypes as SARS virus, but still exists in avariant group. The receptor-binding gene regionrn . et z rn a ral ~ anvariant group. The receptor-binding gene regionappears to be very similar to that of the SARS-CoV and it is believed that the same receptorwould be used for cell entry. |7

**4.1 Virion structure and itsgenome**

Coronaviruses are structurally enveloped,belonging to the positive-strand RNA virusescategory that has the largest known genomes ofRNA. The structures of the coronavirus aremore spherical in shape, but their structure hasthe potential to modify their morphology inresponse to environmental conditions, beingpleomorphic. The capsular membrane whichrepresents the outer envelope usually hasglycoprotein projection and covers the nucleus,comprising a matrix protein containing apositive-strand RNA. Since the structurepossesses 5'-capped and 3'-polyadenylatedends, it remains identical to the cellularmRNAs.'® The structure is comprised ofhemagglutinin esterase (HE) (present only insome beta-coronaviruses), spike (S), smallmembrane (E), membrane (M) and nucleocapsid(N), as shown (Figure 1). The envelopecontaining glycoprotein is responsible forattachment to the host cell, which possesses theprimary anti-genic epitopes mainly thoseprimary anti-genic epitopes mainly thoserecognised by neutralising antibodies. The spikeS-protein being in a spike form is subjected to astructural rearrangement process so that fusingthe outer membrane of the virus with the host-cell membrane becomes easier.'\* 7° RecentSARS-CoV work has also shown that themembrane exopeptidase ACE enzyme(angiotensin-converting enzyme) functions as aCOVID-19 receptor to enter the human cell.\*'-HEFIGURE 1

**4.2 Viral replication**

Usually replication of coronavirus occurs withinthe cytoplasm and is closely associated withendoplasmic reticulum and other cellularmembrane organelles. Human coronavirusesare thought to invade cells, primarily throughdifferent receptors. For 229E and OC43, aminopeptidase-N (AP-N) and a sialic acid containingreceptor, respectively, were known to functionin this role. After the virus enters the host celland uncoating process occurs, the genome istranscribed, and then, translated. Acharacteristic feature of replication is that allmRNAs form an enclosed group of typical 3’ends; only the special portions of the 5’ endsare translated. In total, about 7 MRNAs areproduced. The shortest MRNA codes and theothers can express the synthesis of anothergenome segment for nucleoprotein. At the cellmembrane, these proteins are collected andgenomic RNA is initiated as a mature particletype by burgeoning from internal cellmembranes.2 2°

**5 PATHOGENESIS**

Coronaviruses are tremendously precise andmature in most of the airway epithelial cells asobserved through both in vivo and in vitroobserved through both in vivo and in vitroexperiments. There is an enhanced nasalsecretion observed along with local oedemabecause of the damage of the host cell, whichfurther stimulates the synthesis ofinflammatory mediators. In addition, thesereactions can induce sneezing, difficultybreathing by causing airway inhibition andelevate mucosal temperature. These viruses,when released, chiefly affect the lowerrespiratory tract, with the signs and symptomsexisting clinically. Also, the virus further affectsthe intestinal lymphocytes, renal cells, liver cellsand T-lymphocytes. Furthermore, the virusinduces T-cell apoptosis, causing the reaction ofthe T-cell to be erratic, resulting in the immunesystem's complete collapse.\*\* 7°

**5.1 Mode of transmission**

In fact it was accepted that the originaltransmission originated from a seafood market,which had a tradition of selling live animals,where the majority of the patients had eitherworked or visited, although up to now theunderstanding of the COVID-19 transmissionrisk remains incomplete. '° In addition, while thenewer patients had no exposure to the marketand still got the virus from the humans presentthere, there is an increase in the outbreak ofthere, there is an increase in the outbreak ofthis virus through human-to-humantransmission, with the fact that it has becomewidespread around the globe. This confirms thefact similar to the previous epidemics, includingSARS and MERS, that this coronavirus exhibitedpotential human-to-human transmission, as itwas recently declared a pandemic by WHO.?°

Respiratory droplets are the major carrier forcoronavirus transmission. Such droplets caneither stay in the nose or mouth or enter thelungs via the inhaled air. Currently, it is knownthat COVID-19’s transmission from one personto another also occurs through touching eitheran infected surface or even an object. With thecurrent scant awareness of the transmissionsystems however, airborne safety measureswith a high-risk procedure have been proposedin many countries. Transmission levels, or therates from one person to another, reporteddiffer by both location and interaction withinvolvement in infection control. It is stated thateven asymptomatic individuals or thoseindividuals in their incubation period can act ascarrier of SARS-CoV2.7” 2° With the data andevidence provided by the CDC, the usualincubation period is probably 3 to 7 days,sometimes being prolonged up to even 2weeks, and the typical symptom occurrenceweeks, and the typical symptom occurrencefrom incubation period to infection takes anaverage of 12.5 days.?

**6 CLINICAL DIAGNOSIS**

The symptoms of COVID-19 remain very similarto those of the other respiratory epidemics inthe past, which include SARS and MERS, buthere the range of symptoms includes mildrhinitis to septic shock. Some intestinaldisturbances were reported with the otherepidemics, but COVID-19 was devoid of suchsymptoms. When examined, unilateral orbilateral involvement compatible with viralpneumonia is observed in the patients, andbilateral multiple lobular and sub-segmentalconsolidation areas were observed in patientshospitalised in the intensive care unit.Comorbid patients showed a more severeclinical course than predicted from previousepidemics. Diagnosis of COVID-19 includes thecomplete history of travel and touch, withlaboratory testing. It is more preferable tochoose serological screening, which can help toanalyse even the asymptomatic infections;several serological tests are in progress forSARS-CoV-2. | 2°

**6.1 Laboratory testing forcoronavirus disease 2019 (COVID-19) in suspected human cases**

The assessment of the patients with COVID-19should be based on the clinical features andalso epidemiological factors. The screeningprotocols must be prepared and followed perthe native context.\*' Collecting and testing ofspecimen samples from the suspectedindividual is considered to be one of the mainprinciples for controlling and managing theoutbreak of the disease in a country. Thesuspected cases must be screened thoroughlyin order to detect the virus with the help ofnucleic acid amplification tests such as reversetranscription polymerase chain reaction (RT-PCR). If a country or a particular region does nothave the facility to test the specimens, thespecimens of the suspected individual shouldbe sent to the nearest reference laboratoriesper the list provided by WHO.°2

It is also recommended that the suspectedpatients be tested for the other respiratorypathogens by performing the routine laboratoryinvestigation per the local guidelines, mainly todifferentiate from other viruses that includeinfluenza virus, parainfluenza virus, adenovirus,respiratory syncytial virus, rhinovirus, humanrespiratory syncytial virus, rhinovirus, humanmetapneumovirus and SARS coronavirus. It isadvisable to distinguish COVID-19 from otherpneumonias such as mycoplasma pneumonia,chlamydia pneumonia and bacterialpneumonia.\*? Several published pieces ofliterature based on the novel coronavirusreported in China declared that stool and bloodsamples can also collected from the suspectedpersons in order to detect the virus. However,respiratory samples show better viability inidentifying the virus, in comparison with theother specimens.\*\*-°°

**6.2 Nucleic acid amplification tests(NAAT) for COVID-19 virus**

The gold standard method of confirming thesuspected cases of COVID-19 is carried out bydetecting the unique sequences of virus RNAthrough reverse transcription polymerase chainreaction (RT-PCR) along with nucleic acidsequencing if needed. The various genes ofvirus identified so far include N, E, S (N:nucleocapsid protein, E: envelope protein gene,S: spike protein gene) and RdRP genes (RNA-dependent RNA polymerase gene).°7

**6.3 Serological testing**

Serological surveys are also considered to beone of the most effective ones in facilitatingoutbreak investigation and it also helps us toderive a retrospective assessment of thedisease by estimating the attack rate.°7According to the recent literature, paired serumsamples can also help clinicians to diagnoseCOVID-19 in case of false negative results inNAAT essays.” The literature also declared thatthe commercial and non-commercial serologicaltests are under consideration in order tosupport the practising clinicians by assistingthem in diagnosis. Similarly, there are studiespublished on COVID-19 which are comprised ofthe serological data on clinical samples.\*® °°

**6.4 Viral sequencing**

Apart from confirming the presence of virus inthe specimens, viral sequencing is also quiteuseful in monitoring the viral genomicmutations, which plays a very significant role ininfluencing the performance of the medicalcountermeasures inclusive of the diagnostictest. Genomic sequencing of the virus can alsohelp further in developing several studiesrelated to molecular epidemiology.°\*

**6.5 specimen colection andstorage**

A Nasopharyngeal and oropharyngeal swabshould be collected using Dacron or polyesterflocked swabs. It should be transported to thelaboratory at a temperature of 4°C and storedin the laboratory between 4 and -70°C on thebasis of the number of days and, in order toincrease the viral load, both nasopharyngealand oropharyngeal swabs should be placed inthe same tube. Bronchoalveolar lavage andnasopharyngeal aspirate should be collected ina sterile container and transported similarly tothe laboratory by maintain a temperature ofA°C.

Sputum samples, especially from the lowerrespiratory tract, should be collected with thehelp of a sterile container and stored, whereastissue from a biopsy or autopsy should becollected using a sterile container along withsaline. However, both should be stored in thelaboratory at a temperature that rangesbetween 4 and -70°C. Whole blood fordetecting the antigen, particularly in the firstweek of illness, should be collected inacollecting tube and stored in the laboratorybetween 4 and -70°C. Urine samples must alsobe collected using a sterile container and storedbetween 4 and -70°C. Urine samples must alsobe collected using a sterile container and storedin the laboratory at a temperature that rangesbetween 4 and -70°C.°\*

**7 PREGNANCY**

Currently, there is a paucity of knowledge anddata related to the consequences of COVID-19during pregnancy.4°\*2 However, pregnantwomen seem to have a high risk of developingsevere infection and complications during therecent 2019-nCoV outbreak.\*'"\*? Thisspeculation was based on previous availablescientific reports on coronaviruses duringpregnancy (SARS-CoV and MERS-CoV) as well asthe limited number of COVID-19 cases.\*'-4°Analysing the clinical features and outcomes of10 newborns (including two sets of twins) inChina, whose mothers are confirmed cases ofCOVID-19, revealed that perinatal infection with2019-nCoV may lead to adverse outcomes forthe neonates, for example, premature labour,respiratory distress, thrombocytopenia withabnormal liver function and even death.” It isstill unclear whether or not the COVID-19infection can be transmitted during pregnancyto the foetus through the transplacentalroute.\*\* A recent case series report, whichassessed intrauterine vertical transmission ofassessed intrauterine vertical transmission ofCOVID-19 infection in nine infants born toinfected mothers, found that none of theinfants tested positive for the virus.\*° Likewise,there was no evidence of intrauterine infectioncaused by vertical transmission in the SARS andMERS epidemics.\*?

The CDC asserts that infants born to motherswith confirmed COVID-19 are consideredpersons under investigation (PUI) and should betemporarily separated from the mother andisolated.\*°

**7.1 Breastfeeding and infant care**

The data available to date is limited and cannotconfirm whether or not COVID-19 can betransmitted through breast milk.\*° Assessingthe presence of COVID-19 in breast milksamples from six patients showed negativeresult.\*° The CDC points out that in case of aconfirmed or suspected COVID-19 infection, thedecision of whether or how to start or continuebreastfeeding should be made by the mother incollaboration with the family and healthcarepractitioners.\*’ Careful precautions need to betaken by the mother to prevent transmitting thedisease to her infant through respiratorydroplets during breastfeeding. This includeswearing a facemask and practising handwearing a facemask and practising handhygiene before feeding the baby. In addition, itis advisable that breast pumps are cleanedproperly after each use and, if possible, ahealthy individual is available to feed theexpressed breast milk to the infant.4+

**7.2 Children and elderly population**

On the basis of the available reports, COVID-19among children accounted for 1-5% of theconfirmed cases, and this population does notseem to be at higher risk for the disease thanadults. There is no difference in the COVID-19symptoms between adults and children.However, the available evidence indicated thatchildren diagnosed with COVID-19 have mildersymptoms than the adults, with a low mortalityrate.\*® 9 On the contrary, older people who areabove the age of 65 years are at higher risk forasevere course of disease. In the United Stated,approximately 31-59% of those with confirmedCOVID-19 between the ages of 65 and 84 yearsold required hospitalisation, 11-31% of themrequired admission to the intensive care unit,and 4-11% died.°°

**8 PREVENTION**

The WHO and other agencies such as the CDChave published protective measures to mitigatethe spread of COVID-19. This involves frequenthand washing with handwash containing 60% ofalcohol and soap for at least 20 seconds.Another important measure is avoiding closecontact with sick people and keeping a socialdistance of 1 metre always to everyone who iscoughing and sneezing. Not touching the nose,eyes and mouth was also suggested. Whilecoughing or sneezing, covering the mouth andnose with a cloth/tissue or the bent elbow isadvised. Staying at home is recommended forthose who are sick, and wearing a facial mask isadvised when going out among people.Furthermore, it is recommended to clean andsterilise frequently touched surfaces such asphones and doorknobs on a daily basis.°' °7Staying at home as much as possible isadvisable for those who are at higher risk forsevere illness, to minimise the risk of exposureto COVID-19 during outbreaks.°°severe illness, to minimise the risk of exposureto COVID-19 during outbreaks.°°

**9 VACCINES**

The strange coronavirus outbreak in theChinese city of Wuhan, now termed COVID-19,and its rapid transmission, threatens peoplearound the world. Because of its pandemicnature, the National Institutes of Health (NIH)and pharmaceutical companies are involved inthe development of COVID-19 vaccines. XuNanping, China’s vice-minister of science andtechnology, announced that the first vaccine isexpected to be ready for clinical trials in Chinaat the end of April 2020.°\* There is no approvedvaccine and treatment for COVID-19 infections.Vaccine development is sponsored andsupported by the Biomedical AdvancedResearch and Development Authority (BARDA),a component of the Office of the AssistantSecretary for Preparedness and Response(ASPR). Sanofi will use its egg-free, recombinantDNA technology to produce an exact geneticmatch to proteins of the virus.°°

**10 RECOMBINANT SUBUNITVACCINE**

Clover Biopharmaceuticals is producing arecombinant subunit vaccine based on thetrimeric S-protein of COVID-19.°° The oralrecombinant vaccine is being expanded byVaxart in tablet formulation, using itsproprietary oral vaccine platform.

**11 CLINICAL MANAGEMENTAND TREATMENT**

In severe COVID-19 cases, treatment should begiven to support vital organ functions. Peoplewho think they may have been exposed toCOVID-19 should contact their healthcareprovider immediately. Healthcare personnelshould care for patients in an Airborne InfectionIsolation Room (AIIR). Precautions must betaken by the healthcare professional, such ascontact precautions and airborne precautionswith eye protection.°°Individuals with a mild clinical presentation maynot require primary hospitalisation. Closemonitoring is needed for the persons infectedwith COVID-19. Elderly patients and those withprevailing chronic medical conditions such asprevailing chronic medical conditions such aslung disease, heart failure, cancer,cerebrovascular disease, renal disease,diabetes, liver disease andimmunocompromising conditions andpregnancy are risk factors for developing severeillness. Management includes implementationof prevention and control measures andsupportive therapy to manage thecomplications, together with advanced organsupport.°”Corticosteroids must be avoided unlessspecified for chronic obstructive pulmonarydisease exacerbation or septic shock, as it islikely to prolong viral replication as detected inMERS-CoV patients.°®

**12 EARLY SUPPORTIVETHERAPY AND MONITORING**

Management of patients with suspected ordocumented COVID-19 consists of ensuringappropriate infection control and supportivecare. WHO and the CDC posted clinical guidancefor COVID-19.°°Immediate therapy of add-on oxygen must bestarted for patients with severe acuterespiratory infection (SARI) and respiratoryrespiratory infection (SARI) and respiratorydistress, shock or hypoxaemia. Patients withSARI can be given conservative fluid therapyonly when there is no evidence of shock.Empiric antimicrobial therapy must be startedto manage SARI. For patients with sepsis,antimicrobials must be administered within 1hour of initial assessments. The WHO and CDCrecommend that glucocorticoids not be used inpatients with COVID-19 pneumonia exceptwhere there are other indications (exacerbationof chronic obstructive pulmonary disease).°?

Patients’ clinical deterioration is closelyobserved with SARI; however, rapidlyprogressive respiratory failure and sepsisrequire immediate supportive careinterventions comprising quick use ofneuromuscular blockade and sedatives,hemodynamic management, nutritionalsupport, maintenance of blood glucose levels,prompt assessment and treatment ofnosocomial pneumonia, and prophylaxisagainst deep venous thrombosis (DVT) andgastrointestinal (GI) bleeding.®° Generally, suchpatients give way to their primary illness tosecondary complications like sepsis ormultiorgan system failure.\*°

**13 CONVALESCENT PLASMATHERAPY**

Guo Yanhong, an official with the NationalHealth Commission (NHC), stated thatconvalescent plasma therapy is a significantmethod for treating severe COVID-19 patients.Among the COVID-19 patients currentlyreceiving convalescent plasma therapy in thevirus-hit Wuhan, one has been discharged fromhospital, as reported by Chinese scienceauthorities on Monday, 17th February 2020 inBeijing. The first dose of convalescent plasmafrom a COVID-19 patient was collected on 1stand 9th February 2020 from a severely illpatient who was given treatment at a hospital inJiangxia District in Wuhan. The presence of thevirus in patients is minimised by the antibodiesin the convalescent plasma. Guiqiang statedthat donating plasma may cause minimal harmto the donor and that there is nothing to beworried about. Plasma donors must be curedpatients and discharged from hospital. Onlyplasma is used, whereas red blood cells (RBC),white blood cells (WBC) and blood platelets aretransfused back into the donor's body. Wangalleged that donor's plasma will totally improveto its initial state after one or 2 weeks from theday of plasma donation of around 200 to 300

**14 ANTIVIRAL THERAPY**

COVID-19 is an infectious disease caused bySARS-CoV-2, which is also termed the novelcoronavirus and is diligently associated with theSARS virus. The Ministry of Science andTechnology from the People’s Republic of Chinadeclared three potential antiviral medicinessuitable for treating COVID-19. Those threemedicines are, namely, Favilavir, chloroquinephosphate and remdesivir. A clinical trial wasconducted to test the efficacy of those threedrugs, and the results proved that out of thethree medicines above only Favilavir is effectivein treating the patients with novel coronavirus.The remaining two drugs were effective intreating malaria.°7Likewise a study carried out in the United Statesby the National Institute of Health proved thatremdesivir is effective in treating the MiddleEast respiratory syndrome coronavirus (MERS-CoV), which is also a type of coronavirus thatwas transmitted from monkeys. The drugremdesivir was also used in the United Statesfor treating the patients with COVID-19. Therehas been a proposal to use the combination ofprotease inhibitors lopinavir-ritonavir fortreating the patients affected by COVID-19.°%Itis also evident that remdesivir was effective intreating the patients who were infected withEbola virus. Per this evidence, China has alreadystarted testing the efficacy of remdesivir intreating the patients with COVID-19, especiallyin Wuhan, where the outbreak occurred.Chloroquine, which is an existing drug which iscurrently used in treating malaria cases, wasgiven to more than 100 patients who wereaffected with novel coronavirus to test itsefficacy.°7

A multicentric study was conducted in China totest the effectiveness of remdesivir in treatingthe patients with COVID-19. Thus, the results ofthe clinical trial proved that remdesivir has aconsiderably acceptable level of efficacy fortreating the patients with COVID-19. Therefore,the National Health Commission of the People'sRepublic of China decided to include remdesivirin the Guidelines for the Prevention, Diagnosisand Treatment of Pneumonia Caused by COVID-49.62

Chloroquine and hydroxychloroquine areexisting anti-malaria drugs also given to morethan 30 patients infected with COVID-19 inGuangdong province and Hunan province totest their effectiveness and efficacy. Thus, theresults of the clinical trial showed that the07:29 ~ Oe 948e All clinicians should keepthemselves updated about recentdevelopments including globalspread of the disease.e Non-essential international travelshould be avoided at this time.e People should stop spreadingmyths and false information aboutthe disease and try to allay panicand anxiety of the public.

**Conclusions**

This new virus outbreak haschallenged the economic, medical andpublic health infrastructure of Chinaand to some extent, of other countriesespecially, its neighbours. Time alonewill tell how the virus will impact ourlives here in India. More so, futureoutbreaks of viruses and pathogens ofzoonotic origin are likely to continue.Therefore, apart from curbing thisoutbreak. efforts should be made to07:9 7 @ Oe ain |themselves while examining suchpatients and practice hand hygiene frequently.

Suspected cases should be referredto government designated centresfor isolation and testing (inMumbai, at this time, it is Kasturbahospital). Commercial kits fortesting are not yet available in India.

Patients admitted with severepneumonia and acute respiratorydistress syndrome should beevaluated for travel history andplaced under contact and dropletisolation. Regulardecontamination of surfacesshould be done. They should betested for etiology using multiplexPCR panels if logistics permit andif no pathogen is identified, referthe samples for testing for SARS-CoV-2.07:9 7 @ Oe ain |

**Practice Points from an IndianPerspective**

At the time of writing this article, therisk of coronavirus in India isextremely low. But that may change inthe next few weeks. Hence thefollowing is recommended:

Healthcare providers should taketravel history of all patients withrespiratory symptoms, and anyinternational travel in the past 2wks as well as contact with sickpeople who have travelled internationally.

They should set up a system oftriage of patients with respiratoryillness in the outpatientdepartment and give them asimple surgical mask to wear.They should use surgical masksthemselves while examining such< @ |07:28 @ Owe ain |ro ro ro a(entertainment parks etc). China is alsoconsidering introducing legislation toprohibit selling and trading of wildanimals [32].

The international response has beendramatic. Initially, there were massivetravel restrictions to China and peoplereturning from China/ evacuated fromChina are being evaluated for clinicalsymptoms, isolated and tested forCOVID-19 for 2 wks even ifasymptomatic. However, now withrapid world wide spread of the virusthese travel restrictions have extendedto other countries. Whether theseefforts will lead to slowing of viralspread is not known.A candidate vaccine is underdevelopment.

**Practice Points from an IndianPerspective**

pandemic flu where patients wereasked to resume work/school onceafebrile for 24 h or by day 7 of illness.Negative molecular tests were notaprerequisite for discharge.At the community level, people shouldbe asked to avoid crowded areas andpostpone non-essential travel to placeswith ongoing transmission. Theyshould be asked to practice coughhygiene by coughing in sleeve/ tissuerather than hands and practice handhygiene frequently every 15-20 min.Patients with respiratory symptomsshould be asked to use surgical masks.The use of mask by healthy people inpublic places has not shown to protectagainst respiratory viral infections andis currently not recommended byWHO. However, in China, the publichas been asked to wear masks in publicand especially in crowded places andlarge scale gatherings are prohibited(entertainment parks etc). China is also07:28 MW Ore 748category A agents (cholera, plague).Patients should be placed in separaterooms or cohorted together. Negativepressure rooms are not generallyneeded. The rooms and surfaces andequipment should undergo regulardecontamination preferably withsodium hypochlorite. Healthcareworkers should be provided with fittested N95 respirators and protectivesuits and goggles. Airbornetransmission precautions should betaken during aerosol generatingprocedures such as intubation, suctionand tracheostomies. All contactsincluding healthcare workers shouldbe monitored for development ofsymptoms of COVID-19. Patients can bedischarged from isolation once theyare afebrile for atleast 3 d and havetwo consecutive negative moleculartests at 1 d sampling interval. Thisrecommendation is different frompandemic flu where patients were07:28 MW Ore avin |mask and practice cough hygiene.Caregivers should be asked to wear asurgical mask when in the same roomas patient and use hand hygiene every15-20 min.

The greatest risk in COVID-19 istransmission to healthcare workers. Inthe SARS outbreak of 2002, 21% ofthose affected were healthcare workers[31]. Till date, almost 1500 healthcareworkers in China have been infectedwith 6 deaths. The doctor who firstwarned about the virus has died too. Itis important to protect healthcareworkers to ensure continuity of careand to prevent transmission ofinfection to other patients. WhileCOVID-19 transmits as a dropletpathogen and is placed in Category B ofinfectious agents (highly pathogenicH5N1 and SARS), by the China NationalHealth Commission, infection controlmeasures recommended are those for07:27 BM Owe ban |

**Prevention [21, 30]**

Since at this time there are noapproved treatments for this infection,prevention is crucial. Severalproperties of this virus makeprevention difficult namely, non-specific features of the disease, theinfectivity even before onset ofsymptoms in the incubation period,transmission from asymptomaticpeople, long incubation period, tropismfor mucosal surfaces such as theconjunctiva, prolonged duration of theillness and transmission even afterclinical recovery.

Isolation of confirmed or suspectedcases with mild illness at home isrecommended. The ventilation at homeshould be good with sunlight to allowfor destruction of virus. Patients shouldbe asked to wear a simple surgicalmask and practice cough hygiene.07:27 BM Owe 748OQ — @ ncbinim.nih.gov/pmc/arti[median 17 d]. In the case series ofchildren discussed earlier, all childrenrecovered with basic treatment and didnot need intensive care [17].

There is anecdotal experience with useof remdeswir, a broad spectrum antiRNA drug developed for Ebola inmanagement of COVID-19 [27]. Moreevidence is needed before these drugsare recommended. Other drugsproposed for therapy are arbidol (anantiviral drug available in Russia andChina), intravenous immunoglobulin,interferons, chloroquine and plasma ofpatients recovered from COVID-19 [21,28, 29]. Additionally, recommendationsabout using traditional Chinese herbsfind place in the Chinese guidelines[21].

**Prevention [21, 30]**

It has been used based on the experience with SARS and MERS. In a historicalcontrol study in patients with SARS,patients treated with lopinavir-ritonavir with ribavirin had better outcomes as compared to those given ribavirin alone [15].In the case series of 99 hospitalized patients with COVID-19 infection fromWuhan, oxygen was given to 76%, non-invasive ventilation in 13%,mechanical ventilation in 4%,extra corporeal membrane oxygenation(ECMO) in 3%, continuous renal replacement therapy (CRRT) in 9%,antibiotics in 71%, antifungals in 15%,glucocorticoids in 19% and intravenousimmunoglobulin therapy in 27% [15].Antiviral therapy consisting ofoseltamivir, ganciclovir and lopinavir-ritonavir was given to 75% of thepatients. The duration of non-invasiveventilation was 4-22 d [median 9 d]07:27 Owe 748prongs, face mask, high flow nasalcannula (HFNC) or non-invasiveventilation is indicated. Mechanicalventilation and even extra corporealmembrane oxygen support may beneeded. Renal replacement therapymay be needed in some. Antibioticsand antifungals are required if co-infections are suspected or proven. Therole of corticosteroids is unproven;while current international consensusand WHO advocate against their use,Chinese guidelines do recommendshort term therapy with low-to-moderate dose corticosteroids inCOVID-19 ARDS [24, 25]. Detailedguidelines for critical caremanagement for COVID-19 have beenpublished by the WHO [26]. There is, asof now, no approved treatment forCOVID-19. Antiviral drugs such asribavirin, lopinavir-ritonavir havebeen used based on the experiencewith SARS and MERS. In a historical07:27 MOrxe 748infections clinically or through routinelab tests. Therefore travel historybecomes important. However, as theepidemic spreads, the travel historywill become irrelevant.

**Treatment [21, 23]**

Treatment is essentially supportive and symptomatic.

The first step is to ensure adequateisolation (discussed later) to preventtransmission to other contacts, patientsand healthcare workers. Mild illnessshould be managed at home withcounseling about danger signs. Theusual principles are maintaininghydration and nutrition andcontrolling fever and cough. Routineuse of antibiotics and antivirals such asoseltamivir should be avoided inconfirmed cases. In hypoxic patients,provision of oxygen through nasalprongs, face mask, high flow nasal07:27 BM Owe ban |OQ — @ ncbinim.nih.gov/pmc/artiglass opacities and sub segmentalconsolidation. It is also abnormal inasymptomatic patients/ patients withno clinical evidence of lowerrespiratory tract involvement. In fact,abnormal CT scans have been used todiagnose COVID-19 in suspect caseswith negative molecular diagnosis;many of these patients had positivemolecular tests on repeat testing [22].

**Differential Diagnosis [21]**

The differential diagnosis includes alltypes of respiratory viral infections[influenza, parainfluenza, respiratorysyncytial virus (RSV), adenovirus,human metapneumovirus, non COVID-19 coronavirus], atypical organisms(mycoplasma, chlamydia) and bacterialinfections. It is not possible todifferentiate COVID-19 from theseinfections clinically or through routineBS0725m Ore avin |consolidation. It is also abnormal inasymptomatic patients/ patients withno clinical evidence of lowerrespiratory tract involvement. In fact,abnormal CT scans have been used todiagnose COVID-19 in suspect caseswith negative molecular diagnosis;many of these patients had positivemolecular tests on repeat testing [22].Differential Diagnosis [21]The differential diagnosis includes alltypes of respiratory viral infections[influenza, parainfluenza, respiratorysyncytial virus (RSV), adenovirus,human metapneumovirus, non COVID-19 coronavirus], atypical organisms(mycoplasma, chlamydia) and bacterialinfections. It is not possible todifferentiate COVID-19 from theseinfections clinically or through routinelab tests. Therefore travel historybecomes important. However, as theepidemic spreads, the travel history07:25 MOre 748epidemic progresses, commercial testswill become available.Other laboratory investigations areusually non specific. The white cellcount is usually normal or low. Theremay be lymphopenia; a lymphocytecount <1000 has been associated withsevere disease. The platelet count isusually normal or mildly low. The CRPand ESR are generally elevated butprocalcitonin levels are usuallynormal. A high procalcitonin level mayindicate a bacterial co-infection. TheALT/AST, prothrombin time, creatinine,D-dimer, CPK and LDH may be elevatedand high levels are associated withsevere disease.

The chest X-ray (CXR) usually showsbilateral infiltrates but may be normalin early disease. The CT is moresensitive and specific. CT imaginggenerally shows infiltrates, groundglass opacities and sub segmental07:5 Owe ban |of persistent local transmission orcontact with patients with similartravel history or those with confirmedCOVID-19 infection. However casesmay be asymptomatic or even withoutfever. A confirmed case is a suspectcase with a positive molecular test.

Specific diagnosis is by specificmolecular tests on respiratory samples(throat swab/ nasopharyngeal swab/sputum/ endotracheal aspirates andbronchoalveolar lavage). Virus mayalso be detected in the stool and insevere cases, the blood. It must beremembered that the multiplex PCRpanels currently available do notinclude the COVID-19. Commercial testsare also not available at present. Inasuspect case in India, the appropriatesample has to be sent to designatedreference labs in India or the NationalInstitute of Virology in Pune. As theepidemic progresses, commercial tests07:5 MOwxs v4iwas linked to a family member and 26children had history oftravel/residence to Hubei province inChina. All the patients were eitherasymptomatic (9%) or had milddisease. No severe or critical caseswere seen. The most commonsymptoms were fever (50%) and cough(38%). All patients recovered withsymptomatic therapy and there wereno deaths. One case of severepneumonia and multiorgandysfunction in a child has also beenreported [19]. Similarly the neonatalcases that have been reported havebeen mild [20].

**Diagnosis [21]**

A suspect case is defined as one withfever, sore throat and cough who hashistory of travel to China or other areasof persistent local transmission orcontact with patients with similartravel histarv or thase with confirmed07:5 Owe ain |Interestingly, disease in patientsoutside Hubei province has beenreported to be milder than those fromWuhan [17]. Similarly, the severity andcase fatality rate in patients outsideChina has been reported to be milder[6]. This may either be due to selectionbias wherein the cases reporting fromWuhan included only the severe casesor due to predisposition of the Asianpopulation to the virus due to higherexpression of ACE, receptors on therespiratory mucosa [11].

Disease in neonates, infants andchildren has been also reported to besignificantly milder than their adultcounterparts. In a series of 34 childrenadmitted to a hospital in Shenzhen,China between January 19th andFebruary 7th, there were 14 males and20 females. The median age was 8 y 11mo and in 28 children the infectionwas linked to a family member and 2607:5 Owe ban |including 1L2, 1L7, 1L10, GCSF, 1P10,MCP1, MIP1A, and TNFa [15]. Themedian time from onset of symptomsto dyspnea was 5 d, hospitalization 7dand acute respiratory distresssyndrome (ARDS) 8 d. The need forintensive care admission was in 25-30% of affected patients in publishedseries. Complications witnessedincluded acute lung injury, ARDS,shock and acute kidney injury.Recovery started in the 2nd or 3rd wk.The median duration of hospital stay inthose who recovered was 10 d. Adverseoutcomes and death are more commonin the elderly and those withunderlying co-morbidities (50-75% offatal cases). Fatality rate in hospitalizedadult patients ranged from 4 to 11%.The overall case fatality rate isestimated to range between 2 and 3%[2].

Interestingly, disease in patientsoutside Hubei province has been07:5 Owe ban |OQ — @ ncbinim.nih.gov/pmc/artiidentified angiotensin receptor 2(ACE,) as the receptor through whichthe virus enters the respiratory mucosa[11].The basic case reproduction rate (BCR)is estimated to range from 2 to 6.47 invarious modelling studies [11]. Incomparison, the BCR of SARS was 2 and1.3 for pandemic flu H1N1 2009 [2].

**Clinical Features [8, 15-18]**

The clinical features of COVID-19 arevaried, ranging from asymptomaticstate to acute respiratory distresssyndrome and multi organdysfunction. The common clinicalfeatures include fever (not in all),cough, sore throat, headache, fatigue,headache, myalgia and breathlessness.Conjunctivitis has also been described.Thus, they are indistinguishable fromathar racniratariy infactinne Tn 9a eitheatBS07:5 Owe ain |UL UPI1TLdS Call opl cdu 1-2 LLt aALLu UepYdsIeon surfaces. The virus can remainviable on surfaces for days infavourable atmospheric conditions butare destroyed in less than a minute bycommon disinfectants like sodiumhypochlorite, hydrogen peroxide etc.[13]. Infection is acquired either byinhalation of these droplets or touchingsurfaces contaminated by them andthen touching the nose, mouth andeyes. The virus is also present in thestool and contamination of the watersupply and subsequent transmissionvia aerosolization/feco oral route isalso hypothesized [6]. As per currentinformation, transplacentaltransmission from pregnant women totheir fetus has not been described [14].However, neonatal disease due to postnatal transmission is described [14].The incubation period varies from 2 to14 d [median 5 d]. Studies haveidentified angiotensin receptor 2(ACF.\ ac tha rarantnr thraich wrhich07:5 Owe 7 Ai

**Epidemiology and Pathogenesis[10, 11]**

All ages are susceptible. Infection istransmitted through large dropletsgenerated during coughing andsneezing by symptomatic patients butcan also occur from asymptomaticpeople and before onset of symptoms[9]. Studies have shown higher viralloads in the nasal cavity as comparedto the throat with no difference in viralburden between symptomatic andasymptomatic people [12]. Patients canbe infectious for as long as thesymptoms last and even on clinicalrecovery. Some people may act assuper spreaders; a UK citizen whoattended a conference in Singaporeinfected 11 other people while stayingin a resort in the French Alps and uponreturn to the UK [6]. These infecteddroplets can spread 1-2 m and deposit< @07:24 @ Owe v7 4iOQ — @ ncbinim.nih.gov/pmc/artiexponentially in other countriesincluding South Korea, Italy and Iran.Of those infected, 20% are in criticalcondition, 25% have recovered, and3310 (3013 in China and 297 in othercountries) have died [2]. India, whichhad reported only 3 cases till 2/3/2020,has also seen a sudden spurt in cases.By 5/3/2020, 29 cases had beenreported; mostly in Delhi, Jaipur andAgra in Italian tourists and theircontacts. One case was reported in anIndian who traveled back from Viennaand exposed a large number of schoolchildren in a birthday party at a cityhotel. Many of the contacts of thesecases have been quarantined.

These numbers are possibly anunderestimate of the infected and deaddue to limitations of surveillance andtesting. Though the SARS-CoV-2originated from bats, the intermediary07:24 mM Ore 748Cases continued to increaseexponentially and modelling studiesreported an epidemic doubling time of1.8 d [10]. In fact on the 12th ofFebruary, China changed its definitionof confirmed cases to include patientswith negative/ pending molecular testsbut with clinical, radiologic andepidemiologic features of COVID-19leading to an increase in cases by15,000 in a single day [6]. As of05/03/2020 96,000 cases worldwide(80,000 in China) and 87 othercountries and 1 internationalconveyance (696, in the cruise shipDiamond Princess parked off the coastof Japan) have been reported [2]. It isimportant to note that while thenumber of new cases has reduced inChina lately, they have increasedexponentially in other countriesincluding South Korea, Italy and Iran.Of those infected, 20% are in criticalnandAitinn ION hares vanatrarn A ana07:24 @ Owe ban |extended to otner clues Ol Hupelprovince. Cases of COVID-19 incountries outside China were reportedin those with no history of travel toChina suggesting that local human-to-human transmission was occurring inthese countries [9]. Airports indifferent countries including India putin screening mechanisms to detectsymptomatic people returning fromChina and placed them in isolation andtesting them for COVID-19. Soon it wasapparent that the infection could betransmitted from asymptomatic peopleand also before onset of symptoms.Therefore, countries including Indiawho evacuated their citizens fromWuhan through special flights or hadtravellers returning from China, placedall people symptomatic or otherwise inisolation for 14 d and tested them forthe virus.

Cases continued to increaseexponentially and modelling studies07:23 @ Owe 748the SARS- CoV. Environmental samplesfrom the Huanan sea food market alsotested positive, signifying that the virusoriginated from there [7]. The numberof cases started increasingexponentially, some of which did nothave exposure to the live animalmarket, suggestive of the fact thathuman-to-human transmission wasoccurring [8]. The first fatal case wasreported on 11th Jan 2020. The massivemigration of Chinese during theChinese New Year fuelled the epidemic.Cases in other provinces of China,other countries (Thailand, Japan andSouth Korea in quick succession) werereported in people who were returningfrom Wuhan. Transmission tohealthcare workers caring for patientswas described on 20th Jan, 2020. By23rd January, the 11 million populationof Wuhan was placed under lock downwith restrictions of entry and exit fromthe region. Soon this lock down was07:23 MOre 94had >95% homology with the batcoronavirus and > 70% similarity withthe SARS- CoV. Environmental samplesfrom the Huanan sea food market alsotested positive, signifying that the virusoriginated from there [7]. The numberof cases started increasingexponentially, some of which did nothave exposure to the live animalmarket, suggestive of the fact thathuman-to-human transmission wasoccurring [8]. The first fatal case wasreported on 11th Jan 2020. The massivemigration of Chinese during theChinese New Year fuelled the epidemic.Cases in other provinces of China,other countries (Thailand, Japan andSouth Korea in quick succession) werereported in people who were returningfrom Wuhan. Transmission tohealthcare workers caring for patientswas described on 20th Jan, 2020. By23rd January, the 11 million populationof Wuhan was placed under lock down07:23 Owe 7 Ai

**Origin and Spread of COVID-19[1, 2, 6]**

In December 2019, adults in Wuhan,capital city of Hubei province and amajor transportation hub of Chinastarted presenting to local hospitalswith severe pneumonia of unknowncause. Many of the initial cases had acommon exposure to the Huananwholesale seafood market that alsotraded live animals. The surveillancesystem (put into place after the SARSoutbreak) was activated andrespiratory samples of patients weresent to reference labs for etiologicinvestigations. On December 31st 2019,China notified the outbreak to theWorld Health Organization and on 1stJanuary the Huanan sea food marketwas closed. On 7th January the viruswas identified as a coronavirus thathad >95% homology with the bat<q @07:23 MOwe v4such instance was in 2002-2003 when anew coronavirus of the B genera andwith origin in bats crossed over tohumans via the intermediary host ofpalm civet cats in the Guangdongprovince of China. This virus,designated as severe acute respiratorysyndrome coronavirus affected 8422people mostly in China and Hong Kongand caused 916 deaths (mortality rate11%) before being contained [4].Almost a decade later in 2012, theMiddle East respiratory syndromecoronavirus (MERS-CoV), also of batorigin, emerged in Saudi Arabia withdromedary camels as the intermediatehost and affected 2494 people andcaused 858 deaths (fatality rate 34%)[5].Origin and Spread of COVID-19[1, 2, 6]In December 2019, adults in Wuhan,capital citv of Hubei province and a<4 ® i07:23 Owe 7 Aiarucle gives a bird’s eye view aboutthis new virus. Since knowledge aboutthis virus is rapidly evolving, readersare urged to update themselvesregularly.HistoryCoronaviruses are enveloped positivesense RNA viruses ranging from 60 nmto 140 nm in diameter with spike likeprojections on its surface giving itacrown like appearance under theelectron microscope; hence the namecoronavirus [3]. Four corona virusesnamely HKU1, NL63, 229E and OC43have been in circulation in humans,and generally cause mild respiratorydisease.There have been two events in the pasttwo decades wherein crossover ofanimal betacorona viruses to humanshas resulted in severe disease. The firstsuch instance was in 2002-2003 when aPe ee a ER 0 Meee |07:23 Owe 7 Aiand Middle East respiratory syndromecoronavirus (MERS-CoV), but has lowerfatality. The global impact of this newepidemic is yet uncertain.

Keywords: 2019-nCOV, SARS-CoV-2,COVID-19, Pneumonia, Review

**Introduction**

The 2019 novel coronavirus (2019-nCoV) or the severe acute respiratorysyndrome corona virus 2 (SARS-CoV-2)as it is now called, is rapidly spreadingfrom its origin in Wuhan City of HubeiProvince of China to the rest of theworld [1]. Till 05/03/2020 around 96,000cases of coronavirus disease 2019(COVID-19) and 3300 deaths have beenreported [2]. India has reported 29cases till date. Fortunately so far,children have been infrequentlyaffected with no deaths. But the futurecourse of this virus is unknown. Thisarticle gives a bird’s eye view about07:23 Owe 9 Ai(uouuiy ULE LiUity Guu ULvoe Wattcomorbidities), it may progress topneumonia, acute respiratory distresssyndrome (ARDS) and multi organdysfunction. Many people areasymptomatic. The case fatality rate isestimated to range from 2 to 3%.Diagnosis is by demonstration of thevirus in respiratory secretions byspecial molecular tests. Commonlaboratory findings include normal/low white cell counts with elevated C-reactive protein (CRP). Thecomputerized tomographic chest scanis usually abnormal even in those withno symptoms or mild disease.Treatment is essentially supportive;role of antiviral agents is yet to beestablished. Prevention entails homeisolation of suspected cases and thosewith mild illnesses and strict infectioncontrol measures at hospitals thatinclude contact and dropletprecautions. The virus spreads fasterthan its twoa ancestors the SARS-CoV

**Abstract**

There is a new public health crisesthreatening the world with theemergence and spread of 2019 novelcoronavirus (2019-nCoV) or the severeacute respiratory syndromecoronavirus 2 (SARS-CoV-2). The virusoriginated in bats and was transmittedto humans through yet unknownintermediary animals in Wuhan, Hubeiprovince, China in December 2019.There have been around 96,000reported cases of coronavirus disease2019 (COVID-2019) and 3300 reporteddeaths to date (05/03/2020). The diseaseis transmitted by inhalation or contactwith infected droplets and theincubation period ranges from 2 to 14d. The symptoms are usually fever,cough, sore throat, breathlessness,fatigue, malaise among others. Thedisease is mild in most people; in some(usually the elderly and those withcamarhiditiac) it maw nrnoracce tainteractions remain largely unclear. Intensive studies onthese virological profiles of SARS-CoV-2 will providethe basis for the development of preventive and thera-peutic strategies against COVID-19. Moreover, contin-ued genomic monitoring of SARS-CoV-2 in new cases isneeded worldwide, as it is important to promptly iden-tify any mutation that may result in phenotypic changesof the virus. Finally, COVID-19 is challenging all humanbeings. Tackling this epidemic is a long-term job whichrequires efforts of every individual, and internationalcollaborations by scientists, authorities and the public.this emerging virus will establish a niche in humansand coexist with us for a long time’. Before clinicallyapproved vaccines are widely available, there is no bet-ter way to protect us from SARS-CoV-2 than personalpreventive behaviours such as social distancing andwearing masks, and public health measures, includingactive testing, case tracing and restrictions on socialgatherings. Despite a flood of SARS-CoV-2 researchpublished every week, current knowledge of this novelcoronavirus is just the tip of the iceberg. The animalorigin and cross-species infection route of SARS-CoV-2are yet to be uncovered. The molecular mechanisms ofSARS-CoV-2 infection pathogenesis and virus-hostby the University of Oxford. In a randomized controlledphase I/II trial, it induced neutralizing antibodies againstSARS-CoV-2 in all 1,077 participants after a secondvaccine dose, while its safety profile was acceptable aswell'®. The NIAID and Moderna co-manufacturedmRNA- 1273, a lipid nanoparticle-formulated mRNAvaccine candidate that encodes the stabilized prefusionSARS-CoV-2 S protein. Its immunogenicity has beenconfirmed by a phase I trial in which robust neutralizingantibody responses were induced in a dose-dependentmanner and increased after a second dose'™. Regardinginactivated vaccines, a successful phase I/II trial involv-ing 320 participants has been reported in China. Thewhole-virus COVID-19 vaccine had a low rate of adversereactions and effectively induced neutralizing antibodyproduction’. The verified safety and immunogenicitysupport advancement of these vaccine candidates tophase III clinical trials, which will evaluate their efficacyin protecting healthy populations from SARS-CoV-2infection.

**Future perspectives**

COVID-19 is the third highly pathogenic human coro-navirus disease to date. Although less deadly than SARSand MERS, the rapid spreading of this highly conta-gious disease has posed the severest threat to globalhealth in this century. The SARS-CoV-2 outbreak haslasted for more than half a year now, and it is likely thatin vitro and in vivo'\*~'\*\*. Compared with convalescentplasma, which has limited availability and cannot beamplified, monoclonal antibodies can be developed inlarger quantities to meet clinical requirements. Hence,they provide the possibility for the treatment and pre-vention of COVID-19. The neutralizing epitopes ofthese monoclonal antibodies also offer important infor-mation for vaccine design. However, the high cost andlimited capacity of manufacturing, as well as the prob-lem of bioavailability, may restrict the wide applicationof monoclonal antibody therapy.

**Vaccines**

Vaccination is the most effective method for a long-termstrategy for prevention and control of COVID-19 inthe future. Many different vaccine platforms againstSARS-CoV-2 are in development, the strategies of whichinclude recombinant vectors, DNA, mRNA in lipid nano-particles, inactivated viruses, live attenuated viruses andprotein subunits'\*-'\*'. As of 2 October 2020, ~174 vac-cine candidates for COVID-19 had been reportedand 51 were in human clinical trials (COVID-19vaccine and therapeutics tracker). Many of these vac-cine candidates are in phase II testing, and some havealready advanced to phase III trials. A randomize4double-blinded phase II trial of an adenovirus typevectored vaccine expressing the SARS-CoV-2 S protein,developed by CanSino Biologicals and the Academy ofMilitary Medical Sciences of China, was conducted in603 adult volunteers in Wuhan. The vaccine has provedto be safe and induced considerable humoral and cel-lular immune response in most recipients after a singleimmunization’. Another vectored vaccine, ChAdOx1,The interferon response is one of the major innateimmunity defences against virus invasion. Interferonsinduce the expression of diverse interferon-stimulatedgenes, which can interfere with every step of virusreplication. Previous studies identified type I interfer-ons as a promising therapeutic candidate for SARS’.In vitro data showed SARS-CoV-2 is even more sen-sitive to type I interferons than SARS-CoV, suggestingthe potential effectiveness of type I interferons in theearly treatment of COVID-19 (REF.'\*’). In China, vaporinhalation of interferon-a is included in the COVID-19treatment guideline'”. Clinical trials are ongoing acrossthe world to evaluate the efficacy of different therapiesinvolving interferons, either alone or in combinationwith other agents”.

**Immunoglobulin therapy.**

Convalescent plasma treat-ment is another potential adjunctive therapy forCOVID-19. Preliminary findings have suggestedimproved clinical status after the treatment’\*’\*. TheFDA has provided guidance for the use of COVID-19convalescent plasma under an emergency investigationalnew drug application. However, this treatment may haveadverse effects by causing antibody-mediated enhance-ment of infection, transfusion-associated acute lunginjury and allergic transfusion reactions.Monoclonal antibody therapy is an effective immuno-therapy for the treatment of some viral infections inselect patients. Recent studies reported specific mon-oclonal antibodies neutralizing SARS-CoV-2 infectionother clinical trials in different phases are still ongoingelsewhere.

**Immunomodulatory agents.**

SARS-CoV-2 triggers astrong immune response which may cause cytokinestorm syndrome \*'. Thus, immunomodulatory agentsthat inhibit the excessive inflammatory response maybe a potential adjunctive therapy for COVID-19.Dexamethasone is a corticosteroid often used in a widerange of conditions to relieve inflammation throughits anti-inflammatory and immunosuppressant effects.Recently, the RECOVERY trial found dexamethasonereduced mortality by about one third in hospitalizedpatients with COVID-19 who received invasive mechan-ical ventilation and by one fifth in patients receivingoxygen. By contrast, no benefit was found in patientswithout respiratory support'’’.

Tocilizumab and sarilumab, two types of interleukin-6(IL-6) receptor-specific antibodies previously used toreat various types of arthritis, including rheumatoidarthritis, and cytokine release syndrome, showed effec-iveness in the treatment of severe COVID-19 by atten-uating the cytokine storm in a small uncontrolled trial”.Bevacizumab is an anti-vascular endothelial growth‘actor (VEGF) medication that could potentially reducepulmonary oedema in patients with severe COVID-19.Eculizumab is a specific monoclonal antibody thatinhibits the proinflammatory complement protein C5.Preliminary results showed that it induced a drop ofinflammatory markers and C-reactive protein levels,suggesting its potential to be an option for the treatmentof severe COVID-19 (REF).and ritonavir had little therapeutic benefit in patientswith COVID-19, but appeared more effective when usedin combination with other drugs, including ribavirin andinterferon beta-1b'\*\*'\*. The Randomized Evaluation ofCOVID-19 Therapy (RECOVERY) trial, a national clin-ical trial programme in the UK, has stopped treatmentwith lopinavir and ritonavir as no significant beneficialeffect was observed in a randomized trial established inMarch 2020 with a total of 1,596 patients'“°. Nevertheless,respectively’. However, this study did not includea control arm, and most of the trials of favilavir werebased on a small sample size. For more reliable assess-ment of the effectiveness of favilavir for treatingCOVID-19, large-scale randomized controlled trialsshould be conducted.

Lopinavir and ritonavir were reported to havein vitro inhibitory activity against SARS-CoV andMERS-CoV™"'’, Alone, the combination of lopinavir

**Inhibition of virus replication.**

Replication inhibitorsinclude remdesivir (GS-5734), favilavir (T-705), riba-virin, lopinavir and ritonavir. Except for lopinavir andritonavir, which inhibit 3CLpro, the other three all targetRdRp'\*\*"\*? (FIG. 5). Remdesivir has shown activity againstSARS-CoV-2 in vitro and in vivo'\*\*\*. A clinical studyrevealed a lower need for oxygen support in patientswith COVID-19 (REF.'°’). Preliminary results of theAdaptive COVID-19 Treatment Trial (ACTT) clinicalrial by the National Institute of Allergy and InfectiousDiseases (NIAID) reported that remdesivir can shortenthe recovery time in hospitalized adults with COVID-19by a couple days compared with placebo, but the differ-ence in mortality was not statistically significant'\*. TheFDA has issued an emergency use authorization for rem-desivir for the treatment of hospitalized patients withsevere COVID-19. It is also the first approved option byhe European Union for treatment of adults and adoles-cents with pneumonia requiring supplemental oxygen.Several international phase III clinical trials are contin-uing to evaluate the safety and efficacy of remdesivir forhe treatment of COVID-19.Favilavir (T-705), which is an antiviral drug devel-oped in Japan to treat influenza, has been approved inChina, Russia and India for the treatment of COVID-19.A clinical study in China showed that favilavir signif-icantly reduced the signs of improved disease signson chest imaging and shortened the time to viralclearance’. A preliminary report in Japan showed ratesof clinical improvement of 73.8% and 87.8% from thestart of favilavir therapy in patients with mild COVID-19at 7 and 14 days, respectively, and 40.1% and 60.3%in patients with severe COVID-19 at 7 and 14 days,Chloroquine and hydroxychloroquine are otherpotential but controversial drugs that interfere withthe entry of SARS-CoV-2. They have been used in theprevention and treatment of malaria and autoimmunediseases, including systemic lupus erythematosus andrheumatoid arthritis. They can inhibit the glycosyla-tion of cellular receptors and interfere with virus—hostreceptor binding, as well as increase the endosomal pHand inhibit membrane fusion. Currently, no scientificconsensus has been reached for their efficacy in thereatment of COVID-19. Some studies showed they caninhibit SARS-CoV-2 infection in vitro, but the clinicaldata are insufficient'\*'”’. Two clinical studies indicatedno association with death rates in patients receivingchloroquine or hydroxychloroquine compared withthose not receiving the drug and even suggest it mayincrease the risk of dying as a higher risk of cardiac arrestwas found in the treated patients'\*\*'\*'. On 15 June 2020,owing to the side effects observed in clinical trials, theUS Food and Drug Administration (FDA) revokedthe emergency use authorization for chloroquine andhydroxychloroquine for the treatment of COVID-19.Another potential therapeutic strategy is to block bind-ing of the S protein to ACE2 through soluble recombi-nant hACE2, specific monoclonal antibodies or fusioninhibitors that target the SARS-CoV-2 S protein'’-'\*(FIG. 5). The safety and efficacy of these strategies needto be aeoter in future clinical trials.

**Inhibition of virus entry.**

SARS-CoV-2 uses ACE2 as thereceptor and human proteases as entry activators; sub-sequently it fuses the viral membrane with the cell mem-brane and achieves invasion. Thus, drugs that interferewith entry may be a potential treatment for COVID-19.Umifenovir (Arbidol) is a drug approved in Russia andChina for the treatment of influenza and other respira-tory viral infections. It can target the interaction betweenthe S protein and ACE2 and inhibit membrane fusion(FIG. 5). In vitro experiments showed that it has activityagainst SARS-CoV-2, and current clinical data revealedit may be more effective than lopinavir and ritonavir intreating COVID-19 (REFS'>'\*\*). However, other clinicalstudies showed umifenovir might not improve the prog-nosis of or accelerate SARS-CoV-2 clearance in patientswith mild to moderate COVID-19 (REFS!”\*!”°). Yet someongoing clinical trials are evaluating its efficacy forCOVID-19 treatment. Camostat mesylate is approvedin Japan for the treatment of pancreatitis and postoper-ative reflux oesophagitis. Previous studies showed that itcan prevent SARS-CoV from entering cells by blockingTMPRSS2 activity and protect mice from lethal infectionwith SARS-CoV in a pathogenic mouse model (wild-type mice infected with a mouse-adapted SARS-CoVstrain)'°'’, Recently, a study revealed that camostatmesylate blocks the entry of SARS-CoV-2 into humanlung cells”. Thus, it can be a potential antiviral drugagainst SARS-CoV-2 infection, although so far there arenot sufficient clinical data to support its efficacy.with COVID-19 showed typical features on initial CT,including bilateral multilobar ground-glass opacitieswith a peripheral or posterior distribution''\*''’. Thus,it has been suggested that CT scanning combinedwith repeated swab tests should be used for individu-als with high clinical suspicion of COVID-19 but whotest negative in initial nucleic acid screening". Finally,SARS-CoV-2 serological tests detecting antibodies toNorS protein could complement molecular diagnosis,particularly in late phases after disease onset or for retro-spective studies''®'\*°'?!, However, the extent and dura-tion of immune responses are still unclear, and availableserological tests differ in their sensitivity and specific-ity, all of which need to be taken into account whenone is deciding on serological tests and interpretingtheir results or potentially in the future test for T cellresponses.

**Therapeutics**

To date, there are no generally proven effective thera-pies for COVID-19 or antivirals against SARS-CoV-2,although some treatments have shown some benefitsin certain subpopulations of patients or for certain endpoints (see later). Researchers and manufacturers areconducting large-scale clinical trials to evaluate var-ious therapies for COVID-19. As of 2 October 2020,there were about 405 therapeutic drugs in developmentfor COVID-19, and nearly 318 in human clinical trials(COVID-19 vaccine and therapeutics tracker). In thefollowing sections, we summarize potential therapeuticsagainst SARS-CoV-2 on the basis of published clinicaldata and experience.viruses in nasal washes, saliva, urine and faeces for upto 8 days after infection, and a few naive ferrets with onlyindirect contact were positive for viral RNA, suggest-ing airborne transmission”. In addition, transmissionof the virus through the ocular surface and prolongedpresence of SARS-CoV-2 viral RNA in faecal sampleswere also documented'\*'’. Coronaviruses can persiston inanimate surfaces for days, which could also be thecase for SARS-CoV-2 and could pose a prolonged risk ofinfection'’. These findings explain the rapid geographicspread of COVID-19, and public health interventions toreduce transmission will provide benefit to mitigate theepidemic, as has proved successful in China and severalother countries, such as South Korea\*?!°4!,

**Diagnosis**

Early diagnosis is crucial for controlling the spread ofCOVID-19. Molecular detection of SARS-CoV-2 nucleicacid is the gold standard. Many viral nucleic acid detec-tion kits targeting ORF1b (including RdRp), N, E orS genes are commercially available'"'°~'. The detectiontime ranges from several minutes to hours dependingon the technology'\*'”!-'"", The molecular detectioncan be affected by many factors. Although SARS-CoV-2has been detected from a variety of respiratory sources,including throat swabs, posterior oropharyngeal saliva,nasopharyngeal swabs, sputum and bronchial fluid,the viral load is higher in lower respiratory tract sam-ples'!°°!'""> In addition, viral nucleic acid was alsofound in samples from the intestinal tract or blood evenwhen respiratory samples were negative''’. Lastly, viralload may already drop from its peak level on diseaseonset”. Accordingly, false negatives can be commonwhen oral swabs and used, and so multiple detectionmethods should be adopted to confirm a COVID-19diagnosis''”''\*. Other detection methods were there-fore used to overcome this problem. Chest CT wasused to quickly identify a patient when the capacity ofmolecular detection was overloaded in Wuhan. Patientsareas. For example, a cohort study in London revea’\_\*44% of the frontline health-care workers from a hospwere infected with SARS-CoV-2 (REF.“).The high transmissibility of SARS-CoV-2 maybe attributed to the unique virological features ofSARS-CoV-2. Transmission of SARS-CoV occurredmainly after illness onset and peaked following dis-ease severity”. However, the SARS-CoV-2 viral loadin upper respiratory tract samples was already high-est during the first week of symptoms, and thus therisk of pharyngeal virus shedding was very high atthe beginning of infection”””. It was postulated thatundocumented infections might account for 79% ofdocumented cases owing to the high transmissibilityof the virus during mild disease or the asymptomaticperiod®. A patient with COVID-19 spreads viruses inliquid droplets during speech. However, smaller andmuch more numerous particles known as aerosol parti-cles can also be visualized, which could linger in the airfor a long time and then penetrate deep into the lungswhen inhaled by someone else”\*-'. Airborne trans-mission was also observed in the ferret experimentsmentioned above. SARS-CoV-2-infected ferrets shedof plasma cytokines, which suggests an immunopatho-logical process caused by a cytokine storm®\*”. In thiscohort of patient, around 2.3% people died withina median time of 16 days from disease onset®\*\*. Menolder than 68 years had a higher risk of respiratory fail-ure, acute cardiac injury and heart failure that led todeath, regardless of a history of cardiovascular disease\*°(FIG. 4). Most patients recovered enough to be releasedfrom hospital in 2 weeks” (FIG. 4).

Early transmission of SARS-CoV-2 in Wuhan inDecember 2019 was initially linked to the HuananSeafood Wholesale Market, and it was suggested asthe source of the outbreak””’’. However, communitytransmission might have happened before that\*\*. Later,ongoing human-to-human transmission propagated theoutbreak’, It is generally accepted that SARS-CoV-2 ismore transmissible than SARS-CoV and MERS-CoV;however, determination of an accurate reproductionnumber (RO) for COVID-19 is not possible yet, as manyasymptomatic infections cannot be accurately accountedfor at this stage. An estimated RO of 2.5 (ranging from1.8 to 3.6) has been proposed for SARS-CoV-2 recently,compared with 2.0-3.0 for SARS-CoV”. Notably, mostof the SARS-CoV-2 human-to-human transmissionearly in China occurred in family clusters, and in othercountries large outbreaks also happened in other set-tings, such as migrant worker communities, slaughter-houses and meat packing plants, indicating the necessityof isolating infected people”’'\*”!-°’. Nosocomial transmis-sion was not the main source of transmission in Chinabecause of the implementation of infection controlmeasures in clinical settings’. By contrast, a high riskof nosocomial transmission was reported in some otheror even die, whereas most young people and childrenhave only mild diseases (non- pneumonia or mildpneumonia) or are asymptomatic’, Notably, the riskof disease was not higher for pregnant women. However,evidence of transplacental transmission of SARS-CoV-2rom an infected mother to a neonate was reported,although it was an isolated case\*\*\*\*. On infection, themost common symptoms are fever, fatigue and drycough'\*\*\*°!, Less common symptoms include sputumproduction, headache, haemoptysis, diarrhoea, anorexia,sore throat, chest pain, chills and nausea and vomiting instudies of patients in China'\*°\*\*', Self-reported olfac-‘ory and taste disorders were also reported by patientsin Italy. Most people showed signs of diseases after anincubation period of 1-14 days (most commonly around5 days), and dyspnoea and pneumonia developed withina median time of 8 days from illness onset”.

In a report of 72,314 cases in China, 81% of thecases were classified as mild, 14% were severe cases thatrequired ventilation in an intensive care unit (ICU) anda 5% were critical (that is, the patients had respiratoryfailure, septic shock and/or multiple organ dysfunctionor failure)”\*°. On admission, ground-glass opacity wasthe most common radiologic finding on chest computedtomography (CT)'\*\*\*°\*!, Most patients also developedmarked lymphopenia, similar to what was observed inpatients with SARS and MERS, and non-survivors devel-oped severer lymphopenia over time'\*\*\*\*\*\*', Comparedwith non-ICU patients, ICU patients had higher levelslower respiratory tracts. Acute viral interstitial pneu-monia and humoral and cellular immune responseswere observed\*\*”’. Moreover, prolonged virus sheddingpeaked early in the course of infection in asymptomaticmacaques”, and old monkeys showed severer intersti-tial pneumonia than young monkeys’\*, which is similarto what is seen in patients with COVID-19. In humanACE2-transgenic mice infected with SARS-CoV-2, typ-ical interstitial pneumonia was present, and viral anti-gens were observed mainly in the bronchial epithelialcells, macrophages and alveolar epithelia. Some humanACE2-transgenic mice even died after infection”.In wide-type mice, a SARS-CoV-2 mouse-adapted strainwith the N501Y alteration in the RBD of the S proteinwas generated at passage 6. Interstitial pneumonia andinflammatory responses were found in both youngand aged mice after infection with the mouse-adaptedstrain”. Golden hamsters also showed typical symptomsafter being infected with SARS-CoV-2 (REF.”). In otheranimal models, including cats and ferrets, SARS-CoV-2could efficiently replicate in the upper respiratory tractbut did not induce severe clinical symptoms\*\*”. As trans-mission by direct contact and air was observed in infectedferrets and hamsters, these animals could be used tomodel different transmission modes of COVID-19(REFS’”-”?), Animal models offer important informationfor understanding the pathogenesis of SARS-CoV-2infection and the transmission dynamics of SARS-CoV-2, and are important to evaluate the efficacy ofantiviral therapeutics and vaccines.

**Clinical and epidemiological features**

It appears that all ages of the population are susceptible toSARS-CoV-2 infection, and the median age of infectionis around 50 years”'\*\*\*\*', However, clinical manifesta-tions differ with age. In general, older men (>60 yearsold) with co-morbidities are more likely to developsevere respiratory disease that requires hospitalizationThe pathogenesis of SARS-CoV-2 infection inhumans manifests itself as mild symptoms to severerespiratory failure. On binding to epithelial cells inthe respiratory tract, SARS-CoV-2 starts replicatingand migrating down to the airways and enters alveo-lar epithelial cells in the lungs. The rapid replication ofSARS-CoV-2 in the lungs may trigger a strong immuneresponse. Cytokine storm syndrome causes acute res-piratory distress syndrome and respiratory failure, whichis considered the main cause of death in patients withCOVID-19 (REFS\*”"!). Patients of older age (>60 years)and with serious pre-existing diseases have a greater riskof developing acute respiratory distress syndrome anddeath" (FIG. 4). Multiple organ failure has also beenreported in some COVID-19 cases\*\*.

Histopathological changes in patients with COVID-19occur mainly in the lungs. Histopathology analysesshowed bilateral diffused alveolar damage, hyalinemembrane formation, desquamation of pneumocytesand fibrin deposits in lungs of patients with severeCOVID-19. Exudative inflammation was also shownin some cases. Immunohistochemistry assays detectedSARS-CoV-2 antigen in the upper airway, bronchiolarepithelium and submucosal gland epithelium, as well asin type I and type II pneumocytes, alveolar macrophagesand hyaline membranes in the lungs'\*°’,Animal models used for studying SARS-CoV-2infection pathogenesis include non-human primates(rhesus macaques, cynomolgus monkeys, marmosetsand African green monkeys), mice (wild-type mice (withmouse-adapted virus) and human ACE2-transgenicor human ACE2-knock-in mice), ferrets and goldenhamsters\*\*\*-“, In non-human primate animal mod-els, most species display clinical features similar to thoseof patients with COVID-19, including virus shedding,virus replication and host responses to SARS-CoV-2infection®”\*”\*. For example, in the rhesus macaquemodel, high viral loads were detected in the upper andCurrently, our knowledge on the animal origin ofSARS-CoV-2 remains incomplete to a large part. Thereservoir hosts of the virus have not been clearly proven.It is unknown whether SARS-CoV-2 was transmittedto humans through an intermediate host and whichanimals may act as its intermediate host. Detection oRaTG13, RmYNO02 and pangolin coronaviruses impliesthat diverse coronaviruses similar to SARS-CoV-2 arecirculating in wildlife. In addition, as previous stud-ies showed recombination as the potential origin osome sarbecoviruses such as SARS-CoV, it cannot beexcluded that viral RNA recombination among differenrelated coronaviruses was involved in the evolution oSARS-CoV-2. Extensive surveillance of SARS-CoV-2-related viruses in China, Southeast Asia and otherregions targeting bats, wild and captured pangolins andother wildlife species will help us to better understandthe zoonotic origin of SARS-CoV-2.

Besides wildlife, researchers investigated the sus-ceptibility of domesticated and laboratory animals toSARS-CoV-2 infection. The study demonstrated exper-imentally that SARS-CoV-2 replicates efficiently in catsand in the upper respiratory tract of ferrets, whereasdogs, pigs, chickens and ducks were not susceptible toSARS-CoV-2 (REF.")). The susceptibility of minks wasdocumented by a report from the Netherlands on anoutbreak of SARS-CoV-2 infection in farmed minks.Although the symptoms in most infected minks weremild, some developed severe respiratory distressand died of interstitial pneumonia“. Both virologi-cal and serological testing found evidence for naturalSARS-CoV-2 infection in two dogs from households withhuman cases of COVID-19 in Hong Kong, but the dogsresidues for receptor binding” (FIG. 3b). In comparisonwith the Guangdong strains, pangolin coronavirusesreported from Guangxi are less similar to SARS-CoV-2,with 85.5% genome sequence identity”. The repeatedoccurrence of SARS-CoV-2-related coronavirus infec-tions in pangolins from different smuggling eventssuggests that these animals are possible hosts of theviruses. However, unlike bats, which carry coronaviruseshealthily, the infected pangolins showed clinical signsand histopathological changes, including interstitialpneumonia and inflammatory cell infiltration in diverseorgans“. These abnormalities suggest that pangolins areunlikely to be the reservoir of these coronaviruses butmore likely acquired the viruses after spillover from thenatural hosts.

An intermediate host usually plays an important rolein the outbreak of bat-derived emerging coronaviruses;for example, palm civets for SARS-CoV and dromedarycamels for MERS-CoV. The virus strains carried by thesetwo intermediate hosts were almost genetically identi-cal to the corresponding viruses in humans (more than99% genome sequence identity)'. Despise an RBD that isvirtually identical to that of SARS-CoV-2, the pangolincoronaviruses known to date have no more than 92%genome identity with SARS-CoV-2 (REF). The avail-able data are insufficient to interpret pangolins as theintermediate host of SARS-CoV-2. So far, no evidencehas shown that pangolins were directly involved in theemergence of SARS-CoV-2.in Yunnan. This novel bat virus, denoted “RmYN02;is 93.3% identical to SARS-CoV-2 across the genome.In the long lab gene, it exhibits 97.2% identity toSARS-CoV-2, which is even higher than for RaTG13(REF). In addition to RaTG13 and RmYN02, phyloge-netic analysis shows that bat coronaviruses ZC45 andZXC21 previously detected in Rhinolophus pusillusbats from eastern China also fall into the SARS-CoV-2lineage of the subgenus Sarbecovirus” (FIG. 2). The dis-covery of diverse bat coronaviruses closely related toSARS-CoV-2 suggests that bats are possible reservoirsof SARS-CoV-2 (REF.\*”). Nevertheless, on the basis ofcurrent findings, the divergence between SARS-CoV-2and related bat coronaviruses likely represents more than20 years of sequence evolution, suggesting that these batcoronaviruses can be regarded only as the likely evolu-tionary precursor of SARS-CoV-2 but not as the directprogenitor of SARS-CoV-2 (REF.\*).

Beyond bats, pangolins are another wildlife hostprobably linked with SARS-CoV-2. Multiple SARS-CoV-2-related viruses have been identified in tissues of Malayanpangolins smuggled from Southeast Asia into southernChina from 2017 to 2019. These viruses from pangolinsindependently seized by Guangxi and Guangdong pro-vincial customs belong to two distinct sublineages\*-\*".The Guangdong strains, which were isolated orsequenced by different research groups from smug-gled pangolins, have 99.8% sequence identity with eachother"'. They are very closely related to SARS-CoV-2,exhibiting 92.4% sequence similarity. Notably, the RBDof Guangdong pangolin coronaviruses is highly similarto that of SARS-CoV-2. The receptor-binding motif(RBM; which is part of the RBD) of these viruses hasonly one amino acid variation from SARS-CoV-2, andit is identical to that of SARS-CoV-2 in all five criticalpolymorphism at nucleotide position 28,144, whichresults in amino acid substitution of Ser for Lys at residue84 of the ORF8 protein. Those variants with this muta-tion make up a single subclade labelled as ‘clade S\*\*™\*.Currently, however, the available sequence data are notsufficient to interpret the early global transmission his-tory of the virus, and travel patterns, founder effects andpublic health measures also strongly influence the spreadof particular lineages, irrespective of potential biologicaldifferences between different virus variants.

**Animal host and spillover**

Bats are important natural hosts of alphacoronavi-ruses and betacoronaviruses. The closest relativeto SARS-CoV-2 known to date is a bat coronavirusdetected in Rhinolophus affinis from Yunnan province,China, named ‘RaTG13’, whose full-length genomesequence is 96.2% identical to that of SARS-CoV-2(REF."'). This bat virus shares more than 90% sequenceidentity with SARS-CoV-2 in all ORFs throughoutthe genome, including the highly variable S and ORF8(REF."). Phylogenetic analysis confirms that SARS-CoV-2closely clusters with RaTG13 (FIG. 2). The high geneticsimilarity between SARS-CoV-2 and RaTG13 supportsthe hypothesis that SARS-CoV-2 likely originated frombats®. Another related coronavirus has been reportedmore recently in a Rhinolophus malayanus bat sampledin Yunnan Thic navel hat virne denated ‘RmMYNN)’To assess the genetic variation of different SARS-CoV-2 strains, the 2019 Novel Coronavirus Resourceof China National Center for Bioinformation aligned77,801 genome sequences of SARS-CoV-2 detected glob-ally and identified a total of 15,018 mutations, including14,824 single-nucleotide polymorphisms (BIGD)\*'.In the S protein, four amino acid alterations, V483A,L455I, F456V and G4765, are located near the bindinginterface in the RBD, but their effects on binding to thehost receptor are unknown. The alteration D614G inthe S1 subunit was found far more frequently than otherS variant sites, and it is the marker of a major subclade ofSARS-CoV-2 (clade G). Since March 2020, SARS-CoV-2variants with G614 in the S protein have replaced theoriginal D614 variants and become the dominant formcirculating globally. Compared with the D614 variant,higher viral loads were found in patients infected withthe G614 variant, but clinical data suggested no signif-icant link between the D614G alteration and diseaseseverity’’. Pseudotyped viruses carrying the S proteinwith G614 generated higher infectious titres than virusescarrying the S protein with D614, suggesting the altera-tion may have increased the infectivity of SARS-CoV-2(REF). However, the results of in vitro experiments basedon pseudovirus models may not exactly reflect naturalinfection. This preliminary finding should be validatedby more studies using wild-type SARS-CoV-2 variants toinfect different target cells and animal models. Whetherthis amino acid change enhanced virus transmissibil-ity is also to be determined. Another marker mutationfor SARS-CoV-2 evolution is the single-nucleotidea polybasic cleavage site (RRAR), which enables effec-tive cleavage by furin and other proteases”. Such anS1-S2 cleavage site is not observed in all related virusesbelonging to the subgenus Sarbecovirus, except for asimilar three amino acid insertion (PAA) in RmYN02,a bat-derived coronavirus newly reported fromRhinolophus malayanus in China” (FIG. 3a). Although theinsertion in RmYNO02 does not functionally represent apolybasic cleavage site, it provides support for the notionthat this characteristic, initially considered unique toSARS-CoV-2, has been acquired naturally”. A structuralstudy suggested that the furin-cleavage site can reducethe stability of SARS-CoV-2 S protein and facilitate theconformational adaption that is required for the bindingof the RBD to its receptor”. Whether the higher trans-missibility of SARS-CoV-2 compared with SARS-CoVis a gain of function associated with acquisition of thefurin-like cleavage site is yet to be demonstrated”®.

An additional distinction is the accessory gene orf8of SARS-CoV-2, which encodes a novel protein showingonly 40% amino acid identity to ORF8 of SARS-CoV.Unlike in SARS-CoV, this new ORF8 protein doesnot contain a motif that triggers intracellular stresspathways”. Notably, a SARS-CoV-2 variant with a382-nucleotide deletion covering the whole of ORF8 hasbeen discovered in a number of patients in Singapore,which resembles the 29- or 415-nucleotide deletions inthe ORF8 region observed in human SARS-CoV variantsfrom the late phase of the 2002-2003 outbreak”. SuchORFS deletion may be indicative of human adaptationafter cross-species transmission from an animal host.6 h6fluaS SC... KC tC CANmM. .. ae: oeand other SARSr-CoVs (FIG. 2). Using sequences of fiveconserved replicative domains in pplab (3C-like protease(3CLpro), nidovirus RNA-dependent RNA polymerase(RdRp)-associated nucleotidyltransferase (NiRAN),RdRp, zinc-binding domain (ZBD) and HELI), theCoronaviridae Study Group of the InternationalCommittee on Taxonomy of Viruses estimated thepairwise patristic distances between SARS-CoV-2 andknown coronaviruses, and assigned SARS-CoV-2 tothe existing species SARSr-CoV”. Although phyloge-netically related, SARS-CoV-2 is distinct from all othercoronaviruses from bats and pangolins in this species.

The SARS-CoV-2 S protein has a full size of1,273 amino acids, longer than that of SARS-CoV(1,255 amino acids) and known bat SARSr-CoVs(1,245-1,269 amino acids). It is distinct from the S pro-teins of most members in the subgenus Sarbecovirus,sharing amino acid sequence similarities of 76.7-77.0% with SARS-CoVs from civets and humans,length to the corresponding proteins in SARS-CoV.Of the four structural genes, SARS-CoV-2 shares morethan 90% amino acid identity with SARS-CoV exceptfor the S gene, which diverges''”’. The replicase genecovers two thirds of the 5’ genome, and encodes a largepolyprotein (pp1lab),which is proteolytically cleaved into16 non-structural proteins that are involved in transcrip-tion and virus replication. Most of these SARS-CoV-2non-structural proteins have greater than 85% aminoacid sequence identity with SARS-CoV”.

The phylogenetic analysis for the whole genomeshows that SARS-CoV-2 is clustered with SARS-CoVand SARS-related coronaviruses (SARSr-CoVs) foundin bats, placing it in the subgenus Sarbecovirus of thegenus Betacoronavirus. Within this clade, SARS-CoV-2is grouped in a distinct lineage together with four horse-shoe bat coronavirus isolates (RaTG13, RMYN02, ZC45and ZXC21) as well as novel coronaviruses recently iden-tified in pangolins, which group parallel to SARS-CoV216 countries and regions from all six continents hadreported more than 20 million cases of COVID-19, andmore than 733,000 patients had died’'. High mortalityoccurred especially when health-care resources wereoverwhelmed. The USA is the country with the largesnumber of cases so far.Although genetic evidence suggests that SARS-CoV-2is a natural virus that likely originated in animals, there isno conclusion yet about when and where the virus firs!entered humans. As some of the first reported casesin Wuhan had no epidemiological link to the seafoodmarket”, it has been suggested that the market may not bethe initial source of human infection with SARS-CoV-2.One study from France detected SARS-CoV-2 by PCRin a stored sample from a patient who had pneumoniaat the end of 2019, suggesting SARS-CoV-2 might havespread there much earlier than the generally knownstarting time of the outbreak in France”\*. However, thisindividual early report cannot give a solid answer to theorigin of SARS-CoV-2 and contamination, and thus afalse positive result cannot be excluded. To address thishighly controversial issue, further retrospective inves-tigations involving a larger number of banked samplesfrom patients, animals and environments need to beconducted worldwide with well-validated assays.

**Genomics, phylogeny and taxonomy**

As a novel betacoronavirus, SARS-CoV-2 shares79% genome sequence identity with SARS-CoV and50% with MERS-CoV™. Its genome organization isshared with other betacoronaviruses. The six functionalopen reading frames (ORFs) are arranged in order from5’ to 3’: replicase (ORFla/ORF 1b), spike (S), envelope(E), membrane (M) and nucleocapsid (N). In addition,seven putative ORFs encoding accessory proteins areinterspersed between the structural genes”. Most ofthe proteins encoded by SARS-CoV-2 have a similarit had spread massively to all 34 provinces of China. Thenumber of confirmed cases suddenly increased, withthousands of new cases diagnosed daily during lateJanuary’. On 30 January, the WHO declared the novelcoronavirus outbreak a public health emergency of inter-national concern'®. On 11 February, the InternationalCommittee on Taxonomy of Viruses named the novelcoronavirus ‘SARS-CoV-2; and the WHO named thedisease ‘COVID-19’ (REF).

The outbreak of COVID-19 in China reached anepidemic peak in February. According to the NationalHealth Commission of China, the total number ofcases continued to rise sharply in early February at anaverage rate of more than 3,000 newly confirmed casesper day. To control COVID-19, China implementedunprecedentedly strict public health measures. The cityof Wuhan was shut down on 23 January, and all traveland transportation connecting the city was blocked.n the following couple of weeks, all outdoor activitiesand gatherings were restricted, and public facilities wereclosed in most cities as well as in countryside'’. Owing tohese measures, the daily number of new cases in Chinastarted to decrease steadily”.

However, despite the declining trend in China, theinternational spread of COVID-19 accelerated from lateFebruary. Large clusters of infection have been reportedfrom an increasing number of countries!\*. The highransmission efficiency of SARS-CoV-2 and the abun-dance of international travel enabled rapid worldwidespread of COVID-19. On 11 March 2020, the WHOofficially characterized the global COVID-19 out-break as a pandemic”’. Since March, while COVID-19in China has become effectively controlled, the casenumbers in Europe, the USA and other regions havejumped sharply. According to the COVID-19 dash-board of the Center for System Science and Engineeringat Johns Hopkins University, as of 11 August 2020,and chest discomfort, and in severe cases dyspnea andbilateral lung infiltration®’. Among the first 27 docu-mented hospitalized patients, most cases were epidemi-ologically linked to Huanan Seafood Wholesale Marketa wet market located in downtown Wuhan, which sellsnot only seafood but also live animals, including poultryand wildlife\*\*. According to a retrospective study, theonset of the first known case dates back to 8 December2019 (REF). On 31 December, Wuhan Municipal HealthCommission notified the public of a pneumonia out:break of unidentified cause and informed the Worl¢Health Organization (WHO) (FIG. 1).

By metagenomic RNA sequencing and virus isola.tion from bronchoalveolar lavage fluid samples frompatients with severe pneumonia, independent teamsof Chinese scientists identified that the causative agent o:this emerging disease is a betacoronavirus that had neverbeen seen before®!''. On 9 January 2020, the result ofthis etiological identification was publicly announcec¢(FIG. 1). The first genome sequence of the novel coro-navirus was published on the Virological website or10 January, and more nearly complete genome sequencesdetermined by different research institutes were therreleased via the GISAID database on 12 January’Later, more patients with no history of exposure tc Huanan Seafood Wholesale Market were identifiedSeveral familial clusters of infection were reported.and nosocomial infection also occurred in health-careacilities. All these cases provided clear evidence fothuman-to-human transmission of the new virus\*’-“As the outbreak coincided with the approach of thelunar New Year, travel between cities before the festiva.acilitated virus transmission in China. This novel coro-navirus pneumonia soon spread to other cities in Hube:province and to other parts of China. Within 1 month