CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.3951321

Available online at: http://www.iajps.com *Review Article*

A REVIEW ON PANDEMIC NOVEL CORONA VIRAL DISEASE (COVID -19)

Mr. Kiran M. Kulkarni[1], Mr. Sachin P. Gurav[1], Mr. Manish S. Kondavar[1], Mr. Shrikant magdum [1]

[1]Department of Pharmaceutical Chemistry, Appasaheb Birnale College Of Pharmacy, Sangli. Maharashtra, India. Corresponding Author- kirankulkarni_1986@rediffmail.com

Article Received: May 2020 **Accepted:** June 2020 **Published:** July 2020

Abstract-

COVID-19, the disease caused by SARS-CoV-2, is a highly Pandemic disease. The World Health Organization has declared the ongoing outbreak to be a global public health emergency. Currently, the research on SARS-CoV-2 is in its primary stages. Based on current published evidence, this present review systematically deals with the epidemiology, clinical characteristics, diagnosis, treatment and prevention of COVID-19. It is hoped that this review will help the public to recognize and deal with SARS-CoV-2, and provide a reference for future studies

Corresponding author:
Mr. Kiran M. Kulkarni,
Department of Pharmaceutical Chemistry,
Appasaheb Birnale College Of Pharmacy, Sangli.
Maharashtra, India.
kirankulkarni_1986@rediffmail.com

QR code

Please cite this article in press Kiran M. Kulkarni et al, ***A Review On Pandemic Novel Corona Viral Disease (COVID -19) .,*** *Indo Am. J. P. Sci, 2020; 07(07).*

1. INTRODUCTION: [1,2]

As 2019 carried to end, news arrived of an epidemic of pneumonia with few cases in seafood whole sale market in Wuhan, China. Initially few cases were detected near 8 December and cluster was revealed approximate to 31 December 2019 when office of WHO in china provide the information. The market was shut downed on 1 January 2020 and authority of china announced a new type of virus as corona virus or COVID-2019. All the active and suspected cases were tested. At that time approximate 300 cases were found positive with four persons has been died. Initially few reports verified human to human circulate, reports of super spreading patients including 15 health-care workers and circulated into different cities of chi-na. Various other countries also assured the news of human to human transmission. After china, it spreads in Europe, Asia and then in the whole world. The day 31 Jan 2020, first case of corona was confirmed in state of Kerala, India where a student has tested positive as she returned from Wuhan, China.

Novel coronavirus-induced pneumonia, which was named as coronavirus disease 2019 (COVID-19) by the WHO on the February11, 2020, has rapidly increased in epidemic scale since it firstappeared in Wuhan, China, in December 2019. On the same day, the international virus classification commission announced that the novel coronavirus was named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 is not the first severe respiratory disease outbreak caused by the coronavirus. Just in the past two decades, coronaviruses have caused three epidemic diseases, namely, COVID-19, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). At present, the cases of COVID-19 have been found in many countries around the world. On the 31st of January 2020, the World Health Organization (WHO) announced that COVID-19 was listed as the Public Health Emergency of International Concern (PHEIC), meaning that it may pose risks to multiple countries and requires a coordinated international response.

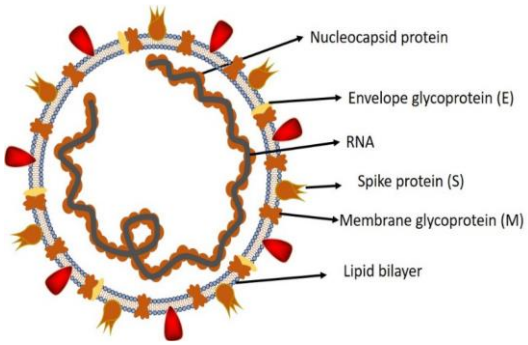

Figure 1: Structure of respiratory syndrome causing human corona virus.

2. CLASSIFICATION [3,4]

Coronaviruses (CoVs) are the largest group of viruses belonging to the Nidovirales order, which includes Corona viridae, Arteriviridae, Mesoniviridae, and Roniviridae families. The Coronavirinae comprise one of two subfamilies in the Coronaviridae family, with the other being the Torovirinae. The Coronavirinaeare further subdivided into four genera, the alpha, beta, gamma, and delta coronaviruses. The viruses were initially sorted into these genera based on serology but are now divided by phylogenetic clustering.

All viruses in the Nidovirales order are enveloped, non-segmented positive-sense RNA viruses. They all contain very large genomes for RNA viruses, with some viruses having the largest identified RNA genomes, containing up to 33.5 kilobase (kb) genomes. Other common features within the Nidovirales order include:

(1) A highly conserved genomic organization, with a large replicase gene preceding structural and accessory genes;

(2) Expression of many non-structural genes by ribosomal frame shifting;

(3) Several unique or unusual enzymatic activities encoded within the large replicase–transcriptase polyprotein;

(4) Expression of downstream genes by synthesis of 3′ nested subgenomicmRNAs.

In fact, the Nidovirales order name is derived from these nested 3′ mRNAs as nido is Latin for “nest.” The major differences within the Nidovirus families are in the number, type, and sizes of the structural proteins. These differences cause significant alterations in the structure and morphology of the nucleocapsids and virions.

Coronaviruses belong to the Corona viridae family in the Nidovirales order. Corona represents crown-like spikes on the outer surface of the virus; thus, it was named as a coronavirus. Coronaviruses are minute in size (65-125nm in diameter) and contain a single-stranded RNA as a nucleic material, size ranging from 26 to 32kbs in length (Fig. 1). The subgroups of coronaviruses family are alpha (α), beta (β), gamma (γ) and delta (δ) coronavirus. The severe acute respiratory syndrome coronavirus (SARS-CoV), H5N1 influenza A, H1N1 2009 and Middle East respiratory syndrome coronavirus (MERS-CoV) cause acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) which leads to pulmonary failure and result in fatality. These viruses were thought to infect only animals until the world witnessed a severe acute respiratory syndrome (SARS) outbreak caused by SARS-CoV, 2002 in Guangdong, China. Only a decade later, another pathogenic coronavirus, known as Middle East respiratory syndrome coronavirus (MERS

CoV) caused an endemic in Middle Eastern countries. Recently at the end of 2019, Wuhan an emerging business hub of China experienced an outbreak of a novel coronavirus that killed more than eighteen hundred and infected over seventy thousand individuals within the first fifty days of the epidemic. This virus was reported to be a member of the β group of coronaviruses.

3. EMERGENCE [5,6]

In 2003, the Chinese population was infected with a virus causing Severe Acute Respiratory Syndrome (SARS) in Guangdong province. The virus was confirmed as a member of the Beta-coronavirus subgroup and was named SARS-CoV. The infected patients exhibited pneumonia symptoms with a diffused alveolar injury which lead to acute respiratory distress syndrome (ARDS). SARS initially emerged in Guangdong, China and then spread rapidly around the globe with more than 8000 infected persons and 776 deceases. A decade later in 2012, couple of Saudi Arabian nationals was diagnosed to be infected with another coronavirus. The detected virus was confirmed as a member of coronaviruses and named as the Middle East Respiratory Syndrome Coronavirus (MERS-CoV). The World health organization reported those MERS coronaviruses infected more than 2428 individuals and 838 deaths. MERS-CoV is a member beta-coronavirus subgroup and phylogenetic ally diverse from other human-CoV. The infection of MERS-CoV initiates from a mild upper respiratory injury while progression leads to severe respiratory disease. Similar to SARS-coronavirus, patients infected with MERS-coronavirus suffer pneumonia, followed by ARDS and renal failure. Recently, by the end of 2019, WHO was informed by the Chinese government about several cases of pneumonia with unfamiliar etiology. The outbreak was initiated from the Wuhan seafood market in Wuhan city of China and rapidly infected more than 50 peoples. The live animals are frequently sold at the Wuhan seafood market such as bats, frogs, snakes, birds, marmots and rabbits. On 12 January 2020, the National Health Commission of China released further details about the epidemic, suggested viral pneumonia. From the sequence-based analysis of isolates from the patients, the virus was identified as a novel coronavirus. Moreover, the genetic sequence was also provided for the diagnosis of viral infection. Initially, it was suggested that the patients infected with Wuhan coronavirus induced pneumonia in China may have visited the seafood market where live animals were sold or may have used infected animals or birds as a source of food. However, further investigations revealed that some individuals contracted the infection even with no record of visiting the seafood market. These observations indicated a humanto the human spreading capability of this virus, which was subsequently reported in more than 100countries in the world. The human to the human spreading of the virus occurs due to close contact with an infected person, exposed to coughing, sneezing, respiratory droplets or aerosols. These aerosols can penetrate the human body (lungs) via inhalation through the nose or mouth.

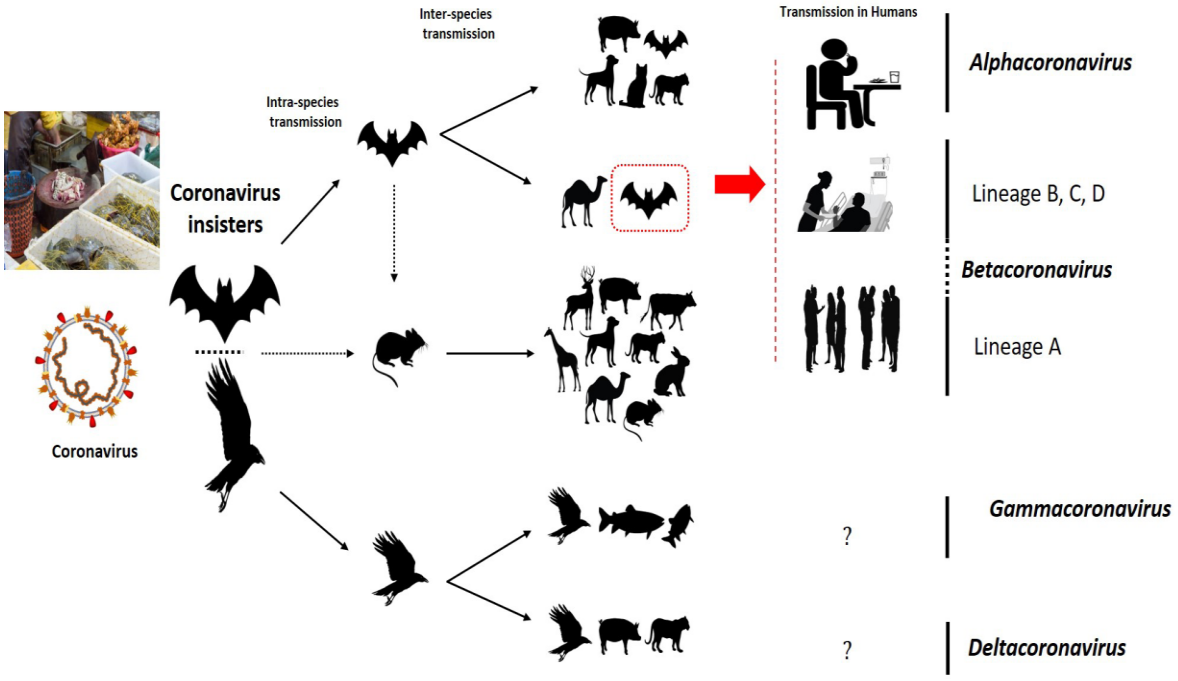

Figure 2: The key reservoirs and mode of transmission of infection

4. Pathogenesis of COVID-19[7,8]

Patients with COVID-19 show clinical manifestations including fever, nonproductive cough, dyspnea, myalgia, fatigue, normal or decreased leukocyte counts, and radiographic evidence of pneumonia, which are similar to the symptoms of SARS-CoV and MERS-CoV infections. Hence, although the pathogenesis ofCOVID-19 is poorly understood, the similar mechanisms of SARSCoV and MERS-CoV still can give us a lot of information on the pathogenesis of SARS-CoV-2 infection to facilitate our recognition of COVID-19.

1) Coronavirus entry and replication

Coronavirus S protein has been reported as a significant determinant of virus entry into host cells. The envelope spike glycoprotein binds to its cellular receptor, ACE2 for SARS-CoVand SARS-CoV-2, CD209L (a C-type lectin, also called L-SIGN) for SARS-CoV, DPP4 for MERS-CoV. The entry of SARS-CoVinto cells was initially identified to be accomplished by direct membrane fusion between the virus and plasma membrane.Belouzard et al. found that a critical proteolytic cleavage event occurred at SARS-CoV S protein at position (S20) mediated the membrane fusion and viral infectivity. MERS-CoV also has evolvedan abnormal two-step furin activation for membrane fusion. Besides membrane fusion, the clathrin-dependent and –independent endocytosis mediated SARS-CoV entry too. After the virus enters the cells, the viral RNA genome is released into the cytoplasm and is translated into two polyproteins and structural proteins, after which the viral genome begins to replicate. The newly formed envelope glycoproteins are inserted into the membrane of the endoplasmic reticulum or Golgi, and the nucleocapsids formed by the combination of genomic RNA and nucleocapsid protein. Then, viral particles germinate into the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). At last, the vesicles containing the virus particles then fuse with the plasma membrane to release the virus.

2) Antigen presentation in coronavirus infection

While the virus enters the cells, its antigen will be presented to the antigen presentation cells (APC), which is a central part of the body's anti-viral immunity. Antigenic peptides are presented by major histocompatibility complex (MHC) or human leukocyte antigen (HLA) in humans; and then recognized by virus-specific cytotoxic T lymphocytes (CTLs). Hence, the understanding of antigen presentation of SARS-CoV-2 will help our comprehension ofCOVID-19 pathogenesis. Unfortunately, there is still lack of any report about it, and we can only get some information from previous researches on SARS-CoV and MERS-CoV. The antigen presentation of SARS-CoV mainly depends on MHC I molecules, but MHC II also contributes to its presentation. Previous research shows numerous HLA polymorphisms correlate to the susceptibility of SARS-CoV, such as HLA-B*4601, HLA-B*0703, HLA-DRB1*1202 and HLA-Cw*0801, whereas the HLA-DR0301, HLA-Cw1502 and HLA-A*0201 alleles are related to the protection from SARS infection. In MERS-CoV infection, MHC II molecules, such as HLA-DRB1*11:01 and HLA-DQB1*02:0, are associated with the susceptibility to MERS-CoV infection. Besides, gene polymorphisms of MBL (mannose-binding lectin) associated with antigen presentation is related to the risk of SARS CoVinfection. These researches will provide valuable clues for the prevention, treatment, and mechanism of COVID-19.

3) Humoral and cellular immunity

Antigen presentation subsequently stimulates the body's humoral and cellular immunity, which are mediated by virus-specific B and T cells. Similar to common acute viral infections, the antibody profile against SARS-CoV virus has a typical pattern of IgM and IgG production. The SARS-specific IgM antibodies disappear at the end of week 12, while the IgG antibody can last for a long time, which indicates IgG antibody may mainly play a protective role, and the SARS-specific IgG antibodies primarily are S-specific and N-specific antibodies. Comparing to humoral responses, there are more researches on the cellular immunity of coronavirus. The latest report shows the number of CD4+ and CD8+ T cells in the peripheral blood of SARS-CoV-2-infected patients significantly is reduced, whereas its status is excessive activation, as evidenced by high proportions of HLA-DR (CD4 3.47%) and CD38 (CD8 39.4%) double positive fractions. Similarly, the acute phase response in patients with SARS-CoV is associated with severe decrease of CD4+ T and CD8+ T cells. Even if there is no antigen, CD4+ and CD8+ memory T cells can persist for four years in a part of SARS-CoV recovered individuals and can perform T cell proliferation, DTH response and production of IFN-γ. Six years after SARS-CoV infection, specific T-cell memory responses to the SARS-CoV S peptide library could still be identified in 14 of 23 recovered SARS patients. The specific CD8+ T cells also show a similar effect on MERS-CoV clearance in mice. These findings may provide valuable information for the rational design of vaccines againstSARS-CoV-2.

4) Cytokine storm in COVID-19

The report in Lancet shows ARDS is the main death cause ofCOVID-19. Of the 41 SARS-CoV-2-infected patients admitted in the early stages of the

outbreak, six died from ARDS. ARDS is the common immune-pathological event for SARS-CoV-2, SARS-CoVand MERS-CoV infections. One of the main mechanisms for ARDS is the cytokine storm, the deadly uncontrolled systemic inflammatory response resulting from the release of large amounts of pro-inflammatory cytokines (IFN-α, IFN-γ, IL-1β, IL-6, IL-12, IL-18, IL-33, TNF-α, TGFβ, etc.) and chemokines (CCL2, CCL3, CCL5, CXCL8,CXCL9, CXCL10, etc.) by immune effector cells in SARS-CoV infection. Similar to those with SARS-CoV, individuals with severe MERS-CoV infection show elevated levels of IL-6, IFN-α, and CCL5, CXCL8, CXCL-10 in serum compared to those with the mild-moderate disease. The cytokine storm will trigger a violent attack by the immune system to the body, cause ARDS and multiple organ failure, and finally lead to death in severe cases of SARS-CoV-2 infection, just like what occurs in SARS-CoV and MERS-CoV infection.

5) Coronavirus immune evasion

To better survive in host cells, SARS-CoV and MERS-CoV use multiple strategies to avoid immune responses. The evolutionarily conserved microbial structures called pathogen-associated molecular patterns (PAMPs) can be recognized by pattern recognition receptors (PRRs). However, SARS-CoV and MERS-CoV can induce the production of double-membrane vesicles that lack PRRs and then replicate in these vesicles, thereby avoiding the host detection of their dsRNA. IFN-I (IFN-α and IFN-β) has a protective effect on SARS-CoV and MERS-CoV infection, but the IFN-I pathway is inhibited in infected mice. Accessory protein 4a of MERS-CoV may block the induction of IFN at the level of MDA5 activation through direct interaction with double-stranded RNA.Besides, ORF4a, ORF4b, ORF5, and membrane proteins of MERS-CoV inhibit nuclear transport of IFN regulatory factor 3 (IRF3) and activation of IFN-β promoter. The antigen presentation can also be affected by the coronavirus. For example, gene expression related to antigen presentation is down-regulated after MERS-CoVinfection. Therefore, destroying the immune evasion of SARS-CoV-2 is imperative in its treatment and specific drug development.

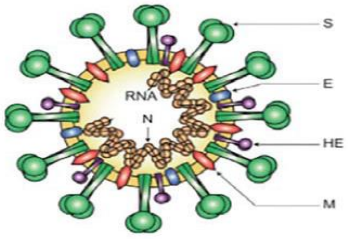

Figure.3. Coronavirus virion structure shown with structural proteins. N: Nucleocapsid protein; S: Spike protein, M: Membrane protein, HE: Hemagglutinin-Esterase and E: Envelope protein

Coronaviruses are enveloped viruses with round and approximately 80 to 120 nm in diameter. Coronaviruses contain positive-strand RNA, with the largest RNA genome (approximately 30 kb) reported to date. The genome RNA is complexed with the basic nucleocapsid (N) protein to form a helical capsid found within the viral membrane. The membranes of all coronaviruses contain at least three viral proteins. These are spike (S), the type I glycoprotein that forms the peplomers on the virion surface, giving the virus its corona- or crown-like morphology in the electron microscope; the membrane (M) protein, a protein that spans the membrane three times and has a short N-terminal ecto domain and a cytoplasmic tail; and small membrane protein (E), a highly hydrophobic protein. The E protein of IBV has a short ectodomain, a transmembrane domain, and a cytoplasmic tail. The E protein of MHV is reported to span the membrane twice, such that both N and C termini are on the interior of the virion. Some group II coronaviruses have an additional membrane protein, hemagglutinin esterase (HE). While the function of HE is not known, it is not an essential protein, and it has been speculated to aid in viral entry and/or pathogenesis in vivo and will be discussed below. HE is not encoded in the SARS-CoV genome. There is an additional group II virion protein called I for internal, as it is encoded within the nucleocapsid open reading frame (ORF). This is a nonessential protein of unknown function. It has recently been shown that the ORF 3a-encoded SARS protein is an additional structural protein. There may be other minor proteins, as yet undetected, included in virions. The genomes of all coronaviruses have a similar structure. The 5′ approximately 20 to 22 kb carries the replicase gene, which encodes multiple enzymatic activities, which will be discussed below. The replicase gene products are encoded within two very large open reading frames, ORFs 1a and 1b, which are translated into two large polypeptides, pp1a and pp1ab, via a frame shifting mechanism involving a pseudo knot structure formed by the genomic RNA. The structural proteins are encoded within the 3′ one-third of the genome, for all coronaviruses, in the order S-E-M-N. (When the HE protein is expressed, it is encoded 5′ to S.) Each group of coronaviruses in addition encodes a group of unique small proteins; while these proteins are nonessential and have been speculated to serve as accessory proteins and to interact or interfere with the host innate immune response; this has not been demonstrated for any of these proteins. There are untranslated regions (UTRs) on both the 5′ and 3′ ends of the genome, which are believed to interact with host and perhaps viral proteins to control RNA replication,

which includes the synthesis of positive- and negative-strand genomic-length RNA. Likewise, there are conserved sequences at the beginning of the transcription sites for each of the multiple sub genomic mRNAs; these are called transcriptional regulatory sequences (previously known as intergenic sequences). Coronavirus transcription has been reviewed recently.

5. Corona virus Life Cycle [9,10,11]

1. Attachment and Entry

The initial attachment of the virion to the host cell is initiated by interactions between the S protein and its receptor. The sites of receptor binding domains (RBD) within the S1 region of a coronavirus S protein vary depending on the virus, with some having the RBD at the N-terminus of S1 (MHV), while others (SARS- CoV) have the RBD at the C-terminus of S1. The S-protein–receptor interaction is the primary determinant for a coronavirus to infect a host species and also governs the tissue tropism of the virus. Many coronaviruses utilize peptidases as their cellular receptor. It is unclear why peptidases are used, as entry occurs even in the absence of the enzymatic domain of these proteins. Many α-coronaviruses utilize aminopeptidase N (APN) as their receptor, SARS-CoV and HCoV-NL63 use angiotensinconvertingenzyme 2 (ACE2) as their receptor, MHV enters through CEACAM1, and the recently identified MERS-CoV binds to dipeptidyl-peptidase 4 (DPP4) to gain entry into human cells.

Following receptor binding, the virus must next gain access to the host cell cytosol. This is generally accomplished by acid dependent proteolytic cleavage of S protein by a cathepsin, TMPRRS2 or another protease, followed by fusion of the viral and cellular membranes. S protein cleavage occurs at two sites within the S2 portion of the protein, with the fi rst cleavage important for separating the RBD and fusion domains of the S protein and the second for exposing the fusion peptide (cleavage at S2′). Fusion generally occurs within acidify ed endosomes, but some coronaviruses, such as MHV, can fuse at the plasma membrane. Cleavage atS2′ exposes a fusion peptide that inserts into the membrane, which is followed by joining of two heptad repeats in S2 forming an anti-parallel six-helix bundle. The formation of this bundle allows for the mixing of viral and cellular membranes, resulting in fusionand ultimately release of the viral genome into the cytoplasm.

2.ReplicaseProtein Expression

The next step in the coronavirus lifecycle is the translation of the replicase gene from the virion genomic RNA. The replicase gene encodes two large ORFs, rep1a and rep1b, which express two coterminal polyproteins, pp1a and pp1ab. In order to expressboth polyproteins, the virus utilizes a slippery sequence (5′-UUUAAAC-3′) and an RNA pseudoknot that cause ribosomal frameshifting from the rep1a reading frame into the rep1b ORF. Inmost cases, the ribosome unwinds the pseudoknot structure, and continues translation until it encounters the rep1a stop codon. Occasionally the pseudoknot blocks the ribosome from continuing elongation, causing it to pause on the slippery sequence, changing the reading frame by moving back one nucleotide, a -1 frame shift, before the ribosome is able to melt the pseudoknot structure and extend translation into rep1b, resulting in the translation of pp1ab. In vitro studies predict the incidence of ribosomal frame shifting is as high as 25 %, but this has not been determined in the context of virus infection. It is unknown exactly why these viruses utilize frameshifting to control protein expression, but it is hypothesized to either control the precise ratio of rep1b and rep1aproteins or delay the production of rep1b products until theproducts of rep1a have created a suitable environment for RNAreplication.

Polyproteins pp1a and pp1ab contain the nsps 1–11 and 1–16, respectively. In pp1ab, nsp11 from pp1a becomes nsp12 following extension of pp1a into pp1b. However, γ coronaviruses do not contain a comparable nsp1. These polyproteins are subsequently cleaved into the individual nsps. Coronaviruses encode eithertwo or three proteases that cleave the replicase polyproteins. Theyare the papain-like proteases (PLpro), encoded within nsp3, and aserine type protease, the main protease, or Mpro, encoded by nsp5. Most coronaviruses encode two PLpros within nsp3, except theγ-coronaviruses, SARS-CoV and MERS-CoV, which only express one PLpro. The PLpros cleave the nsp1/2, nsp2/3, andnsp3/4 boundaries, while the Mpro is responsible for the remaining11 cleavage events.

Next, many of the nsps assemble into the replicase–transcriptase complex (RTC) to create an environment suitable for RNA synthesis, and ultimately are responsible for RNA replication and transcription of the sub-genomic RNAs. The nsps also contain other enzyme domains and functions, including those importantfor RNA replications, for example nsp12 encodes the RNA dependent RNA polymerase (RdRp) domain; nsp13 encodes the RNA helicase domain and RNA 5′-triphosphatase activity; nsp14encodes the exoribonuclease (ExoN) involved in replication fidelityand N7-methyltransferase activity; and nsp16 encodes2′-O-methyltransferase activity. In addition to the replication functions other activities, such as blocking innate immune responses (nsp1; nsp16-2′-O-methyl transferase; nsp3-deubiquitinase) have

been identified for some of the nsps, while others have largely unknown functions (nsp3-ADP-ribose-1″-phosphatase; nsp15-endoribo-nuclease (NendoU)). Interestingly, ribonucleases nsp15-NendoU and nsp14-ExoN activities areunique to the Nidovirales order and are considered genetic markers for these viruses.

3.Replicationand Transcription

Viral RNA synthesis follows the translation and assembly of the viral replicase complexes. Viral RNA synthesis produces both genomic and sub-genomic RNAs. Sub-genomic RNAs serve asm RNAs for the structural and accessory genes which reside down streamof the replicase polyproteins. All positive-sense sub-genomic RNAs are 3′ co-terminal with the full-length viral genome andthus form a set of nested RNAs, a distinctive property of the order Nidovirales . Both genomic and sub-genomic RNAs are produced through negative-strand intermediates. These negative-strand intermediates are only about 1 % as abundant as their positive sense counterparts and contain both poly-uridylate and anti-leader sequences.

Many cis-acting sequences are important for the replication ofviral RNAs. Within the 5′ UTR of the genome are seven stem-loop structures that may extend into the replicase 1a gene. The3′ UTR contains a bulged stem-loop, a pseudoknot, and a hypervariable region. Interestingly, the stem-loop and the pseudoknot at the 3′ end overlap, and thus cannot form simultaneously. Therefore, these different structures are proposed to regulate alternate stages of RNA synthesis, although exactly which stages are regulated and their precise mechanism of action are still unknown.

Perhaps the most novel aspect of coronavirus replication ishow the leader and body TRS segments fuse during production ofsub-genomic RNAs. This was originally thought to occur during positive-strand synthesis, but now it is largely believed to occur during the discontinuous extension of negative-strand RNA. The current model proposes that the RdRp pauses at any one ofthe body TRS sequences (TRS-B); following this pause the RdRpeither continues elongation to the next TRS or it switches to amplifying the leader sequence at the 5′ end of the genome guided by complementarity of the TRS-B to the leader TRS (TRS-L). Many pieces of evidence currently support this model, including the presence of anti-leader sequence at the 3′ end of the negative strand sub-genomic RNAs. However, many questions remainto fully define the model. Finally, coronaviruses are also known for their ability to recombine using both homologous and non-homologous recombination. The ability of these viruses to recombine is tied to thestrand switching ability of the RdRp. Recombination likely plays a prominent role in viral evolution and is the basis for targeted RNA recombination, a reverse genetics tool used to engineer viral recombinants at the 3′ end of the genome.

4.Assembly and Release

Following replication and sub-genomic RNA synthesis, the viral structural proteins, S, E, and M are translated and inserted into the endoplasmic reticulum (ER). These proteins move along the secretory pathway into the endoplasmic reticulum–Golgi intermediate compartment (ERGIC). There, viral genomes encapsulated N protein bud into membranes of the ERGIC containing viral structural proteins, forming mature virions. The M protein directs most protein–protein interactions required for assembly of coronaviruses. However, M protein is not sufficient for virion formation, as virus-like particles (VLPs) cannot be formed by M protein expression alone. When M protein is expressed along with E protein VLPs are formed, suggesting thesetwo proteins function together to produce coronavirus envelopes. N protein enhances VLP formation, suggesting that fusionof encapsulated genomes into the ERGIC enhances viral envelopment. The S protein is incorporated into virions at this step,but is not required for assembly. The ability of the S protein to traffic c to the ERGIC and interact with the M protein is critical forits incorporation into virions. While the M protein is relatively abundant, the E protein is only present in small quantities in the virion. Thus, it is likely that protein interactions provide the impetus for envelope maturation. It is unknown how E protein assists M protein in assembly of the virion, and several possibilities have been suggested. Some work has indicated a role for the E protein in inducing membrane curvature, although others have suggested that E protein prevents the aggregation of M protein. The E protein may also have a separate role in promoting viral release by altering the host secretory pathway. The M protein also binds to the nucleo capsid and this interaction promotes the completion of virion assembly. These interactions have been mapped to the C-terminus of the endodomain of with CTD of the N-protein. However, it is unclear exactly how the nucleocapsid complexed with virion RNA traffics to the ERGIC to interact with M protein and become incorporated intothe viral envelope. Another outstanding question is how the N protein selectively packages only positive-sense full-length genomes among the many different RNA species produced during infection. A packaging signal for MHV has been identifi ed in the nsp15 coding sequence, but mutation of this signal does not appear to affect virus production, and a mechanism for how this packaging signal works has not been determined. Furthermore, most coronaviruses do

not contain similar sequences at this locus, indicating that packaging may be virus specific.

Following assembly, virions are transported to the cell surface in vesicles and released by exocytosis. It is not known if the virions use the traditional pathway for transport of large cargo from the Golgi or if the virus has diverted a separate, unique pathway for its own exit. In several coronaviruses, S protein that does not get assembled into virions transits to the cell surface where it mediates cell–cell fusion between infected cells and adjacent, uninfected cells. This leads to the formation of giant, multinucleated cells, which allows the virus to spread within an infected organism without being detected or neutralized by virus-specific c antibodies.

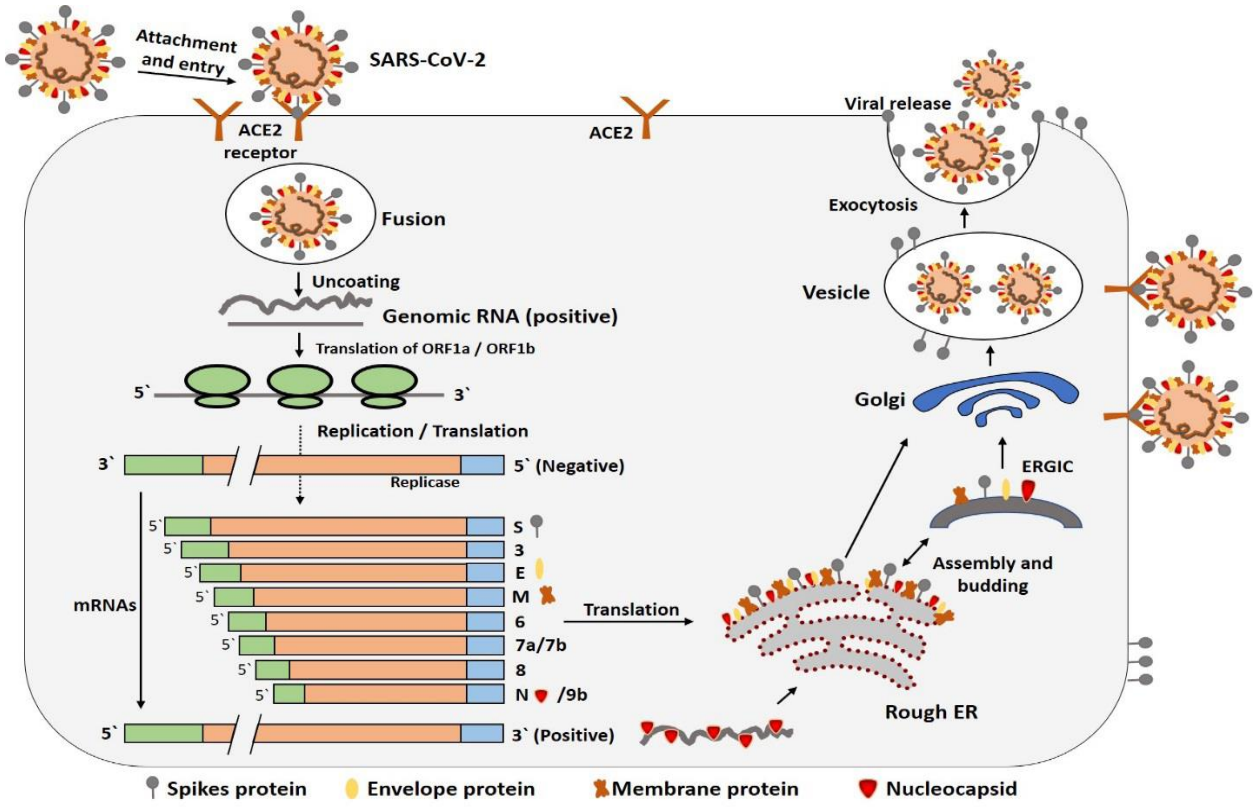

Figure No 4 The life cycle of SARS-CoV-2 in host cells

5. SYMPTOMS OF COVID-19[12-18]

Doctors are learning new things about this virus every day. So far, COVID-19 may not initially cause any symptoms for some people.

People may carry the virus for 2 days or up to 2 weeks Trusted Source before you notices symptoms.

Some common symptoms that have been specifically linked to COVID-19 include:

- Shortness of breath
- having a cough that gets more severe over time
- a low-grade fever that gradually increases in temperature
- Fatigue

Less common symptoms include:

- chills
- repeated shaking with chills
- Sore throat
- Headache
- Muscle aches and pain
- Loss of taste
- Loss of smell

These symptoms may become more severe in some people. Calling emergency medical services if you or someone you care for have any of the following symptoms:

- Trouble breathing
- Blue lips or face
- Persistent pain or pressure in the chest
- Confusion
- Excessive drowsiness

The full list of symptoms is still being investigated by the Centers for Disease Control and Prevention (CDC) Trusted Source.

People are at high risk for contracting SARS-CoV-2 if they come into contact with someone who's carrying it, especially if you've been exposed to their saliva or been near them when they've coughed or sneezed.

Without taking proper preventive measures, people are also at high risk if:

- live with someone who has contracted the virus
- are providing home care for someone who has contracted the virus
- have an intimate partner who has contracted the virus

Older people and people with certain health conditions have a higher risk for severe complications if they contract the virus. These health conditions include:

- Lung conditions, such as COPD and asthma
- Certain heart conditions
- Immune system conditions, such as HIV
- Cancer that requires treatment
- Severe obesity
- Other health conditions, if not well-managed, such as diabetes, kidney disease, or liver disease

Pregnant women have a higher risk of complications from other viral infections, but it's not yet known if this is the case for COVID-19.
The CDC Trusted Sources states that pregnant people seem to have the same risk of contracting the virus as adults who aren't pregnant. Transmitting the virus from mother to child during pregnancy isn't likely, but the newborn is capable of contracting the virus after birth.

6. DIAGNOSIS OF COVID-19[19-23]

Clinical diagnosis of COVID-19 is mainly based on epidemiological history, clinical manifestations and some auxiliary examinations, such as nucleic acid detection, CT scan, immune identification technology (Point-of-care Testing (POCT) of IgM/IgG, enzyme-linked immunosorbent assay (ELISA)) and blood culture. However, the clinical symptoms and signs of patients infected with SARS-CoV-2 are highly atypical, including respiratory symptoms, cough, fever, dyspnea, and viral pneumonia. Therefore, auxiliary examinations are necessary for the diagnosis of COVID-19, just as the epidemiological history

1.Nucleic acid detection technology
The two commonly used nucleic acid detection technologies for SARS-CoV-2 are real-time quantitative polymerase chain reaction (RT-qPCR) and high-throughput sequencing. The authoritative identification method for SARS-CoV-2 is virus blood culture and high-throughput sequencing of the whole genome. However, the application of high-throughput sequencing technology in clinical diagnosis is limited because of its equipment dependency and high cost. So RT-qPCR is the most common, effective and straightforward method for detecting pathogenic viruses in respiratory secretions and blood. After the outbreak of SARS-CoV-2 in China, many companies soon launched RT-qPCR test kits for clinical diagnosis.

The Chinese Center for Disease Control and Prevention (China CDC) recommends the use of specific primers and probes in the ORF1ab and N gene regions for SARS-CoV-2 detection by RT-qPCR. The patient is defined as having a laboratory-confirmed infection when both targets are positive. The negative control samples were all confirmed as negative ones, while samples from two SARS CoV-2 infected patients were confirmed as positive ones in respiratory specimens by this method. Another study showed that the positive rate of SARS-CoV- 2was 91.7% in the patients' self-collected saliva by using RTqPCR (non-probes SYBR based fluorescence signal), which suggests that saliva is a promising non-invasive specimen for the diagnosis, monitoring, and infection control of patients with SARS-CoV-2 infection. RT-qPCR detection also showed high sensitivity and specificity for SARS-CoV and MERS-CoV infection. However, five patients with negative results of RT-qPCR for SARS-CoV-2 may present with positive chest CT findings, and repeated swab tests (RT-qPCR) eventually confirmed that all patients were infected by SARS-CoV-2. The detection of SARS-CoV using RT-qPCR can only achieve a sensitivity of 50- 79%, depending on the protocol used the sample type and number of clinical specimens collected. Thus, it is essential to improve the detection rate of RT-qPCR for SARS-CoV-2 infection. Besides, RT-qPCR has some other shortcomings, including certain biological safety hazards brought by the retention and operation of patient samples, cumbersome nucleic acid detection operations, and long waiting time for results.

2.CT scans and other diagnostic methods
For the diagnosis of COVID-19, although RT-qPCR is specific, its false-negative rate cannot be ignored because of the severe consequences of missed diagnosis. So many clinicians proposed CT scans should be one necessary auxiliary diagnostic method because it is more sensitive. For individuals with a high clinical suspicion of SARS-CoV-2 infection with negative RT-qPCR screening, a combination of repeated RT-qPCR tests and chest CT scan may be helpful. Especially the high-resolution CT (HRCT) for the chest is essential for early diagnosis and evaluation of disease severity of patients with SARS-CoV-2. Several studies have analyzed chest CT images of patients infected with SARS-CoV-2. The typical CT images show bilateral pulmonary parenchymal ground-glass and consolidative pulmonary opacities, sometimes with a rounded morphology and a peripheral lung distribution. Lung involvement with a peripheral predominance was

also seen in patients with SARS-CoV and MERS-CoV infections, and the chest CT showed that disease progressed with ground-glass opacities and consolidation, which is similar to that of SARS-CoV-2 infection. According to those findings, CT scans have a great clinical diagnostic value for COVID-19, especially in the high prevalence area of SARS-CoV-2 infection. However, CT scans also have some shortcomings, such as indistinguishability from other viral pneumonia and the hysteresis of abnormal CT imaging.

Given the shortcomings of the currently used nucleic acid detection and CT scans for the diagnosis of COVID-19, clinical laboratories should apply some immunological detection kits that target viral antigens or antibodies as soon as possible. Currently, POCT of IgM/IgG and ELISA kits for SARS-CoV-2 have been developed and pre-tested by some companies and have shown higher detection rates than nucleic acid detection, but there is still no product or published article. The sensitivity of SARS-CoV N-based IgG ELISA (94.7%) is significantly higher than that of SARS-CoV S based IgG ELISA (58.9%), but the sensitivity of SARS-CoV-2 IgG/

IgM remains to be studied. Hence, developing other sensitive and specific auxiliary methods is necessary and urgent for the diagnosis of COVID-19.

7. TREATMENT STRATEGIES FOR COVID-19[21-24]

Just like SARS-CoV and MERS-CoV, there is currently no clinically proven specific antiviral agent available for SARS-CoV-2 infection. The supportive treatment, including oxygen therapy, conservation fluid management, and the use of broad-spectrum antibiotics to cover secondary bacterial infection, remains to bethe most important management strategy. According to the research on molecular mechanisms of coronavirus infection and the genomic organization of SARS-CoV-2, there are several potential therapeutic targets to repurpose the existing antiviral agents or develop effective interventions against this novel coronavirus.

1.Virally targeted inhibitors

i. Remdesivir, an adenosine analogue that can target the RNAdependent RNA polymerase and block viral RNA synthesis, has been a promising antiviral drug against a wide array of RNA viruses (including SARS/MERS-CoV) infections in cultured cells, mice and nonhuman primate models. The Washington Department of Health administrated remdesivir intravenously first and found that remdesivir might have potential protection from SARS-CoV-2 infection.

ii. Remdesivir and chloroquine have been demonstrated to inhibit SARS-CoV-2 effectively in vitro.

iii. Other nucleoside analogues, such as favipiravir, ribavirin and galidesivir, may be potentially clinically applicable against SARS-CoV-2.

iv. Chymotrypsin-like (3C-like protease, 3CLpro) and papain-like protease (PLP) are non-structural proteins, which have an essential function for coronaviral replication and can inhibit the host innate immune responses.

v. So 3CLpro inhibitors, such as cinanserin and flavonoids, and PLP inhibitors, such as diarylheptanoids, are other attractive choices to fight against SARS-CoV-2. ACE2 mediates SARS-CoV-2 entry into the cell as a functional receptor of coronaviruses. So blocking the binding of S protein with ACE2 is also a meaningful strategy against SARS-CoV-2 infection.

vi. Various other anti-virals are currently being evaluated against infection. Nafamostat, Nitazoxanide, Ribavirin, Penciclovir, Favipiravir, Ritonavir, AAK1, Baricitinib, and Arbidol exhibited moderate results when tested against infection in patients and in-vitro clinical isolates.

2.Antibody and plasma therapy

It has also been reported that there are many convalescent patients donating plasma against SARS-CoV-2, just as SARS-CoV and MERS-CoV trials. It has preliminary acquired favorable results in acute, severe SARS-CoV-2 patients. Moreover, the generation of recombinant human monoclonal antibody (mAb) is a fairly straightforward path to neutralize SARS-CoV.

i.CR3022, a SARS coronavirus-specific human monoclonal antibody, can bind potently with the receptor-binding domain (RBD) of SARS-CoV-2 and has the potential to be developed as candidate therapeutics of SARS-CoV-2 infections.

ii.Other monoclonal antibodies neutralizing SARS-CoV, such as m396, CR3014, could be an alternative for the treatment of SARS-CoV-2.

3.Vaccines

Effective SARS-CoV-2 vaccines are essential for reducing disease severity, viral shedding and transmission, thus helping to control the coronavirus outbreaks. There are several vaccination strategies against SARS-CoV, MERS-CoV tested in animals, including a live attenuated virus, viral vectors, inactivated virus, subunit vaccines, recombinant DNA, and proteins vaccines. These studies are in progress, but it requires months to years to develop the vaccines for SARS-CoV-2. Currently, there may be many promising targets for SARS-CoV-2, but more laboratory and clinical evidence still should be explored. The WHO is working with Chinese scientists to launch

more than 80 clinical trials on potential treatments for SARS-CoV-2. Traditional Chinese medicine seems to have some effects in the supportive treatments. Some new pharmaceutical drugs, including HIV drugs and stem cells, were testified in those clinical trials. Although research teams all over the world are working to investigate the key features, pathogenesis and treatment options, it is deemed necessary to focus on competitive therapeutic options and cross-resistance of other vaccines. For instance, there is a possibility that vaccines for other diseases such as rubella or measles can create cross-resistance for SARS-CoV-2. This statement of cross-resistance is based on the observations that children in china were found less vulnerable to infection as compared to the elder population, while children are being largely vaccinated for measles in China. Owing to the lack of effective therapeutics or vaccines, the best measures to control human corona viruses remain a strong public health surveillance system coupled with rapid diagnostic testing and quarantine when necessary. For international outbreaks, cooperation of governmental entities, public health authorities, and health care providers is critical. During veterinary outbreaks that are readily transmitted, such as PEDV, more drastic measures such as destruction of entire herds of pigs may be necessary to prevent transmission of these deadly viruses.

8. INFECTION CONTROL AND PREVENTION [25,26]

To decrease the damage associated with COVID-19, public health and infection control measures are urgently required to limit the global spread of the virus. Experience from the early phase of SARS-CoV-2 pneumonia strongly highlighted that travel history, rather than chest radiography, is of paramount importance for early detection and isolation of SARS-CoV-2 pneumonia cases. It is essential to limit human-to-human transmission in order to reduce secondary infections among close contacts and health- care workers and to prevent transmission amplification events and further international spread from China. Based on previous experience of management of MERS and SARS infections, the WHO recommend infection control interventions to reduce the general risk of transmission of acute respiratory infections, including avoiding close contact with people suffering from acute respiratory infections, frequent hand washing especially after direct contact with ill people or their environment, and avoiding unprotected contact with farm or wild animals. Moreover, people with symptoms of acute respiratory infection should practice cough etiquette, which is to maintain distance, cover coughs and sneezes with disposable tissues or clothing, and wash hands, and within healthcare facilities enhanced standard infection prevention and control practices are recommended in hospitals, especially in emergency departments. All efforts are being made to slow the spread of the illness in order to provide time to better prepare healthcare systems and the general public, to better characterize COVID-19 to guide public-health recommendations, and to develop timely diagnostics, therapeutics and vaccines. Finally, although the improvement of internet communication largely enhances the availability and dissemination of knowledge, the internet also has the potential for the development and spread of misinformation or fake news. Governments should be responsible for providing accurate knowledge and clarifying misinformation to help the public face this novel infection.

9. FUTURE PROSPECTS [27]

The overall goal of the strategic preparedness and response plan is to stop further transmission of 2019-nCoV within China and to other countries, and to mitigate the impact of the outbreak in all countries.

Taking the above into account, the strategic objectives of the plan are to:

• Limit human-to-human transmission, including reducing secondary infections among close contacts and healthcare workers, preventing transmission amplification events, and preventing further international spread from China;

• Identify, isolate, and care for patients early, including providing optimized care for infected patients;

• Identify and reduce transmission from the animal source;

• Address crucial unknowns regarding clinical severity, extent of transmission and infection, treatment options, and accelerate the development of diagnostics, therapeutics, and vaccines;

• Communicate critical risk and event information to all communities, and counter misinformation;

• Minimize social and economic impact through multi-sectoral partnerships.

These objectives can be achieved by:-

A) Rapidly establishing international coordination to deliver strategic, technical, and operational support through existing mechanisms and partnerships;

B) Scaling up country preparedness and response operations, including strengthening readiness to rapidly identify, diagnose and treat cases; identification and follow-up of contacts when feasible (with priority given to high-risk settings such as healthcare facilities); infection prevention and control in healthcare settings; implementation of health measures for travelers; and awareness

raising in the population though risk communication and community engagement.

C) Accelerating priority research and innovation to support a clear and transparent global process to set research and innovation priorities to fast track and scale-up research, development, and the equitable availability of candidate therapeutics, vaccines, and diagnostics. This will build a common platform for standardized processes, protocols and tools, to facilitate multidisciplinary and collaborative research integrated with the response.

The response strategy is based on several planning assumptions. Owing to the considerable uncertainty surrounding the extent of the outbreak within China, the transmissibility of the virus, and the clinical spectrum of the disease, it will be necessary to regularly update these assumptions as gaps in our knowledge of the disease are filled. The current response plan assumes that human-to-human transmission takes place, and that it may be amplified in specific settings, including healthcare facilities. We also assume that human-to-human transmission is widespread within Hubei, and possibly other population centers in China. It is expected that cases will continue to be exported to other countries while the outbreak continues in China. While the response emphasis will be to rapidly identify and isolate imported cases, there is a risk of clusters of cases caused by localized community transmission outside China. In some cases, countries may require operational assistance to strengthen their capacity to detect and respond to these imported cases.

CONCLUSION:

The novel coronavirus originated from the Wuhan seafood market at Wuhan, China where bats, snakes, raccoon dogs, palm civets, and other animals are sold, and rapidly spread up to 109 countries. The zoonotic source of SARS-CoV-2 is not confirmed, however, sequence-based analysis suggested bats as the key reservoir. DNA recombination was found to be involved at spike glycoprotein which assorted SARS-CoV with the RBD of another Beta CoV, thus could be the reason for cross-species transmission and rapid infection. According to phylogenetic characters, SARS-CoV is closer to SARS-like bat CoVs. Until now, no promising clinical treatments or prevention strategies have been developed against human coronaviruses.

However, the researchers are working to develop efficient therapeutic strategies to cope with the novel coronaviruses. Various broad-spectrum antivirals previously used against influenza, SARS and MERS coronaviruses have been evaluated either alone or in combinations to treat COVID-19 patients, mice models, and clinical isolates. Remdesivir, Lopinavir, Ritonavir, and Oseltamivir significantly blocked the COVID-19 infection in infected patients. It can be concluded that the homologous recombination event at the S protein of RBD region enhanced the transmission ability of the virus. While the decision of bring back the nationals from infected area by various countries and poor screening of passengers, become the leading cause of spreading virus in others countries.

Most importantly, human coronaviruses targeting vaccines and antiviral drugs should be designed that could be used against the current as well as future epidemics. There are many companies working for the development of effective SARS-CoV-2 vaccines, such as Moderna Therapeutics, Inovio Pharmaceuticals, Novavax, Vir Biotechnology, Stermirna Therapeutics, Johnson & Johnson, VIDO-InterVac, GeoVax-BravoVax, Clover Biopharmaceuticals, CureVac, and Codagenix. But there is a need for rapid human and animal-based trails as these vaccines still require 3 to 10 months for commercialization. There must be a complete ban on utilizing wild animals and birds as a source of food. Beside the development of most efficient drug, a strategy to rapidly diagnose SARS-CoV-2 in suspected patient is also required.

The signs and symptoms of SARS-CoV-2 induced COVID-19 are a bit similar to influenza and seasonal allergies (pollen allergies). Person suffering from influenza or seasonal allergy may also exhibit temperature which can be detected by thermo-scanners; hence the person will become suspected. Therefore, an accurate and rapid diagnostic kit or meter for detection of SARS-CoV-2 in suspected patients is required, as the PCR based testing is expensive and time consuming. Different teams of Chinese doctors should immediately sent to Eurpean and other countries, to control the over spread of COVID-19, because Chinese doctors have efficiently controlled the outbreak in china and limited the mortality rate to less than 3% only. The therapeutic strategies used by Chinese, should also be followed by other countries.

9. REFERENCES:

1. Lu H, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology
in Wuhan China: the mystery and the miracle. Journal of Medical Virology
2020, Jan.16.
2. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission
dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. New
England Journal of Medicine 2020, Jan 29.

3. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of
global health concern. Lancet 2020.
4. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics
of 138 hospitalized patients with 2019 novel coronavirus-infected
pneumonia in Wuhan, China. JAMA 2020, Feb 7.
5. Lee J, Chowell G, Jung E. A dynamic compartmental model for the Middle
East respiratory syndrome outbreak in the Republic of Korea: a retrospective
analysis on control interventions and superspreading events. J Theor Biol
2016
6. Lee JY, Kim YJ, Chung EH, et al. The clinical and virological features of
the first imported case causing MERS-CoV outbreak in South Korea, 2015.
BMC Infect. Dis. 2017.
7. Tan W, Zhao X, Ma X, et al. A novel coronavirus genome identified in a
cluster of pneumonia cases—Wuhan, China 2019−2020. China CDC Weekly
2020
8. Chen ZM, Fu JF, Shu Q, Chen YH, Hua CZ, Li FB, et al. Diagnosis and
treatment recommendations for pediatric respiratory infection caused by the
2019 novel coronavirus. World J. Pediatr. 2020, Feb, 5.
9. Spann, K. M., K. C. Tran, B. Chi, R. L. Rabin, and P. L. Collins. 2004.
Suppression of the induction of alpha, beta, and gamma interferons by the
NS1 and NS2 proteins of human respiratory syncytial virus in human
epithelial cells and macrophages. Journal of Virology.
10. Sperry, S., L. Kazi, R. Graham, R. Baric, S. Weiss, and M. Denison. 2005.
Single amino acid substitutions in nonstructural ORF1b-nsp14 and ORF2a
30kDa proteins of the murine coronavirus MHV-A59 are attenuating in
mice. Journal of Virology.
11. Spiegel, M., A. Pichlmair, L. Martinez-Sobrido, J. Cros, A. Garcia-Sastre,
O. Haller, and F. Weber. 2005. Inhibition of beta interferon induction by
severe acute respiratory syndrome coronavirus suggests a two-step model for
activation of interferon regulatory factor 3. Journal of Virology.
12. Subbarao, K., J. McAuliffe, L. Vogel, G. Fahle, S. Fischer, K. Tatti, M.
Packard, W. J. Shieh, S. Zaki, and B. Murphy. 2004. Prior infection and
passive transfer of neutralizing antibody prevent replication of severe acute
respiratory syndrome coronavirus in the respiratory tract of mice. Journal of
Virology.
13. Nicholls, J. M., L. L. Poon, K. C. Lee, W. F. Ng, S. T. Lai, C. Y. Leung, C.
M. Chu, P. K. Hui, K. L. Mak, W. Lim, K. W. Yan, K. H. Chan, N. C.
Tsang, Y. Guan, K. Y. Yuen, and J. S. Peiris. 2003. Lung pathology of fatal
severe acute respiratory syndrome. Lancet
14. Nie, Q. H., X. D. Luo, J. Z. Zhang, and Q. Su. 2003. Current status of severe
acute respiratory syndrome in China. World J. Gastroenterol.
15. Poon, L. L., O. K. Wong, K. H. Chan, W. Luk, K. Y. Yuen, J. S. Peiris, and
Y. Guan. 2003. Rapid diagnosis of a coronavirus associated with severe
acute respiratory syndrome (SARS). Clinical Chemistry.
16. Prentice, E., J. McAuliffe, X. Lu, K. Subbarao, and M. R. Denison. 2004.
Identification and characterization of severe acute respiratory syndrome
coronavirus replicase proteins. Journal of Virology.
17. Phillips, J. J., M. M. Chua, G. F. Rall, and S. R. Weiss. 2002. Murine
coronavirus spike glycoprotein mediates degree of viral spread,
inflammation, and virus-induced immunopathology in the central nervous
system. Journal of Virology.
18. Poon, L. L., Y. Guan, J. M. Nicholls, K. Y. Yuen, and J. S. Peiris. 2004. The
aetiology, origins, and diagnosis of severe acute respiratory syndrome.
Lancet Infectious Diseases.
19. Poon, L. L., C. S. Leung, K. H. Chan, K. Y. Yuen, Y. Guan, and J. S. Peiris.
2005. Recurrent mutations associated with isolation and passage of SARS
coronavirus in cells from non-human primates. Journal of Virology.
20. Su S, Wong G, Shi W, et al. Epidemiology, genetic recombination, and
pathogenesis of coronaviruses. Trends Microbiol 2016.
21. Ismail MM, Tang AY, Saif YM. Pathogenicity of turkey coronavirus in
turkeys and chickens. Avian Dis. 2003.
22. Zhou P, Fan H, Lan T, et al. Fatal swine acute diarrhoea syndrome caused
by an HKU2-related coronavirus of bat origin. Nature 2018.
23. Peiris JS, Guan Y, Yuen KY. Severe acute respiratory syndrome. Nat Med
2004.
24. Chan-Yeung M, Xu RH. SARS: epidemiology. Respirology 2003.

25. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with
pneumonia in China, 2019. N Engl J Med 2020; published online Jan 24.
26. Chan JFW, Yuan S, Kok KH, et al. A familial cluster of pneumonia
associated with the 2019 novel coronavirus indicating person-to-person
transmission: a study of a family cluster. Lancet 2020; published online Jan
27. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with
2019 novel coronavirus in Wuhan, China. Lancet 2020; published online Jan