

Review Article

Analysis of SARS-CoV-2 and factors predicting next spillover of its more contagious variant.

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Abstract

Background: At the beginning of 2020, the world has started experiencing the epidemic of a novel coronavirus; by the mid of March 2020, it has been declared a pandemic. The disease has been named COVID-19, and the virus is labelled as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) based on the type of infection it is causing. Coronaviruses are not new to us, and there are 15 different coronaviruses known to us. In the last 20 years, this is the fourth coronavirus pandemic, and SARS-CoV-2 seems to be the deadliest among all, with the ability to continue producing more contagious variants.

Methodology: In this review, we used EMBASE, MEDLINE and PubMed database search engines (retrieval systems) for the words SARS-CoV-2, its origin, transmission, tropism, zoonotic, vaccines, and the factors that contribute to its contagious and virulent nature.

Results: Accumulating evidence indicated that the pandemic might have a massive impact on both physical and mental health, particularly on those predisposed to COVID-19. Approved vaccines are in use around the globe, they may show different effects in future. We observe signs of constant mutation of SARS-CoV-2, recently SARS-CoV-2 variants such as delta and C.1.2.

Conclusion: In this review, we endeavour to reveal the factors which can impede the ability of this highly lethal virus to reproduce into more contagious variants.

Keywords

Coronavirus, Contagious Variants, SARS-CoV-2 Spillover, Zoonosis, Tropism



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Introduction

On January 30, 2020, the World Health Organization declared the SARS-CoV-2 epidemic and, on March 11, 2020, as a pandemic. Now, SARS-CoV-2 brought the world economies to their knees, and we now know that pathogens can be as destructive as nuclear weapons. COVID-19 is causing a major once-in-a-century global pandemic¹. The recurrent spillovers of coronaviruses in humans, along with the detection of numerous coronaviruses in bats, including many SARS-related coronaviruses (SARSr-CoVs), suggest that future zoonotic transmission events may continue to occur. To date, 66 vaccines in clinical trials on humans, and 20 have reached the final stages of testing, 8 vaccines are in early or limited use, 2 vaccines approved for full use, and 3 vaccines are abandoned after trials. At least 90 preclinical vaccines are under active investigation in animals². Most human emerging infectious diseases are zoonotic, with viruses that originate in wild mammals of particular interest, i.e., HIV, Ebola, and SARS³⁻⁵. Understanding the patterns of viral diversity in wildlife and determinants of successful cross-species transmission, or spillover, are, therefore, crucial goals for pandemic surveillance programs⁶.

However, few analytical tools exist to spot which host species are likely to harbor the subsequent human virus or which viruses can cross species boundaries⁷⁻⁹. Viral zoonoses are an enormous threat to public health and global security. They have caused the bulk of current pandemics in people, but our understanding of the factors that drive viral diversity in mammals, viral host range, and cross-species transmission to humans remains poor.

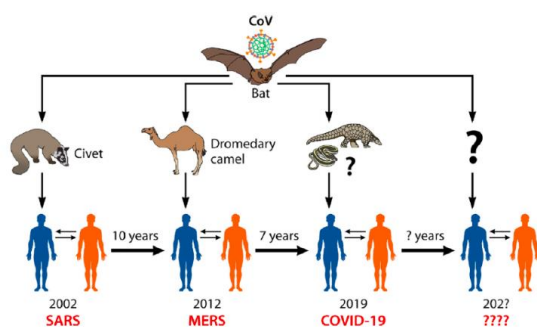
Recent studies have described broad patterns of pathogen-host range¹⁰ and various host or microbial factors that facilitate cross-species transmission¹¹ or have focused on factors promoting pathogen and parasite sharing within specific mammalian taxonomic groups, including primate^{9,12-15}. There has been no comprehensive, species-level analysis of viral sharing between humans and other mammals to date. Transmission

of infectious pathogens from one host to another results in infectious diseases such as COVID 19. Host shifts resulted in multiple pandemics such as HIV and Spanish flu. Host and pathogen phylogenies showed evidence of host shifts. Viral tropism, Type 3 interferon and lysosomal proteases play an important role in zoonosis¹⁶.

The virus infection may be a profound challenge to host survival. The virus's ability to duplicate and spread is countered by the antiviral defense mechanisms that are mounted by the host. Virus infection is often either productive (that is, progeny viruses are produced, new cells are infected, and therefore the virus transmission continues) or abortive (that is, progeny viruses are not produced, or virus dissemination is blocked). For many viruses, the initial infection results can vary widely, counting on the location of entry, the cell types infected, the responses of local sentinel immune cells, the architecture and vasculature of the tissues involved. The summation of those variable elements during virus infection has led to the concept of viral tropism. Basically, viral tropism refers to the power of a given virus to productively infect a specific cell (cellular tropism), tissue (tissue tropism) or host species (host tropism), for instance, if a selected virus can productively infect rabbits but no other vertebrate hosts like humans, that virus would be said to possess a number tropism limited to rabbits¹⁷.

The first line of defense for resisting pathogen infections generally depends on the innate immune reaction. Interferons (IFNs), which are host-encoded secreted proteins and are classified into three types (I, II & III), often join multiple immune interplays and perform both the induction and regulation of innate and adaptive antiviral mechanisms when viruses infect the host. Once viral infections occur, the expression of type I IFN (generally focusing on IFN- α & IFN- β) will function as a pivotal innate antiviral defense response. The antiviral activity performed by type I IFNs directly inhibits viral replication and keeps long-term immunity¹⁸. Similarly, if the same virus could productively infect rabbit macrophages but not the opposite rabbit cells, that virus would be said to

possess a cellular tropism limited to macrophages. Importantly, this definition of tropism can only be applied to viruses with lytic replication cycles that produce progeny viruses. Viruses that establish latent infections can successfully enter cells and maintain the power to supply infectious viruses, but they never actually undergo productive replication while they continue to be latent¹⁹. There are 15 different coronaviruses, ranging from 120-160 nm in size and half of them affecting humans. SARS-CoV-2 is the seventh coronavirus known to infect humans; SARS-CoV, Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2 can cause severe disease, whereas HKU1, OC43, NL63, and 229E are associated with mild symptoms, out of these seven viruses, only NL63, and 229E are alpha and rest are beta coronaviruses. SARS-CoV-2 may originate from bat reservoirs as assumed for many other coronaviruses. Over 30 new viruses have been discovered in the last 4 decades. The most distressing viruses are zoonotic, which modified themselves for tropism because of climate change.



There is a chance of another viral pandemic due to the eighth coronavirus, but no one can predict when the next spillover is going to take place²⁰.

In the past, zoonotic coronaviruses readily traffic into new host species, as evidenced by the emergence of SARS-CoV in 2002-2003, MERS CoV in 2012 and SARS-CoV-2, the causative agent of the COVID-19 pandemic in 2019. SARS CoV2 infection has caused many illnesses and thousands of deaths worldwide²¹.

Small animal model systems are vital for understanding COVID-19 disease mechanisms and for measuring their impact to improve global

health. Mouse models not only provide critical insights into the pathogenic mechanisms of CoV disease but can function as high throughput preclinical evaluation platforms to identify high-performance antivirals and vaccines essential for downstream human trials^{22,23}. SARS-CoV-2 enters host cells through the binding of the cellular receptor angiotensin-converting enzyme 2 (ACE2). Unfortunately, standard laboratory mice do not support infection with SARS-CoV-2 because of the spike protein's incompatibility to the murine ortholog (mACE2) of the human receptor, complicating model development²⁴.

Recently, the first K18-hACE2 SARS-CoV-2 infection was examined. Mice exhibited no clinical symptoms or weight loss until 4 dpi. By day 5, mice had 10% weight loss with variable clinical presentation, ranging from reduced activity to increased respiration and lethargy. Infected mice also had moderate viral lung titers, suggesting productive infection. CoV2 tropism towards cells of different tissues depends upon the availability of biochemical stimulus such as ectopeptidases²⁵.

Viral Tropism

One of the foremost outstanding features of viruses is their tropism, including species and tissue tropism. Viral entry into host cells is among the foremost important determinants of viral tropism and depends upon the type III interferon group of antiviral cytokines²⁶. They are also known as IFN-gammas 1,2,3 & 4 and directly performing an antiviral immune response at epithelial surfaces in the early stages of viral infection and could be a good therapeutic target²⁷.

Enveloped virus entry via endocytosis pathway

Entry of enveloped viruses involves two steps: receptor binding and membrane fusion. Enveloped viruses often hijack the endocytosis pathway: they enter endosomes, proceed to lysosomes, and then fuse the viral and lysosomal membranes. The lysosomes play critical roles in cell metabolism by breaking down biomolecules and cellular debris and by providing nutrients for other cellular functions. The lysosomal protease activities are central to the functions of lysosomes. They are also

required to activate the membrane fusion of a spread of viruses, including coronaviruses and filoviruses. Understanding the correlation between lysosomal protease activities and viral tropism has important implications for investigating viral pathogenesis, developing antiviral strategies, and identifying zoonotic strains with pre-pandemic potential¹⁹.

An envelope-anchored spike protein guides coronavirus entry into host cells. It first binds to a receptor on the host cell surface for viral attachment through its S1 subunit then fuses viral and host membranes through its S2 subunit. The membrane fusion step by coronavirus spikes requires two prior cleavages by host proteases, the first at the S1-S2 boundary (i.e., the S1-S2 site) and thus the second within S2 (i.e., the S2' site) relying on the virus, the spike-processing proteases may come from different stages of the coronavirus infection cycle²⁸.

Lysosomal proteases

It had been previously reported that MERS-CoV spike could be processed by furin after viral endocytosis in virus-targeted cells, but this finding was not supported by a recent study²⁹. The protease activation pattern of SARS-CoV entry is analogous thereto of MERS-CoV, except that SARS-CoV spike can also be processed by extracellular proteases (e.g., elastase) after virus release³⁰. It has been suggested that the tissue tropisms of MERS-CoV and SARS-CoV are correlated with the tissue distributions of proprotein convertases, extracellular proteases, and cell surface proteases within the host; as an example, the availability of trypsin-like proteases within the tract has been suggested to be a determinant of the respiratory tropism of SARS-CoV³¹.

However, coronavirus entry also depends on lysosomal proteases, and it is not clear whether the species and tissue tropisms of coronaviruses are correlated with different lysosomal protease activities from different hosts or tissue cells. Both MERS-CoV and SARS-CoV are thought to be originated from bats. SARS-like coronaviruses

isolated from bats and SARS-CoV isolated from humans are genetically almost like each other; bat SARS-like coronaviruses recognize the same receptor, angiotensin-converting enzyme 2 (ACE2), as human SARS-CoV [27]. MERS-like coronaviruses isolated from bats and MERS-CoV isolated from humans so far are also genetically similar, albeit not as similar as between bat SARS-like coronaviruses and human SARS-CoV. Several MERS-like coronaviruses from bats, including HKU4, recognize the same receptor, dipeptidyl peptidase 4 (DPP4), as MERS-CoV. Moreover, human lysosomal proteases activate only MERS-CoV spike, but not HKU4 spike, for viral entry into human cells, while bat lysosomal proteases activate both MERS-CoV and HKU4 spikes for viral entry into bat cells³². Furthermore, the expression of lysosomal proteases in human lung cells could be less than in human liver cells, leading to inefficient activation of MERS-CoV spike by lysosomal proteases in human lung cells. Apparently, this enzyme is essential for viral entry; that is why the liver was the main site for MERS-CoV. These results suggest that lysosomal protease activities may differ among the cells from various hosts or even between the cells from the same host species, restricting coronavirus entry and their tropism³³.

However, these studies did not consider the contribution from the host receptors, even though homologues receptors from different host species may vary in their function as coronavirus receptor or that the same receptor protein could even be expressed at different levels in several tissues within one host species. Moreover, these studies were administered at the cellular level and did not provide direct biochemical evidence to demonstrate that lysosomal proteases from human and bat cells process coronavirus spikes differently. Therefore, factor-controlled viral entry data and direct biochemical data are both needed to work out the correlation between lysosomal protease activities and coronavirus tropism^{19,31}.

By the end of 2020, about 300,000 infants were born to women who were infected by SARS-CoV-2. Millions more are going to be born into families who have experienced tremendous stress and

upheaval due to the pandemic, even though they are not infected; while the long-term effects of COVID-19 on infants is yet to be seen, scientist can find some insight from the past, including the 1918 flu pandemic and former coronavirus illnesses like SARS in 2002 and MERS in 2012. The 1918 influenza pandemic had long-term impacts on the cohort exposed in utero, which experienced earlier adult mortality and more diabetes, ischemic heart condition and depression after the age of 50. It is possible that the COVID-19 pandemic may also have long-term impacts on the cohort that was in utero during the pandemic due to the exposure to maternal infection and the stress of the pandemic environment.

Maternal viral infection

Maternal viral infections can affect fetuses through multiple pathways, from direct transmission through the placenta to inflammatory responses that disturb in-utero metabolism and negatively impact growth. While direct maternal-fetal transmission of the virus and severe congenital disabilities appear to have been rare during previous coronavirus outbreaks, there was an increase in the preterm deliveries and low birth weight during both the 2002 SARS and 2009 H1N1 influenza outbreaks, which may be due to enhanced inflammation of infected mothers.

Even though studies in relation to COVID-19 and pregnancy are still in their early stages, there are some concerning results that merit a closer look in ongoing investigations. Increased preterm birth rates could also be linked to maternal SARS-CoV-2 infections, and the severity of illness is related to the formation of blood clots and the probability of stillbirth.

In addition to the direct risks posed by infection, the COVID-19 pandemic has also increased levels of stress, unemployment, food insecurity, and violence, and lack of or disrupted prenatal care. For these reasons, the scientist suggests that cohort studies also include non-infected mothers and youngsters and compare the COVID-19 cohort to children born before or after the pandemic and include various socioeconomic measures. "The

inclusion of data on social and economic stresses will allow comparisons between countries taking different measures to scale back the spread of the virus." These comparisons may give us further insights beyond the outcomes of COVID-19, such as socioeconomic and social policies, which can reduce the risk of preterm and stillbirth³⁴⁻³⁷.

Animal testing

Animals can be infected or act as spreaders or can be used for clinical studies for the SARS CoV virus. A few studies investigated the putative role of livestock within the present COVID-19 pandemic³⁸ have demonstrated that among seven animal species tested, the ferret, cat and dog could be experimentally infected by the intranasal route, probably through the viral receptor angiotensin-converting enzyme 2 (ACE2). Contrary to dogs and ferrets (which were less affected), cats were found to be more susceptible to the experimental infection, particularly during their juvenile post-weaning life (70-100 days). Shi et al. also reported that one out of three naïve cats exposed to infected cats developed infection³⁹. This data revealed that intra-species respiratory droplet transmission could occur in cats, a recent study of 102 pet cats living in Wuhan, China, and picked up during the pandemic period, reported a prevalence of 14.7% seroconverted cats⁴⁰. Given the susceptibility of cats inoculated intranasally with 105 pfu or by direct aerosol transmission from the infected cats, it is possible that the infected and seroconverted cats identified in Wuhan, China⁴¹ had either been intuned with the patients whose viral load was high or have had more contacts with the infected cats. Therefore, despite the susceptibility of some animal species revealed in experimental conditions and developing juvenile cat as a possible model of SARS-CoV-2 reproduction, there are evidence of significantly low rates of COVID-19 infection in companion dogs and cats, even with repeated contacts and closed proximity to infected humans, indicating a low possibility of SARS-CoV-2 transmission between humans and pets⁴². However, the availability of reliable animal models is critical for pathogenesis studies, preclinical evaluation of vaccines and for the evaluation of therapeutic targets^{43,44}.

Several animal species were predicted by in silico analysis supported comparisons of the entry receptor for SARS-CoV and SARS-CoV-2, angiotensin-converting enzyme 2 (ACE2). These predictions are relevant because the ACE2 receptor sequence is a critical factor for governing the susceptibility for infection⁴⁵. The interaction of the viral spike (S) glycoprotein receptor-binding domain (RBD) with ACE2 was examined and confirmed in vivo^{46,47}. Productive SARS-CoV-2 infection was shown in non-human primates, which developed into respiratory disease mimicking moderate disease conditions observed in humans^{26,48,49}. Mice are not naturally prone to get infected with SARS-CoV-2, but mouse-adapted virus strains are developed and utilized in BALB/c mice⁵⁰. Moreover, transgenic mice expressing human ACE2 receptors represent a lethal SARS-CoV-2 infection model, exhibiting significant weight loss and robust virus replication within the respiratory tract, including the lungs.

Ferrets have provided valuable data of SARS-CoV-2 infection and successful transmission to nearby animals without clinical signs⁵¹.

Origin of SARS-CoV-2

The origin of SARS-CoV-2 and its emergence within the human population remains quite mysterious. Numerous cases were linked to the Huanan seafood and wild-animal market within Wuhan's town, raising the likelihood of zoonotic origin⁴. Sequence analyses showed that the genome of SARS-CoV-2 shares 79.5%, 89.1%, 93.3%, and 96.2% nucleotide sequence identity between human SARS-CoV, bat CoV ZC45, bat CoV RmYN02, and bat CoV RaTG13, respectively, suggesting that SARS-CoV-2 probably has bat origins^{3,10,7}.

This finding is not surprising as bats are notorious for serving as natural reservoirs for two other deadly human coronaviruses (hCoVs), SARS-CoV and MERS-CoV, which previously initiated global outbreaks^{8,9}.

Although SARS-CoV-2 may have originated from the bats but, bat CoVs are unlikely to leap on to humans directly due to a general ecological separation. Other mammalian species may have served as intermediate hosts, where the progenitor virus acquires critical mutations for efficient zoonotic transmission to humans. During SARS-CoV and MERS-CoV's emergence, palm civet and dromedary camel act as intermediate hosts, respectively. Pangolin CoVs (PCoVs) form two phylogenetic lineages, PCoV-GX and PCoV-GD^{11,12,52,53}.

The lineage PCoV-GD was primarily found to carry a receptor-binding motif within the spike (S) protein, like SARS-CoV-2; however, the genomes of pangolin SL-CoVs share only 85.5% to 92.4% nucleotide identities with the genome of SARS-CoV-2.

CoVs isolated from the intermediate host's palm civet and dromedary camel share 99.6% and 99.9% genome sequence identities, with their human counterpart¹³. Therefore, pangolins tested in these studies are not the direct intermediate hosts for SARS-CoV-2.

S protein-driven cellular entry, triggered by receptor recognition, is the first determinant of host range, cell, tissue tropism, and coronaviruses¹⁴. The S protein of SARS-CoV-2 is a glycoprotein, which can be cleaved into S1 and S2 subunits during biogenesis at the polybasic furin cleavage site (RRAR)^{15,21-24}.

Coronavirus genome structure and life cycle

Coronavirus responsible for COVID-19 could be a spherical or pleomorphic enveloped particle-containing single-stranded (positive-sense) RNA connected to a nucleoprotein within a capsid comprised of matrix protein. The envelope bears club-shaped glycoprotein projections. Some coronaviruses also contain a heme agglutinin-esterase protein (HE)⁵⁴.

Coronaviruses possess the most important genomes (26.4e31.7 kb) among all known RNA viruses, with G þ C contents varying from 32% to

43%. Variable numbers of small ORFs are present between the various conserved genes (ORF1ab, Spike, envelope, membrane and nucleocapsid) and downstream to the nucleocapsid gene in several coronavirus lineages. The viral genome contains distinctive features, including a single N-terminal fragment within the spike protein⁵⁴.

All the structural and accessory proteins are translated from the RNAs of CoVs. Four main structural proteins contain spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins are encoded by ORFs 10, 11 on one-third of the genome near the 3' terminus^{55,56}. Besides these four main structural proteins, different CoVs encode unique structural and accessory proteins, such as HE protein, 3a/b protein, and 4a/b protein. These mature proteins are responsible for several essential functions in genome maintenance and virus replication⁵⁵.

There are three or four viral proteins within the coronavirus membrane. The most abundant structural protein is that the membrane (M) glycoprotein; it spans the membrane bilayer 3 times, leaving a brief NH₂-terminal domain outside the virus and an extended COOH terminus (cytoplasmic domain) inside the virion. The spike protein (S) is a type of membrane glycoprotein that contains peplomers, the primary inducer of neutralizing antibodies in S protein. M plays a predominant role within the intracellular formation of virus particles without requiring S. In the presence of tunicamycin, coronavirus grows and produces non-infectious virions that contain M but without S proteins^{57,54}.

Comparison of SARS-CoV2, SARS-CoV & MERS-CoV

The 5' UTR and 3' UTR are involved in inter- and intramolecular interlinkage and are minimalist crucial for RNA eRNA interlinkage and binding of viral and cellular proteins⁵⁸. At the 5' end, the first ORF of the whole genome length encoding non-structural proteins with the size of 29844bp (7096aa), 29751bp (7073aa) and 30119bp (7078) are present in COVID-19, SARS-CoV; and MERS-CoV, respectively. Comparison of the spike proteins

exhibits a clear difference, i.e., 1273aa, 21493aa 1270aa in COVID-19, SARS-CoV, and MERS-CoV, respectively. Genetically, SARS-CoV-2 is like SARS-CoV (about 79%) and MERS-CoV (about 50%). The arrangement of nucleocapsid protein, envelope protein, and membrane protein among beta coronaviruses are different^{58,59}.

Virus host cell binding

Previous studies have shown that furin cleavage is not essential for coronavirus-cell membrane fusion but enhances cell-to-cell fusion^{24,38,41,43}, and increases the fitness of sequence variants within the quasi-species population of bovine CoV [60]. Several recent studies indicated that cleavage at the S1/S2 boundary by furin in virus-producing cells could be a critical primary step that facilitates conformation change triggered by receptor binding during virus entry and subsequent fusion-activating cleavage at the S2 site, which is found immediately upstream of fusion peptide within the S2 subunit^{23,43,44}. Also, furin cleavage in hemagglutinin was found to convert an avirulent avian influenza virus isolate into a highly pathogenic isolate⁶¹. Interestingly, this cleavage site is not present within the S protein of SARS-CoV, bat SL-CoVs, or pangolin SL-CoVs identified so far; additionally, to furin-mediated cleavage in virus-producing cells, SARS-CoV-2 S protein is further cleaved for fusion by the cell surface protease TMPRSS2 and lysosomal proteases, e.g., cathepsin L, during virus entry of target cells. During cell entry, S1 binds to the cellular receptor, subsequently triggering a cascade of events leading to S2-mediated membrane fusion between host cells and coronavirus particles.

S1 protein contains an independently folded domain called the receptor binding domain, which harbours this domain primarily involved in-tuned with the receptor. Human angiotensin-converting enzyme 2 (hACE2) has been identified because of the cellular receptor for both SARS-CoV-2 and SARS-CoV. Additionally to hACE2, ACE2 from horseshoe bat (*Rhinolophus Alcyone*) was found to support cell entry of SARS-CoV-2 S-mediated stomatitis virus-based pseudotyped virus. By using the infectious virus, it is also shown that ACE2s from

Chinese horseshoe bat (*Rhinolophus sinicus*), civet, and swine, but not the mouse, could have functional receptors. However, during this infection system, the entry step was including other measures during the virus life cycle, i.e., viral genome replication, translation, virion assembly, and budding; thus, the receptor activity of these animals ACE2 orthologs was not directly investigated.

Basically, SARS CoV2 spike protein (S1) due to the presence of RRAR (between S1 and S2) make a correct orientation to attach itself with ACE2 through TMPRSS2 and other enzymes and remove ACE2 from the cell membrane. ACE 2 receptor removal not only results in the virus (via S2) to enter, into the cell through endocytosis and hijack the ribosomal system to multiply itself faster⁶².

ACE-2 has a greater binding affinity for SARS-CoV-2, though this receptor is involved in the fusion and entry of this virus. Other enzymes which are responsible for the breakdown of the protein and peptidases may help CoV-2 to enter and multiply in the human epithelial cells, mainly from the respiratory tract⁶³.

As we mentioned earlier that SARS-CoV-2 enters, into the host cell only after the spike protein S1 attached and removed the ACE-2 receptors from the cell membrane with the help of transmembrane serine proteases (TTSPs) present in the epithelial cells of the nose, trachea, bronchioles, and alveoli [64]. The S protein of SARS-CoV-2 is a heavily glycosylated type I membrane protein with numerous sites such as disulfide and N-linked glycation for its perfusion. S1 binds to the host cell receptor ACE2 through the receptor-binding domain (RBD), and S2 is cleaved by a serine protease TMPRSS2 or endosomal cysteine proteases cathepsin, triggered the dissociation of S1 and irreversible refolding of S2 into a post-fusion conformation. This S2 conformation elicited the viral entry into the cell Virus binding to the host receptor is optimized by the presence of 12 nucleotide Furin site at the S1/S2 junction⁶⁵.

Potential animal hosts

To look for potential animal hosts, researcher have examined the receptor activity of ACE2s from 14 mammal species, including human, rhesus, Chinese horseshoe bat (*Rhinolophus sinicus*), Mexican freetail (*Tadarida brasiliensis*), rat, mouse, palm cat, raccoon dog, badger, hog badger, canine, feline, rabbit, and pangolin for SARS-CoV-2 and a mutant virus lacking the furin cleavage site within the S protein.

The findings showed that multiple animal ACE2 proteins could function receptors for SARS-CoV- 2 and, therefore, the SARS-CoV-2 mutant. ACE2 proteins of human/rhesus monkey and rat/mouse exhibited the absolute best and lowest receptor activities, respectively, with the other ACE2s showing intermediate activity. Among the 14 ACE2s tested, hACE2 and rhesus ACE2 are the foremost efficient receptors, suggesting that SARS-CoV-2 has already been well-adapted to humans. Additionally, ACE2s of other animals, except mouse and rats, could also support SARS-CoV-2 entry. Although these data were obtained using an HIV-1-based pseudo typed virus, for ACE2 of *R. sinicus* bat, civet, and mouse, the data is consistent with in vitro infection data using an infectious virus¹⁰.

Downregulation of ACE 2 receptor

Entry of SARS-CoV2 into the cells through membrane fusion distinctly down-regulates ACE2 receptors results in the severity of the disease. A decreasing number of ACE2 receptors reduces the conversion of angiotensin 2 into angiotensin 1-7, responsible for vasodilation through binding to G-protein coupled Mas receptors. In the absence of ACE2, the availability of angiotensin 2 will increase, causing further vasoconstrictions, enhanced inflammation, and thrombosis⁶⁶. During SARS CoV2 infection, activation of Mas receptor or administration of Angiotensin 1-7 or their analogs may limit the detrimental effects of the disease⁶⁷. While the virus replicated poorly in dogs, it replicated efficiently in cats and was able to transmit to unaffected cats that were housed with the infected animals⁶⁸; another study revealed that 14.7% of cat serum samples collected within the town of Wuhan after the outbreak were positive for

antibody against SARS-CoV-2, demonstrating that tons of cats were infected during the episode, presumably from infected humans in close contact⁶⁹. Domestic cats are also susceptible to SARS-CoV infection⁷⁰, and human-to-cat transmission was evident during the SARS-CoV outbreak in 2003 in Hong Kong Law S⁷¹. These findings were also in agreement with our results that ACE2s of cat and dog could function receptors for SARS-CoV. There is no evidence that infected pets can transmit the virus back to humans; however, this could be possible and can be investigated. It would be best out of an abundance of caution when one is infected to possess both human and pets quarantined. Animal models are essential for the study of pathogenesis, vaccinology, and therapeutics of viral pathogens. Rodents are probably the foremost standard and amenable animal models thanks to low cost, easy handling, defined genetics, and, therefore, the likelihood of scalability (50). Genetically engineered mice expressing hACE2 were previously developed as an animal model for SARS-CoV^{72,73}. This model has been tested recently for SARS-CoV-2 and is susceptible to SARS-CoV-2 infection and respiratory illness⁷⁴, a typical clinical feature of COVID-19 patients⁷⁵. Human ACE2-transgenic mice, therefore, represent useful animal models. However, because of the high demand for these mice and the discontinuance of the model thanks to the disappearance of SARS-CoV within the human population after 2004, it is expected that this mouse model goes to be briefly supplied⁷⁶. Therefore, multiple animal models could even be needed to be aside from the traditional model, as rabbit ACE 2 could also be a more efficient receptor than other animal's ACE 2 receptors for both SARS-CoV-2 and SARS-CoV. Therefore, it is getting to be worth assessing the rabbit as a valuable animal model for further studies.

Factors contributing to the immunity against SARS-COV-2 in humans

1. Race

SARS CoV-2 has excessively affected various communities and races. Infection and death rates

for COVID-19 are 2 to 3 times higher in some populations than their counterparts in the country. Transmembrane serine protease 2 (TMPRSS2) is used by SARS-CoV-2 to facilitate its entry and spread. This protease enzyme is present on the epithelial lining of the nose and bronchi and activates the spike proteins so they can stick to the ACE2 receptors to enter the cell. Studies showed significantly higher expression of TMPRSS2 in some races as compared to others. Inhibitors of this enzyme such as camostat mesylate may be a good therapeutic target⁷⁷.

2. RAAS

According to current knowledge, there are two main axes of the Renin-Angiotensin-Aldosterone System (RAAS), which counteract each other in terms of vascular control: The classical vasoconstrictive axis, renin/angiotensin-converting enzyme/angiotensin II/angiotensin II receptor type 1 (AT1R), and the opposing vasorelaxant axis, angiotensin-converting enzyme 2/angiotensin-(1-7)/Mas receptor (MasR)⁷⁸.

3. COVID 19 and immunity

Adaptive immunity occurs within seven to ten days of SARS CoV2 infection. B and T-cells are essential for the development of adaptive immunity to SARS-CoV-2. Development of a long-term immunological memory against SARS-CoV-2 in the B-cell and T-cell is essential for long-lasting protection against the virus. It was generally observed that by day 5 to 7, IgM and IgA antibodies were present in the serum of Covid 19 patients and IgG by day 7 to 10 from the onset of symptoms. In general, serum IgM and IgA antibodies decline after approximately 28 days, and IgG peak around 49 days. T cells are activated within the first week of SARS-CoV-2 infection, and virus-specific CD4+ and CD8+T memory cells peaked within 2 weeks but remain detectable at lower levels for 100 or more days. The presence of SARS-CoV-2-specific memory CD4+T cells in 100% and CD8+T cells in 70% were observed in patients recovering from COVID-19. Although severe COVID-19 is characterized by high-viral titers, dysregulated innate inflammatory cytokine and chemokine responses and prolonged lymphopenia

do not appear to contribute to acute COVID-19 severity. Recent reports have demonstrated a decline in IgG neutralizing antibodies to SARS-CoV-2 in convalescence, raising apprehension of susceptibility to reinfection⁷⁹.

4. Gut health and immunity

Many factors influence our immunity, 80% of the immune system is in the gut, particularly in the specialized immune tissues called Peyer's patches that are found on the wall of small intestines. The gut microbiota not only plays an important role in ensuring that the digestive system functions efficiently but also a crucial role in maintaining a healthy immune system. It also must be balanced, ideally consisting of approximately 85% good bacteria and 15% bad bacteria, to function optimally^{80,81}.

5. Cytokine storms

An abnormally strong proinflammatory response known as "cytokine storm" may play an important role in the severity of COVID-19. Levels of IL-6 are elevated in severe COVID-19 but still lower than the levels usually observed⁸².

6. Vitamin D

Literature indicated a direct relationship between vitamin D deficiency and increased risk of having COVID-19⁸³.

7. Corticosteroids

Administration of systemic corticosteroids lowers the mortality in critically ill patients with COVID-19⁸⁴.

8. CoV-2 infection without symptoms

Millions of individuals around the world are dying due to SARS CoV-2 infection; however, there are thousands of people who are infected with CoV-2 without any symptoms. This difference among mankind could be due to their genetic makeup⁸⁵.

9. Palmitoylation

The cytoplasmic portion of the S glycoprotein contains four cysteine-rich amino acid clusters. Individual cysteine clusters were altered via cysteine-to-alanine amino acid replacement, and

the modified S glycoproteins were tested for their transport to cell surfaces and ability to cause cell fusion in transient transfection assays. Mutagenesis of the cysteine cluster I, located immediately proximal to the predicted transmembrane, the domain did not appreciably reduce cell-surface expression, although S-mediated cell fusion was reduced by more than 50% in comparison to the wild-type S. Similarly, mutagenesis of the cysteine cluster II located adjacent to cluster I reduced S-mediated cell fusion by more than 60% compared to the wild-type S, while cell-surface expression was reduced by less than 20%. Mutagenesis of cysteine clusters III and IV did not appreciably affect S cell-surface expression or S-mediated cell fusion. The wild-type S was palmitoylated, as evidenced by the efficient incorporation of 3H-palmitic acid in wild-type S molecules. Palmitoylation of the membrane-proximal cysteine clusters I and II may be important for S-mediated cell fusion⁸⁶.

10. Environment

In this review, we discussed genetic and epigenetic factors responsible for severe, mild and asymptomatic COVID-19 infection through animal biochemistry, and we believe that this information may help in the future prevention of infection by more lethal mutant strains of coronaviruses⁸⁷.

Conclusion

The potential of wild animals to function as natural reservoirs or intermediate hosts for SARS-CoV-2 and its progenitor, the danger of zoonotic transmission of animal SL-CoVs to humans, and, therefore, thorough virus surveillance in wild animals is crucial to stop another pandemic. After discussing the present situation of COVID-19, we went through the cycle of zoonosis, types of coronaviruses endorsed with their tropism and discuss the steps taken by the enveloped viruses to enter their respective host cells via endocytosis pathways using lysosomal proteases, essential for their entry and multiplication. Our understanding of the relationship between viral tropism and lysosomal proteases in different tissues and species leads to the enzymes essential for the viral tropism of organs in humans. While analyzing animal

models for clinical trials, 70-100 days old post-weaning cats came across as a suitable model and at the same time, on another note, we discussed the therapeutic measures required to protect newborns of infected mothers. We reviewed the origin of SARS-CoV-2, intermediate host, virus-host cell-binding processes and the evolving list of potential animal hosts. We also focused on ACE 2 receptors and how downregulation of ACE-2Rs causes the severity of Covid 19 disease, and why some people are asymptomatic. In the end, we examine the factors enhancing immunity and how alterations in human behavior and climate change can predict the zoonotic transmission and the possible time frame for the next spell.

Future Concern

Since March of 2020, we are in a global pandemic. Vaccines are around, but it will take many months, if not years when everyone is vaccinated. One of the major concerns is about those who got infected while they are pregnant and may pass the toxins of COVID-19 to the newborn. In the USA alone, about half a million babies will bear by the infected mothers, which may result in preterm or stillbirth, as reflected from the data collected after the outbreak of SARS CoV and H1N1. The third trimester is typically regarded as critical when the highest placental antibody transfer occurs^{88,89}. Comparing the efficiency of antibody transfer in first, second, and third-trimester native conditions with SARS-CoV-2 will be an important area for future study, as women infected in the first and second trimesters begin to deliver. Assessment of maternal and neonatal SARS-CoV-2 viral load, Transplacental antibody transfer, and placental pathology in pregnancies during the COVID-19 pandemic are required. Further concerns are the emergence of new, more contagious SARS-CoV-2 variants, as shown to increase the virus's ability to infect cells and the development and availability of effective vaccines⁹⁰.

A total of 1108 cases infected with SARS-CoV-2 VUI 202012/01 have been detected in the United Kingdom as of December 13, 2020. The variant is defined by the presence of a range of 14 mutations resulting in amino acid changes and three

deletions. Some of these mutations may influence the transmissibility of the virus in humans: One of the mutations identified (N501Y) is altering an amino acid within the six key residues in the receptor-binding domain (RBD). Sequence analysis revealed that the N501Y mutation of the virus reported in the United Kingdom and South Africa originated separately. Another mutation of biological significance, P681H, has been found in the RBD. Finally, the deletion at position 69/70 has been found to affect the performance of some diagnostic PCR assays that use an S gene target. The new VUI-202012/01 variant has been identified in several countries, including Australia, Denmark, Italy, Iceland, and the Netherlands. Preliminary reports by the United Kingdom are that this variant is more transmissible than previous circulating viruses, with an estimated increase of between 40% and 70% in transmissibility. As one of the mutations (N501Y) is in the receptor-binding domain, the authorities are urgently investigating the neutralization activity of sera from recovered and vaccinated patients against this variant to determine if there is any impact on vaccine performance⁹¹.

Conflicts of Interest

The authors have declared that no competing interests exist.

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References

1. Fagoonee I, Pellicano R. COVID-19 brings the world economy to its knees. *Minerva medica*. 2020;111(4):297-299.
2. Carl Zimmer, J.C.a.S.-L.W., Covid-19 Vaccine Tracker_ Latest Updates -. *The New York Times*, 2020.
3. Decaro N, Cordonnier N, Demeter Z, Egberink H, Elia G, Grellet A, Le Poder S, Mari V, Martella V, Ntasis V, Von Reitzenstein M. European surveillance for

- pantropic canine coronavirus. *J Clin Microbiol.* 2013;51(1):83-88.
4. Decaro NC. Buonavoglia, Canine coronavirus: not only an enteric pathogen. *Vet Clin North Am Small Anim Pract.* 2011;41(6):1121-1132.
 5. Decaro N, Mari V, Campolo M, Lorusso A, Camero M, Elia G, Martella V, Cordioli P, Enjuanes L, Buonavoglia C. Recombinant canine coronaviruses related to transmissible gastroenteritis virus of Swine are circulating in dogs. *J Virol.* 2009;83(3):1532-1537.
 6. Becker DJ, Washburne AD, Faust CL, Pulliam JR, Mordecai EA, Lloyd-Smith JO, Plowright RK. Dynamic and integrative approaches to understanding pathogen spillover. *Philos Trans R Soc Lond B Biol Sci.* 2019;374(1782):20190014.
 7. Buonavoglia C, Decaro N, Martella V, Elia G, Campolo M, Desario C, Castagnaro M, Tempesta M. Canine coronavirus highly pathogenic for dogs. *Emerg Infect Dis J.* 2006;12(3):492.
 8. Chen S, Liu D, Tian J, Kang H, Guo D, Jiang Q, Liu J, Li Z, Hu X, Qu L. Molecular characterization of HLJ-073, a recombinant canine coronavirus strain from China with an ORF3abc deletion. *Arch Virol.* 2019;164(8):2159-2164.
 9. Pinto LD, Barros IN, Budaszewski RF, Weber MN, Mata H, Antunes JR, Boabaid FM, Wouters AT, Driemeier D, Brandão PE, Canal CW. Characterization of pantropic canine coronavirus from Brazil. *Vet J.* 2014;202(3):659-662.
 10. Decaro N, Buonavoglia C. An update on canine coronaviruses: viral evolution and pathobiology. *Vet Microbiol.* 2008. 132(3-4): 221-234.
 11. Marinaro M, Mari V, Bellacicco AL, Tarsitano E, Elia G, Losurdo M, Rezza G, Buonavoglia C, Decaro N. Prolonged depletion of circulating CD4+ T lymphocytes and acute monocytosis after pantropic canine coronavirus infection in dogs. *Virus Res.* 2010;152(1-2):73-78.
 12. Awuni JA, Bianco A, Dogbey OJ, Fusaro A, Yingar DT, Salviato A, Ababio PT, Milani A, Bonfante F, Monne I. Avian influenza H9N2 subtype in Ghana: virus characterization and evidence of co-infection. *Avian Pathol.* 2019;48(5):470-476.
 13. Klimov AI, Garten R, Russell C, Barr IG, Besselaar TG, Daniels R, Engelhardt OG, Grohmann G, Itamura S, Kelso A. and McCauley J. WHO recommendations for the viruses to be used in the 2012 Southern Hemisphere Influenza Vaccine: epidemiology, antigenic and genetic characteristics of influenza A(H1N1)pdm09, A(H3N2) and B influenza viruses collected from February to September 2011. *Vaccine.* 2012;30(45):6461-6471.
 14. Antón A, Pozo F, Niubó J, Casas I, Pumarola T. Influenza A(H1N1)pdm09 virus: viral characteristics and genetic evolution. *Enfermedades Infecciosas y Microbiología Clínica.* 2012;30:10-17.
 15. Fereidouni SR, Starick E, Grund C, Globig A, Mettenleiter TC, Beer M, Harder T. Rapid molecular subtyping by reverse transcription polymerase chain reaction of the neuraminidase gene of avian influenza A viruses. *Vet Microbiol.* 2009;135(3-4):253-260.
 16. Maginnis MS. Virus-Receptor Interactions: The Key to Cellular Invasion. *J Mol Biol.* 2018;430(17):2590-2611.
 17. McFadden G, Mohamed MR, Rahman MM, Bartee E. Cytokine determinants of viral tropism. *Nat Rev Immunol.* 2009;9(9):645-55.
 18. Zhou JH, Wang YN, Chang QY, Ma P, Hu Y, Cao X. Type III Interferons in Viral Infection and Antiviral Immunity. *Cell Physiol Biochem.* 2018;51(1):173-185.
 19. Zheng Y, Shang J, Yang Y, Liu C, Wan Y, Geng Q, Wang M, Baric R, Li F. Lysosomal Proteases Are a Determinant of Coronavirus Tropism. *J Virol.* 2018;92(24).
 20. Ye ZW, Yuan S, Yuen KS, Fung SY, Chan CP, Jin DY. Zoonotic origins of human coronaviruses. *Int J Biol Sci.* 2020;16(10):1686-1697.
 21. Hattori J, Shiino T, Gatanaga H, Yoshida S, Watanabe D, Minami R, Sadamasu K, Kondo M, Mori H, Ueda M, Tateyama M. Trends in transmitted drug-resistant HIV-1 and demographic characteristics of newly diagnosed patients: nationwide surveillance from 2003 to 2008 in Japan. *Antiviral Res.* 2010;88(1):72-79.
 22. De Jong JC, Rimmelzwaan GF, Fouchier RA, Osterhaus AD. Influenza virus: a master of metamorphosis. *J Infect.* 2000;40(3):218-228.
 23. Steinhauer DA, Skehel JJ. Genetics of influenza viruses. *Annu Rev Genet.* 2002;36:305-332.
 24. Matrosovich MN, Matrosovich TY, Gray T, Roberts NA, Klenk HD. Neuraminidase is important for the initiation of influenza virus infection in human airway epithelium. *J Virol.* 2004;78(22):12665-12667.
 25. Oladunni FS, Park JG, Pino PA, Gonzalez O, Akhter A, Allué-Guardia A, Olmo-Fontánez A, Gautam S, Garcia-Vilanova A, Ye C, Chiem K. Lethality of SARS-CoV-2 infection in K18 human angiotensin-converting enzyme 2 transgenic mice. *Nat Commun.* 2020;11(1):6122.
 26. Hu B, Zeng LP, Yang XL, Ge XY, Zhang W, Li B, Xie JZ, Shen XR, Zhang YZ, Wang N, Luo DS. Discovery of a

- rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS Pathog.* 2017;13(11):e1006698.
27. Sun J, He WT, Wang L, Lai A, Ji X, Zhai X, Li G, Suchard MA, Tian J, Zhou J, Veit M. COVID-19: Epidemiology, Evolution, and Cross-Disciplinary Perspectives. *Trends Mol Med.* 2020;26(5):483-495.
 28. Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, Wang F, Li D, Yang M, Xing L, Wei J. Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma. *JAMA.* 2020;323(16):1582-1589.
 29. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. *JAMA.* 2020;324(8):782-793.
 30. Tang T, Bidon M, Jaimes JA, Whittaker GR, Daniel S. Coronavirus membrane fusion mechanism offers a potential target for antiviral development. *Antiviral Res.* 2020;178:104792.
 31. Millet JK, Whittaker GR. Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis. *Virus Res.* 2015;202:120-134.
 32. Yang L, Wu Z, Ren X, Yang F, He G, Zhang J, Dong J, Sun L, Zhu Y, Du J, Zhang S. Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus. *Proc Natl Acad Sci U S A.* 2014;111(34):12516-12521.
 33. Yang, L., et al., Novel SARS-like betacoronaviruses in bats, China, 2011. *Emerg Infect Dis.* 2013;19(6):989-991.
 34. Easterlin MC, Crimmins EM, Finch CE. Will prenatal exposure to SARS-CoV-2 define a birth cohort with accelerated aging in the century ahead? *J Dev Orig Health Dis.* 2020:1-5.
 35. Kwon JY, Aldo P, You Y, Ding J, Racicot K, Dong X, Murphy J, Glukshtad G, Silasi M, Peng J, Wen L. Relevance of placental type I interferon beta regulation for pregnancy success. *Cell Mol Immunol.* 2018. 15(12): p. 1010-1026.
 36. Mosby LG, Rasmussen SA, Jamieson DA, 2009 pandemic influenza A (H1N1) in pregnancy: a systematic review of the literature. *Am J Obstet Gynecol.* 2011;205(1):10-18.
 37. Alfaraj SH, Al-Tawfiq JA, Memish ZA, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection during pregnancy: Report of two cases & review of the literature. *J Microbiol Immunol Infect.* 2019;52(3):501-503.
 38. Gong L, Li J, Zhou Q, Xu Z, Chen L, Zhang Y, Xue C, Wen Z, Cao Y. A New Bat-HKU2-like Coronavirus in Swine, China, 2017. *Emerg Infect Dis.* 2017;23(9).
 39. Ge XY, Li JL, Yang XL, Chmura AA, Zhu G, Epstein JH, Mazet JK, Hu B, Zhang W, Peng C, Zhang YJ. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature.* 2013;503(7477):535-538.
 40. Zhao K, Shi W, Han F, Xu Y, Zhu L, Zou Y, Wu X, Zhu H, Tan F, Tao S, Tang X. Specific, simple and rapid detection of porcine circovirus type 2 using the loop-mediated isothermal amplification method. *Virol J.* 2011;8.
 41. Pan Y, Tian X, Qin P, Wang B, Zhao P, Yang YL, Wang L, Wang D, Song Y, Zhang X, Huang YW. Discovery of a novel swine enteric alphacoronavirus (SeACoV) in southern China. *Vet Microbiol.* 2017;211:15-21.
 42. Zhou L, Sun Y, Wu JL, Mai KJ, Chen GH, Wu ZX, Bai Y, Li D, Zhou ZH, Cheng J, Wu RT. Development of a TaqMan-based real-time RT-PCR assay for the detection of SADS-CoV associated with severe diarrhea disease in pigs. *J Virol Methods.* 2018;255:66-70.
 43. Menachery VD, Eisfeld AJ, Schäfer A, Josset L, Sims AC, Proll S, Fan S, Li C, Neumann G, Tilton SC, Chang J. Pathogenic influenza viruses and coronaviruses utilize similar and contrasting approaches to control interferon-stimulated gene responses. *MBio.* 2014;5(3):e01174-14.
 44. Cook JK, Jackwood M, Jones RC. The long view: 40 years of infectious bronchitis research. *Avian Pathol.* 2012;41(3):239-250.
 45. Jaiswal G, Kumar V. In-silico design of a potential inhibitor of SARS-CoV-2 S protein. *PLoS One.* 2020;15(10):e0240004.
 46. Hou Y, Peng C, Yu M, Li Y, Han Z, Li F, Wang LF, Shi Z. Angiotensin-converting enzyme 2 (ACE2) proteins of different bat species confer variable susceptibility to SARS-CoV entry. *Arch Virol.* 2010;155(10):1563-1569.
 47. Benton DJ, Wrobel AG, Xu P, Roustan C, Martin SR, Rosenthal PB, Skehel JJ. Receptor binding and priming of the spike protein of SARS-CoV-2 for membrane fusion. *Nature.* 2020;588(7837):327-330.
 48. Liu P, Jiang JZ, Wan XF, Hua Y, Li L, Zhou J, Wang X, Hou F, Chen J, Zou J, Chen J. Are pangolins the intermediate host of the 2019 novel coronavirus (SARS-CoV-2)? *PLoS Pathog.* 2020;16(5):e1008421.

49. Zhang T, Wu Q, Zhang Z. Probable Pangolin Origin of SARS-CoV-2 Associated with the COVID-19 Outbreak. *Curr Biol.* 2020;30(7):1346-1351 e2.
50. Xiao K, Zhai J, Feng Y, Zhou N, Zhang X, Zou JJ, Li N, Guo Y, Li X, Shen X, Zhang Z. Isolation of SARS-CoV-2-related coronavirus from Malayan pangolins. *Nature.* 2020;583(7815):286-289.
51. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, Hu Y, Tao ZW, Tian JH, Pei YY, Yuan ML. A new coronavirus associated with human respiratory disease in China. *Nature.* 2020;579(7798):265-269.
52. Fraser C, Donnelly CA, Cauchemez S, Hanage WP, Van Kerkhove MD, Hollingsworth TD, Griffin J, Baggaley RF, Jenkins HE, Lyons EJ, Jombart T. Pandemic potential of a strain of influenza A (H1N1): early findings. *Science.* 2009;324(5934):1557-61.
53. Team NS. OIAHNVI. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team, 2009: p. 12.
54. Woo PC, Huang Y, Lau SK, Yuen KY. Coronavirus genomics and bioinformatics analysis. *viruses.* 2010;2(8):1804-1820.
55. van Boheemen S, de Graaf M, Lauber C, Bestebroer TM, Raj VS, Zaki AM, Osterhaus AD, Haagmans BL, Gorbelenya AE, Snijder EJ, Fouchier RA. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. *MBio.* 2012;3(6):e00473-12.
56. Czub M, Weingartl H, Czub S, He R, Cao J. Evaluation of modified vaccinia virus Ankara based recombinant SARS vaccine in ferrets. *Vaccine.* 2005;23(17-18):2273-2279.
57. Cornelis AM, De Haan LK, Paul S. Masters, Harry Vennema, And Peter J. M. Rottie, Coronavirus Particle Assembly: Primary Structure Requirements of the Membrane Protein. *J Virol.* 1998:6838-6850.
58. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet.* 2020;395(10224):565-574.
59. Mousavizadeh L, Ghasemi S. Genotype and phenotype of COVID-19: Their roles in pathogenesis. *J Microbiol Immunol Infect.* 2020.
60. Avgousti DC, Herrmann C, Kulej K, Pancholi NJ, Sekulic N, Petrescu J, Molden RC, Blumenthal D, Paris AJ, Reyes ED, Ostapchuk P. A core viral protein binds host nucleosomes to sequester immune danger signals. *Nature.* 2016;535(7610): 173-177.
61. Roussan DA, Haddad R, Khawaldeh G. Molecular survey of avian respiratory pathogens in commercial broiler chicken flocks with respiratory diseases in Jordan. *Poult Sci.* 2008;87(3):444-448.
62. Jaimes JA, Millet JK, Whittaker GR. Proteolytic Cleavage of the SARS-CoV-2 Spike Protein and the Role of the Novel S1/S2 Site. *iScience.* 2020;23(6):101212.
63. Bosch BJ, Smits SL, Haagmans BL. Membrane ectopeptidases targeted by human coronaviruses. *Curr Opin Virol.* 2014;6:55-60.
64. Rahman N, Basharat Z, Yousuf M, Castaldo G, Rastrelli L, Khan H. Virtual Screening of Natural Products against Type II Transmembrane Serine Protease (TMPRSS2), the Priming Agent of Coronavirus 2 (SARS-CoV-2). *Mol.* 2020;25(10).
65. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veasler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell.* 2020;181(2):281-292.
66. Verdecchia P, Cavallini C, Spanevello A, Angeli F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. *Eur J Intern Med.* 2020;76:14-20.
67. Magalhaes GS, Rodrigues-Machado MD, Motta-Santos D, Campagnole-Santos MJ, Santos RA. Activation of Ang-(1-7)/Mas Receptor Is a Possible Strategy to Treat Coronavirus (SARS-CoV-2) Infection. *Front Physiol.* 2020;11:730.
68. Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, Liu R, He X, Shuai L, Sun Z, Zhao Y. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Sci.* 2020;368:1016-1020.
69. Shi J, Deng G, Kong H, Gu C, Ma S, Yin X, Zeng X, Cui P, Chen Y, Yang H, Wan X. H7N9 virulent mutants detected in chickens in China pose an increased threat to humans. *Cell Res.* 2017;27(12):1409-1421.
70. Okba NM, Widjaja I, van Dieren B, Aebischer A, van Amerongen G, de Waal L, Stittelaar KJ, Schipper D, Martina B, van den Brand JM, Beer M. Particulate multivalent presentation of the receptor binding domain induces protective immune responses against MERS-CoV. *Emerg Microbes Infect.* 2020;9(1):1080-1091.
71. Law S, Leung AW, Xu C. Severe acute respiratory syndrome (SARS) and coronavirus disease-2019 (COVID-19): From causes to preventions in Hong Kong. *Int J Infect Dis.* 2020;94:156-163.
72. Gurumurthy CB, Quadros RM, Richardson GP, Poluektova LY, Mansour SL, Ohtsuka M. Genetically

- modified mouse models to help fight COVID-19. *Nat Protoc.* 2020;15(12):3777-3787.
73. Jiang RD, Liu MQ, Chen Y, Shan C, Zhou YW, Shen XR, Li Q, Zhang L, Zhu Y, Si HR, Wang Q. Pathogenesis of SARS-CoV-2 in Transgenic Mice Expressing Human Angiotensin-Converting Enzyme 2. *Cell.* 2020;182(1):50-58 e8.
 74. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol.* 2021; 19(3): 141-145.
 75. Shah SJ, Barish PN, Prasad PA, Kistler A, Neff N, Kamm J, Li LM, Chiu CY, Babik JM, Fang MC, Abe-Jones Y. Clinical features, diagnostics, and outcomes of patients presenting with acute respiratory illness: A retrospective cohort study of patients with and without COVID-19. *EClinicalMedicine.* 2020;27:100518.
 76. Hongjing Gu QC, Yang G, He L, Fan H, Deng Y-Q. Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy. *Science.* 2020;369:1603-1607.
 77. Bunyavanich S, Grant C, Vicencio A. Racial/Ethnic Variation in Nasal Gene Expression of Transmembrane Serine Protease 2 (TMPRSS2). *JAMA.* 2020;324(15):1567-1568.
 78. Povlsen AL, Grimm D, Wehland M, Infanger M, Krüger M. The Vasoactive Mas Receptor in Essential Hypertension. *J Clin Med.* 2020;9(1).
 79. Stephens DS, McElrath MJ. COVID-19 and the Path to Immunity. *JAMA.* 2020;324(13):1279-1281.
 80. Wu HJ, Wu E, The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes.* 2012;3(1):4-14.
 81. Eisenstein M. The microbial ambassadors of the immune system. *Nature* 2020.
 82. Kox M, Waalders NJ, Kooistra EJ, Gerretsen J, Pickkers P. Cytokine Levels in Critically Ill Patients With COVID-19 and Other Conditions. *JAMA*, 2020.
 83. Meltzer DO, Best TJ, Zhang H, Vokes T, Arora V, Solway J. Association of Vitamin D Status and Other Clinical Characteristics With COVID-19 Test Results. *JAMA Netw Open.* 2020;3(9):e2019722.
 84. Sterne JA, Murthy S, Diaz JV, Slutsky AS, Villar J, Angus DC, Annane D, Azevedo LC, Berwanger O, Cavalcanti AB, Dequin PF. Association Between Administration of Systemic Corticosteroids and Mortality Among Critically Ill Patients With COVID-19: A Meta-analysis. *JAMA*, 2020;324(13):1330-1341.
 85. Byambasuren O, Cardona M, Bell K, Clark J, McLaws ML, Glasziou P. Estimating the extent of asymptomatic COVID-19 and its potential for community transmission: Systematic review and meta-analysis. *JAMMI.* 2020;5(4): 223-234.
 86. Petit CM, Chouljenko VN, Iyer A, Colgrove R, Farzan M, Knipe DM, Kousoulas KG. Palmitoylation of the cysteine-rich endodomain of the SARS-coronavirus spike glycoprotein is important for spike-mediated cell fusion. *Viol.* 2007;360(2):264-274.
 87. Azuma K, Yanagi U, Kagi N, Kim H, Ogata M, Hayashi M. Environmental factors involved in SARS-CoV-2 transmission: effect and role of indoor environmental quality in the strategy for COVID-19 infection control. *Environ Health Prev Med.* 2020;25(1):66.
 88. Pentšuk N, van der Laan JW. An interspecies comparison of placental antibody transfer: new insights into developmental toxicity testing of monoclonal antibodies. *Birth Defects Res B Dev Reprod Toxicol.* 2009;86(4):328-344.
 89. Ciobanu AM, Colibaba S, Cimpoca B, Peltecu G, Panaitescu AM. Thrombocytopenia in pregnancy. *Maedica.* 2016;11(1):55.
 90. Jeyanathan M, Afkhami S, Smaill F, Miller MS, Lichty BD, Xing Z. Immunological considerations for COVID-19 vaccine strategies. *Nat Rev Immunol.* 2020;20(10):615-632.
 91. Volz E, Mishra S, Chand M, Barrett JC, Johnson R, Geidelberg L, Hinsley WR, Laydon DJ, Dabrera G, O'Toole Á, Amato R. Transmission of SARS-CoV-2 Lineage B. 1.1. 7 in England: Insights from linking epidemiological and genetic data. *MedRxiv.* 2021:2020-12.