Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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BRIEF REVIEW

Is SARS-CoV-2 Also an Enteric Pathogen With Potential Fecal–Oral Transmission? A COVID-19 Virological and Clinical Review

Siyuan Ding[1] and T. Jake Liang[2]

[1]Department of Molecular Microbiology, Washington University in St Louis, St Louis, Missouri and [2]Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland

In as few as 3 months, coronavirus disease 2019 (COVID-19) has spread and ravaged the world at an unprecedented speed in modern history, rivaling the 1918 flu pandemic. Severe acute respiratory syndrome coronavirus-2, the culprit virus, is highly contagious and stable in the environment and transmits predominantly among humans via the respiratory route. Accumulating evidence suggest that this virus, like many of its related viruses, may also be an enteric virus that can spread via the fecal–oral route. Such a hypothesis would also contribute to the rapidity and proliferation of this pandemic. Here we briefly summarize what is known about this family of viruses and literature basis of the hypothesis that severe acute respiratory syndrome coronavirus-2 is capable of infecting the gastrointestinal tract and shedding in the environment for potential human-to-human transmission.

Coronaviruses (CoVs) are ubiquitous in nature and infect a wide range of animals, causing diseases involving the respiratory, gastrointestinal (GI), and neurological systems.[1] Before 2000, only 2 species of CoVs (HCoV-229E and HCoV-OC43) were known to infect humans and cause mild respiratory illness.[2] Two other species of human CoVs (HCoV-NL63 and HCoV-HKU1) were isolated in the early 2000s. Since then, the human race has encountered the following 3 novel CoV outbreaks: severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, Middle East respiratory syndrome (MERS)-CoV in 2012, and the ongoing SARS-CoV-2, all of which have jumped species from animals to humans and are associated with severe respiratory disease and high mortality. Zoonotic infections involving nonhuman species as intermediate hosts, such as invertebrate vectors, rodents, and bats, are common in nature.[3] But cross-species jumps from animals to humans with altered tropism as a result of genetic alterations are less frequent. At present, it is not clear what accounts for this seemingly increased pace of cross-species transmission of such virulent pathogens that can cause devastating disease in humans. Manipulation of the environment by mankind, increased human–animal contacts, and globalization may have facilitated conditions for cross-species infection.[4]

At the time this review was completed (April 23, 2020), coronavirus disease 2019 (COVID-19) has emerged as a world pandemic. Globally SARS-CoV-2 has infected 2,667,532 people, of which 850,116 cases are identified in the United States alone. Currently, no clinically approved specific antivirals (except the recently demonstrated benefit of remdesivir), other therapeutic remedies, or vaccines are available for this disease. Although spread of the virus among humans is predominantly through respiratory droplets, questions remain regarding other potential modes of transmission that may contribute to the initial cross-species infection, a large number asymptomatic cases, and the rapid and unusual pattern of dissemination across the globe. In this review, we provide a summary of the molecular biology of the virus, evidence for its infection of cells within the gastrointestinal and hepatotropic/biliary tracts, and implications for potential fecal–oral transmission of the virus. For more extensive review of the virus and its associated diseases, we refer to other review articles and brief summaries.[5–9]

Molecular Biology and Tropism of Coronavirus

CoVs belong to the *Coronaviridae* family within the *Nidovirales* order. They are enveloped, nonsegmented, positive-sense RNA viruses with a large genome of approximately 30 kb. Figure 1 illustrates the schematic replication cycle of the virus. The initial attachment of the CoV to the host cell is mediated by interactions between the spike glycoprotein (S) and its cognate receptor. This molecular interaction is a major determinant of species, tissue, and cell tropism of a CoV. Many CoVs utilize cell-surface peptidases as their receptors, but the peptidase activity seems to be dispensable for viral entry.[10] Many alphacoronaviruses use aminopeptidase N.[11,12] In the case of SARS-CoV and SARS-CoV-2, angiotensin I converting enzyme 2 (ACE2) mediates entry into host cells,[13–15] whereas dipeptidyl-peptidase 4 (DPP4) is the receptor for MERS-CoV.[16] Of note, ACE2 is an X-linked gene and has sex-specific expression profiles[17] that may contribute to the observed more severe clinical manifestations in males compared to females with COVID-19.[18] Smokers and individuals with chronic obstructive pulmonary disease have higher ACE2 expression levels.[19] Innate immune signaling such as interferon also seems to regulate ACE2 levels and thus susceptibility to SARS-CoV-2 infection.[20] In the context of

Abbreviations used in this paper: ACE2, angiotensin I converting enzyme 2; CoV, coronavirus; COVID-19, coronavirus disease 2019; DPP4, dipeptidyl-peptidase 4; GI, gastrointestinal; MERS, Middle East respiratory syndrome; S, spike glycoprotein; SARS, severe acute respiratory syndrome; TMPRSS2, transmembrane serine protease 2.

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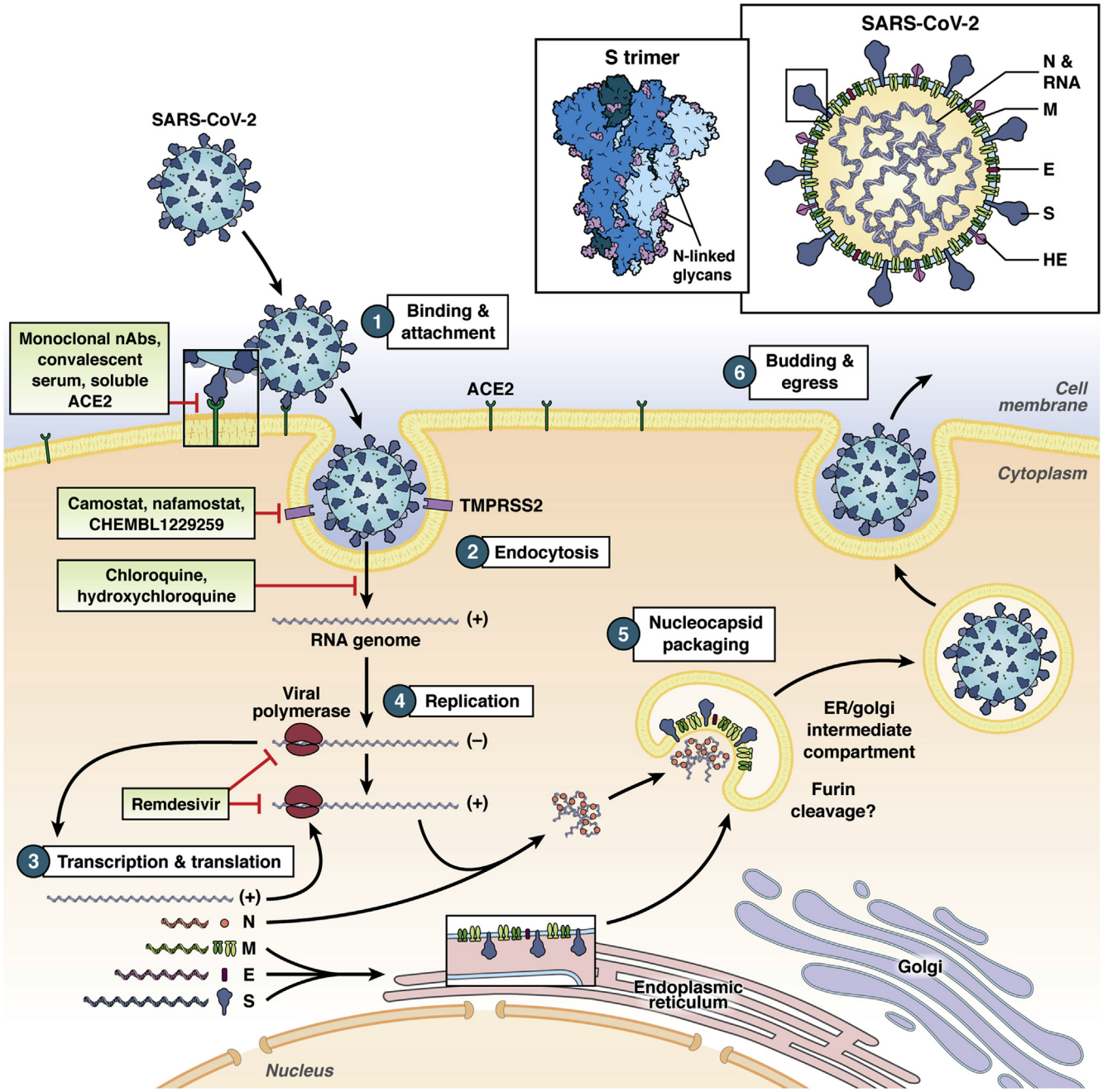


Figure 1. A simplified diagram of the SARS-CoV-2 replication cycle (with potential pharmacological inhibitors under investigation depicted at respective steps). The virion and its associated viral proteins are shown schematically at the *top*. The structure of the S trimer is depicted. (*1*) Interaction between viral S protein and host ACE2 mediates virus binding to the host cell. (*2*) S protein is cleaved by host serine proteases, such as TMPRSS2, allowing the fusion of viral membrane with the host membrane and single-stranded RNA (ssRNA) (+) genome release into the cytoplasm. (*3*) Transcription and translation of viral proteins from genomic and subgenomic RNAs. (*4*) Replication occurs within the replicative membranous compartment, where and new ssRNA(+) are synthesized. (*5*) Virus assembly at the endoplasmic reticulum (ER), the intermediate compartments, and/or the Golgi complex. (*6*) Release of new virions by exocytosis. E, envelope protein; HE, hemagglutinin-esterase glycoprotein; M, membrane protein; N, nucleocapsid protein.

the GI tract, patients with enteric virus infections and other inflammatory conditions may have a different cytokine profile and thus distinct ACE2 levels in the gut. In addition, genetic polymorphisms in the *ACE2* gene have been associated with diabetes and hypertension.[21,22] Whether they are linked to clinical outcomes in COVID-19 patients remains to be tested and may shed light on the role of genetic predisposition to more severe diseases.

Interestingly, these viral receptors have been the targets of drug development for cardiac disease, hypertension, and diabetes, with ACE inhibitors blocking the renin-angiotensin-aldosterone system and gliptins inhibiting the

DPP4 action to improve glucose control. While the enzymatic actions of these peptidases are dispensable for viral infection, these inhibitors can result in the up-regulation of the protein, offering an intriguing hypothesis that patients with hypertension and diabetes are often on these drugs and thus may be more susceptible to more severe COVID-19 disease.[4]

Multiple Cryo-EM structures of the recombinant S receptor binding domain and ACE2 complex have already been solved with an unprecedented speed.[23–26] These data indicate that the receptor-binding domain of S binds tightly to human and bat ACE2, suggestive of a zoonotic origin. A recent study demonstrated that the virus can indeed be transmitted to cats (including recent news of transmission to tigers in the Bronx Zoo) and ferrets, but not dogs, chickens, or pigs,[24] although porcine ACE2 mediates viral entry in cell culture.[14] This broad species tropism raises concerns of potential transmission from domestic pets to humans and vice versa. From a structural and immunogen design perspective, more information is needed regarding the native S protein trimeric state on virions, how the trimer interacts with ACE2, and how such interaction is disrupted by neutralizing antibodies. A recent publication on the crystal structure of an antibody in complex with the receptor-binding domain of the SARS-CoV-2 S protein provides important molecular insight into antibody recognition of the virus and a potential strategy for vaccine development.[27] Besides ACE2, other potential entry factors, such as CD147[28] and integrins,[29] are currently under investigation.

After receptor engagement, SARS-CoV-2 gains access into the host cell. Like other human CoVs, this process is generally accomplished by acid-dependent proteolytic cleavage of S protein proteases such as cathepsins, which exposes the fusion domain of the S protein in the endosome,[30] or by transmembrane serine protease 2 (TMPRSS2) at the plasma membrane.[31,32] For MERS-CoV, furin-mediated cleavage and fusion also occurs during virus entry,[33] and may be relevant for SARS-CoV-2.[34] This step takes place before fusion of the viral and cellular membranes and is also a key determinant of tissue and species tropism of the virus.[35] This acid-dependent process may explain the proposed efficacy of chloroquine or hydroxychloroquine, as a lysosomotropic agent, in the treatment of COVID-19[36] (Figure 1). Recent publications suggest that, similar to SARS-CoV, trypsin and TMPRSS2 also prime SARS-CoV-2 S protein for efficient infection.[15,37] Overexpression of TMPRSS2 in African green monkey Vero-E6 cells significantly enhanced SARS-CoV-2 infectivity.[38] Serine protease inhibitor camostat blocked SARS-CoV-2 entry into host cells in a dose-dependent manner,[15] making it and other similar inhibitors, such as nafamostat[39] and Pharos compounds (CHEMBL1229259), candidate small-molecule inhibitors in the treatment of COVID-19 patients (Figure 1).

Besides the respiratory tract, including oral mucosa,[40] the GI tract, in particular the small intestine, has high expression levels of ACE2 and TMPRSS2 in both humans[41] and mice.[42] Multiple data sets of single-cell RNA-sequencing analysis indicated that mature absorptive enterocytes from ileum and colon have high ACE2 messenger RNA expression levels.[43–45] ACE2 protein levels have been validated by immunostaining in human small intestinal epithelium.[46] On the luminal surface of intestinal epithelial cells, ACE2 associates with the neutral amino acid transporter B0AT1 and regulates intestinal microflora.[47,48] SARS-CoV-2 infection of the GI tract, by altering the levels of ACE2 at the brush border, can cause microbial dysbiosis and inflammation. In addition, high ACE2 expression is also evident in cholangiocytes and, to a lesser extent, hepatocytes, and suggests possible hepatobiliary infection by SARS-CoV-2.[49]

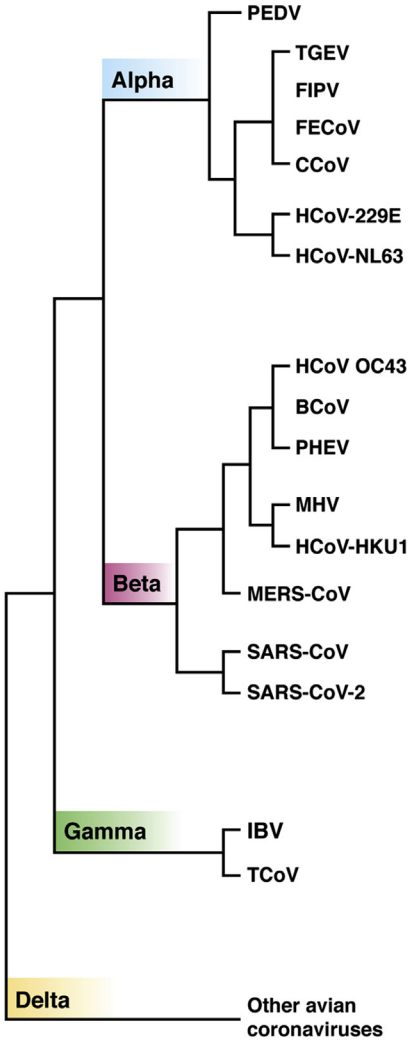
Human and Animal Coronaviruses With Intestinal and Hepatic Involvement

As major human pathogens of medical significance, CoVs cause a variety of diseases in animals.[50] For instance, the prototypic mouse hepatitis virus infects the lung and spreads systemically to the GI tract, liver, and brain, causing gastroenteritis, hepatitis, and encephalitis. Transmissible gastroenteritis virus and porcine epidemic diarrhea virus cause severe gastroenteritis in young piglets, leading to significant morbidity and mortality. Avian infectious bronchitis virus is a major cause of economic loss within the poultry industry. Bat CoV infects the GI and respiratory tracts of the bats without apparent diseases. Feline enteric CoV causes a mild or asymptomatic infection in domestic cats. It is clear from the names of these animal CoVs that they are entero-pathogens and the primary symptoms point to an intestinal tropism. Figure 2 illustrates the species and tissue tropisms of the common human and animal CoVs and diseases they are known to cause.

The receptors of the human pathogens, HCoV-229E, SARS-CoV, and MERS-CoV, are aminopeptidase N (also known as CD13), ACE2, and DPP4 (also known as CD26), respectively, all brush-border enzymes highly expressed on the apical surface of mature enterocytes.[51] GI involvements were frequently reported in both SARS-CoV and MERS-CoV infections. During the SARS outbreak, up to 76% of patients with SARS developed diarrhea, usually within the first week of illness.[52] Intestinal biopsy demonstrated active SARS-CoV replication within both the small and large intestines and infectious virus was isolated from intestinal tissue but not fecal specimens.[53] In 2012, during the MERS outbreak, one-quarter of patients with MERS-CoV reported GI symptoms, including diarrhea and abdominal pain, before the manifestation of respiratory symptoms[54] and active shedding of viral RNA could be detected in the stool of these patients, although no infectious virus was recovered.[55] MERS virus was shown to actively replicate in primary human intestinal enteroids and can be transmitted enterically to human DPP4 transgenic mice with replication in intestinal epithelium, enterocolitis, and subsequent spread to other organs.[56]

Frequent liver involvement has also been reported in SARS and MERS infections, mostly with mild to moderate elevations of aminotransferases (more than 2 times the upper limit of normal).[57] Viral RNA and particles have been detected in the liver of SARS patients on autopsy.[58] Overall,

A



B

Virus	Host	Respiratory	Enteric	Hepatic	Neurologic
PEDV			√		
TGEV		√	√		
FIPV		√	√	√	√
FECoV		√	√		
CCoV			√		
HCoV-229E		√			
HCoV-NL63		√			
HCoV-OC43		√	√		√
BCoV		√	√		
PHEV		√			√
MHV		√	√	√	√
HCoV-HKU1		√			
MERS-CoV		√	√	?	
SARS-CoV		√	√	?	
SARS-CoV-2		√	?	?	
IBV		√		√	
TCoV			√		
Other avian CoVs		?	?	?	?

Figure 2. Coronaviruses and their associated diseases. (*A*) Common human and animal CoVs are shown in relative genetic distance by phylogenetic analysis (not drawn to scale). Coronaviruses can be divided into 4 genera: alpha, beta, gamma, and deltacoronaviruses (division shown on the *branches* of the *phylogenetic tree*). BCoV, bovine coronavirus; CCoV, canine coronavirus; FECoV, feline enteric coronavirus; FIPV, feline infectious peritonitis virus; IBV, infectious bronchitis virus; PEDV, porcine epidemic diarrhea virus; PHEV, porcine hemagglutinating encephalomyelitis virus; TCoV, turkey coronavirus; TGEV, transmissible gastroenteritis virus. HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2 are human CoVs. Hundreds of bat CoVs (not shown on the phylogenetic tree here) have been isolated and many of them are closely related to these human and animal CoVs, suggesting that bats are the original source of these viruses. SARS-CoV has been proposed to jump from bat to civet to human, SARS-CoV-2 from bat to pangolin to human, and MERS-CoV from bat to camel to human. The main hosts and involvement of organ systems of these CoVs[100] are shown in (*B*).

it is not clear whether liver injury is indeed the result of direct viral infection, inflammation-mediated damage, or drug-induced injury.

The majority of human enteric viruses, including rotaviruses, noroviruses, and astroviruses, are characterized by a nonenveloped, naked capsid,[59] which presumably can tolerate digestive enzymes and the harsh environment of gastric fluids and bile in the duodenum. This naturally begs the question: how can enveloped CoVs survive the low pH of the stomach and withstand the detergent effect of bile salts in the small bowel? Known enteric CoVs, like transmissible gastroenteritis virus, can resist these harsh conditions by heavy glycosylation of S protein, evolving intrinsic resistance to low pH and digestive enzymes, and forming a tight complex with mucins.[60] Both SARS-CoV and MERS-CoV are intrinsically capable of enduring harsh conditions: SARS-CoV is viable for up to 2 weeks after drying and up to 5 days in room temperature and low humidity.[61] MERS-CoV appears to be similarly hardy.[62] SARS-CoV RNA was detected in sewage of hospitals treating patients with SARS and the virus remained infectious up to 2 weeks in sewage water under experimental condition.[63] At least 2 recent studies suggest that SARS-CoV-2 seems to have a remarkably similar stability in the environment.[64,65]

SARS-CoV-2: Another Enteric Coronavirus?

Similar to SARS-CoV and MERS-CoV, there is mounting evidence that SARS-CoV-2 infection also involves the GI tract. In early reports from the city of Wuhan, 2%–10% of patients with COVID-19 had GI symptoms, such as abdominal pain, diarrhea, nausea, or vomiting.[66–70] A recent meta-analysis of >4000 East Asian patients with COVID-19 described up to 20% had GI symptoms and viral RNA was detected in the stool of almost 50% of patients.[71] Notable GI symptoms had also been reported in several other studies and a meta-analysis, and can either precede or follow respiratory symptoms.[72,73] In another cohort study that included more than 200 individuals, digestive symptoms were observed in 50.5% of patients, and those with GI symptoms took longer to be discharged from the hospital.[74]

In addition to clinical symptoms, SARS-CoV-2 RNA was detected in the endoscopic specimens of the esophagus, stomach, duodenum, and rectum from several patients.[72] Substantial amounts of SARS-CoV-2 RNA have been consistently detected in stool specimens from COVID-19 patients.[71] The first reported case of COVID-19 in the United States experienced diarrhea and tested positive for viral RNA in his feces but not serum.[75] These results were subsequently confirmed by other studies. In some cases, by day 5 of admission, more anal swabs tested positive for viral RNA than oral swabs.[76] Stool samples from patients tested positive somewhere between 36% and 53% of all confirmed cases.[8,71] Prolonged presence of SARS-CoV-2 viral RNA was noted in fecal samples.[77,78] The stool specimens of many patients remained positive after a negative nasopharyngeal swab test.[79] Persistent fecal viral shedding was also observed in SARS-CoV-2–infected children.[80] Although detection of high copy numbers of viral RNA in the stool does not equate to shedding of infectious viruses or transmission of the disease, these findings raise the possibility that SARS-CoV-2 may also be an enteric virus and can be transmitted via the fecal–oral route.

Vis-à-vis direct evidence of viral infection of gut tissues, SARS-CoV-2 antigen was positively stained in the intestinal epithelium of 1 COVID-19 patient[78] and high viral loads were observed in the intestines of infected macaques.[81] In 1 study, no infectious virus was successfully isolated from the feces of COVID-19 patients,[82] but the cohort size is small and it is unclear how sensitive the cell assay system was. In a recently published ferret model, fecal shedding was seen in naïve animals in direct or indirect contact with the infected host.[83] However, respiratory transmission was not specifically blocked, making it difficult to attribute the transmission to fecal–oral route.

Evidence for direct liver involvement by SARS-CoV-2 is less clear. Recent studies on COVID-19 patients from Asia showed the presence of liver injury, indicated by elevated aminotransferases, ranged from 15%–50% of the patients.[66,67,70] But the elevations were mostly mild except for patients with more severe COVID-19,[84] who might be experiencing drug-induced or sepsis/shock-related liver injury. Limited postmortem pathology of the liver of COVID-19 patients showed moderate microvesicular steatosis and mild lobular and portal activity, which are nonspecific changes.[85] In a US-based study, serum aminotransferase levels were also not significantly altered in COVID-19 patients.[86] Further studies are necessary to define whether SARS-CoV-2 is indeed a hepato- or cholangiotropic virus that can cause direct liver injury or be secreted into the bile.

Many key questions remain before we can definitively address whether SARS-CoV-2 is an enteric virus that can be transmitted via fecal–oral route. Figure 3 illustrates the predominant respiratory route of transmission and potential fecal–oral spread of SARS-CoV-2 in human populations. Does SARS-CoV-2 actively infect human enteriods and intestinal epithelial cells in vivo? Are GI symptoms caused by viral replication in the enterocytes and its interaction with GI mucosal immunity? Are infectious viruses shed in the fecal samples and can they be recovered in a laboratory setting? If so, is the fecal viral load sufficiently high for human transmission? How long can the excreted virus persist in the environment, more specifically in the sewage system? Can fecally shedded virus infect animals that may serve as a reservoir for spread? During transmission, can gut be the first site of infection or does the virus spread to the gut from the respiratory or other tissues?

Future Research Opportunities in COVID-19

From both clinical and public health standpoints, it is critical to fully understand SARS-CoV-2's route of transmission. If high levels of infectious viruses are present in the intestinal lumen of infected patients, especially in asymptomatic patients, this poses risks to gastroenterologists,[87] endoscopy personnel, and other patients during endoscopy and colonoscopy. For the general public, infectious viral particles in the feces shed by infected people, if aerosolized, have great implications in confined environments, such as cruise ships, hospitals, individual households, and densely populated housing, especially in regions with poor sanitation.

In basic research, much work is needed to examine the full extent of GI and liver aspects of COVID-19. Primary human intestinal epithelial cells, hepatocytes, or cholangiocytes, as well as donor-derived human intestinal enteroids[88] and liver organoids, will be valuable models to study SARS-CoV-2 infectivity and replication. So far, there is 1 report of SARS-CoV-2 infection of cholangiocytes[49] and the physiological relevance is unclear. If infection is observed in gut epithelial cells, it would be of great interest to understand the apical vs basolateral polarity of infection and secretion, whether and how the epithelium barrier integrity is disrupted, and whether M cells in the gut mediate systemic spread, like other enteric viruses.[89] A genetically tractable animal model, for instance, several lines of transgenic mice that encode human ACE2[90–93] will be important to dissect the relative contribution of different transmission routes. Murine models would also help investigate the disease in vivo, such as potential intestinal and/or hepatic injury, effects on various digestive functions and

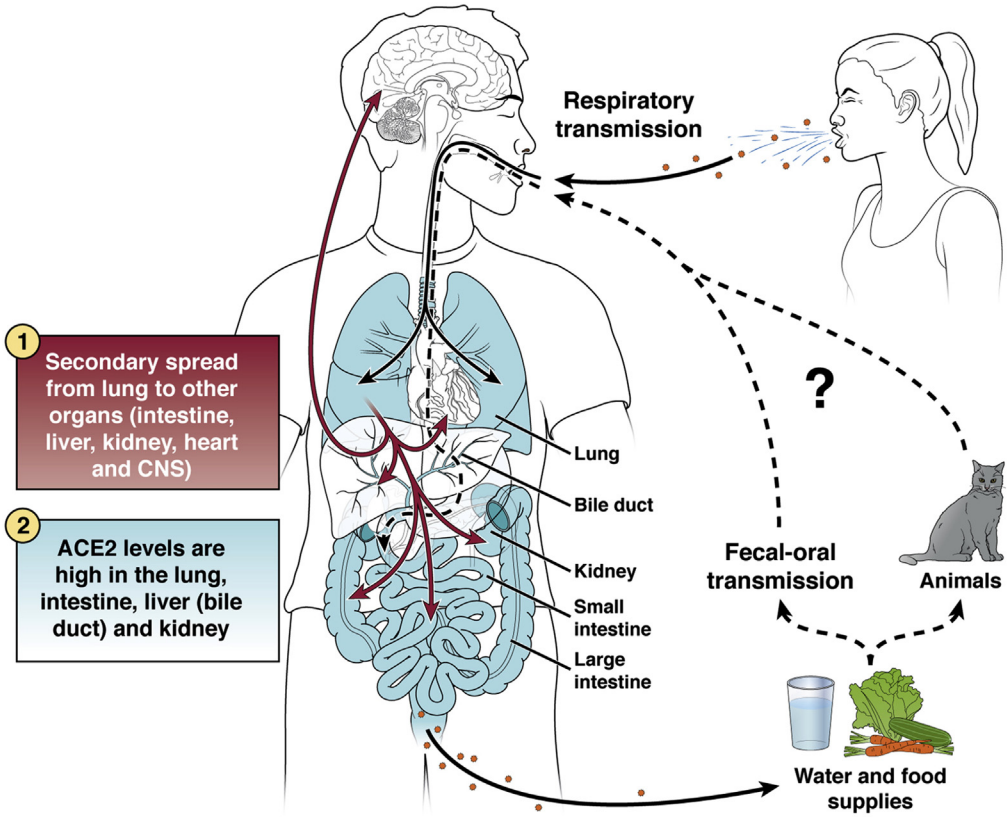


Figure 3. Modes of transmission of SARS-CoV-2 in humans. The primary mode of human-to-human transmission is airborne for SARS-CoV-2. The virus likely first infects the respiratory epithelium and spreads to the rest of the body via circulation. Other potential organs of involvement include intestine, hepatobiliary system, heart, kidney, or central nervous system, many of which express high levels of ACE2, the main receptor for viral entry. Whether the virus can directly infect the intestine bypassing the respiratory system is unknown. Either way, the virus may infect, replicate, and shed from the enterocytes and possibly hepatocytes/cholangiocytes and be excreted as fecal materials into the environment, contaminating water and food supplies. Whether the virus can be transmitted directly to other humans via fecal–oral route or infect household pets, like cats, or wildlife first before passing to humans remain key questions.

interactions with gut and systemic immunity. Other larger animal models, such as ferrets,[83,94] cats,[95] and macaques,[81,83,95] all recently shown to be infectible by SARS-CoV-2 with similar disease, will also be valuable models to study the biology of infection and testing of vaccine and drug therapies. Finally, host innate immunity to SARS-CoV-2, including interferon signaling[96] and the inflammasome-related cytokines,[97] in particular those pathways at the mucosal surfaces,[98,99] the short- and long-term adaptive immune responses against this virus, and how these antiviral activities vary in children, adults and the elderly, are largely unknown and fertile for future research.

Conclusions

As COVID-19 continues to have a devastating impact on countless people's lives, we do not know with certainty what the future holds. Whether the virus will persist in human populations with recurrent bouts of outbreaks, like influenza or other emerging infections; attenuate after accumulating mutations to the likes of HCoV-OC43 and HCoV-229E common cold CoVs; or disappear after the primary outbreak, like SARS-CoV, remain vital questions for the coming year. Regardless of the answers, it is indisputable that future pandemic like this one will likely occur again. We are at the crossroads of science, medicine, and societal policies; our actions and commitments will profoundly shape the future of our world. For now, lessons learned from studying this virus, its infection modes, pathogenesis, and disease manifestations will not only be invaluable for developing effective vaccines and therapies against this emerging disease, but will also better prepare our world for future pandemics.

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Author names in bold designate shared co-first authorship.

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Correspondence
Address correspondence to: T. Jake Liang, MD, Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bldg10-9B16, 10 Center Drive, Bethesda, MD. e-mail: jliang@nih.gov; and Siyuan Ding, PhD, Department of Molecular Microbiology, Washington University in St Louis, Campus Box 8230, 660 S. Euclid Avenue, St Louis, MO 63110. e-mail: siyuan.ding@wustl.edu.

CRediT Authorship Contributions
Siyuan Ding, PhD (Writing – original draft: Equal; Writing – review & editing: Equal). T. Jake Liang, M.D. (Writing – original draft: Equal; Writing – review & editing: Equal).

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