


REVIEW

Potential intestinal infection and faecal-oral transmission of human coronaviruses

Tingting Ning¹ | Si Liu¹ | Junxuan Xu¹ | Yi Yang¹ | Nan Zhang¹ | Sian Xie¹ | Li Min¹ | Shutian Zhang¹ | Shengtao Zhu¹ | Youchun Wang² 

¹Department of Gastroenterology, Beijing Friendship Hospital, Capital Medical University, National Clinical Research Center for Digestive Disease, Beijing Digestive Disease Center, Beijing Key Laboratory for Precancerous Lesion of Digestive Disease, Beijing, China

²Division of HIV/AIDS and Sexually Transmitted Virus Vaccines, National Institutes for Food and Drug Control (NIFDC), Beijing, China

Correspondence

Shengtao Zhu, Beijing Friendship Hospital, Capital Medical University, Beijing, 100050, China.

Email: zhushengtao@ccmu.edu.cn

Youchun Wang, Division of HIV/AIDS and Sexually Transmitted Virus Vaccines, National Institutes for Food and Drug Control, Beijing, 102629, China.

Email: wangyc@nifdc.org.cn

Funding information

National Natural Science Foundation of China; Beijing Friendship Hospital; Capital Medical University

Abstract

Human coronaviruses (HCoVs) were first described in 1960s for patients experiencing common cold. Since then, increasing number of HCoVs have been discovered, including those causing severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and the circulating coronavirus disease 2019 (COVID-19), which can cause fatal respiratory disease in humans on infection. HCoVs are believed to spread mainly through respiratory droplets and close contact. However, studies have shown that a large proportion of patients with HCoV infection develop gastrointestinal (GI) symptoms, and many patients with confirmed HCoV infection have shown detectable viral RNA in their faecal samples. Furthermore, multiple *in vitro* and *in vivo* animal studies have provided direct evidence of intestinal HCoV infection. These data highlight the nature of HCoV GI infection and its potential faecal-oral transmission. Here, we summarise the current findings on GI manifestations of HCoVs. We also discuss how HCoV GI infection might occur and the current evidence to establish the occurrence of faecal-oral transmission.

KEYWORDS

faecal-oral transmission, human coronaviruses, intestinal infection, MERS-CoV, SARS-CoV, SARS-CoV-2

1 | INTRODUCTION

According to the International Committee on Taxonomy of Viruses, coronaviruses (CoVs) are classified under the order *Nidovirales*, family *Coronaviridae*, and subfamily *Orthocoronavirinae* (Figure 1). They are a group of enveloped viruses with non-segmented, single-stranded, and positive-sense RNA genomes.¹ Apart from infecting vertebrates (such as pigs and chickens), seven CoVs can infect human hosts and are called human CoVs (human coronaviruses (HCoVs)).² Severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV), Middle East

respiratory syndrome (MERS) coronavirus (MERS-CoV), and SARS coronavirus 2 (SARS-CoV-2) are highly pathogenic (HP) HCoVs, which have caused regional and global outbreaks.² HCoV-229 E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1 are common HCoVs that usually cause mild illness.²

Although HCoVs primarily manifest as respiratory infections, airway exposure is intuitively assumed to be the infection route. However, epidemiological studies, biological evaluation of the viruses, and bioinformatic prediction collectively suggest that humans might also acquire HCoV infection via the gastrointestinal (GI) tract.

Abbreviations: ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019; CoVs, coronaviruses; CPE, cytopathic effect; dpi, days post infection; DPP4, dipeptidyl peptidase four; FaSGF, fasting-state gastric fluid; FaSIF, fasting-state intestinal fluid; FeSGF, fed-state gastric fluid; FeSIF, fed-state intestinal fluid; GI, gastrointestinal; hACE2, human ACE2; hAPN, human aminopeptidase N; HCoVs, human coronaviruses; HP, highly pathogenic; MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sgRNA, subgenomic RNA.

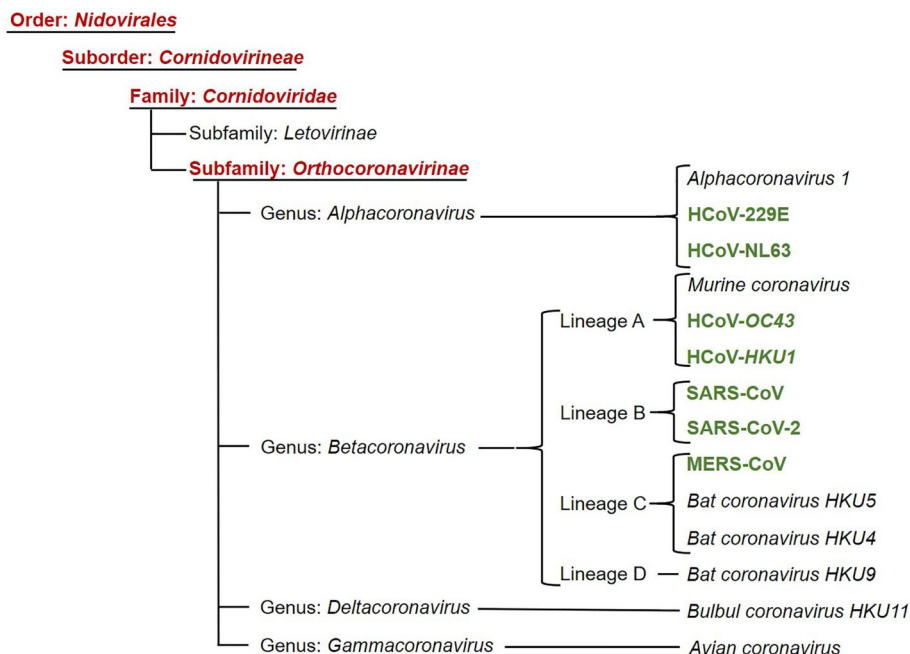


FIGURE 1 Taxonomy of human coronaviruses (HCoVs): the updated classification scheme of HCoVs and other coronaviruses. The seven known HCoVs are in green. Abbreviation: MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV, Severe acute respiratory syndrome coronavirus

In this review, we revisit the GI symptoms and shedding of the virus into the faeces of patients infected with HCoVs, supporting the possibility of faecal-oral transmission.

2 | GASTROINTESTINAL SYMPTOMS OF HUMAN CORONAVIRUSES

2.1 | SARS-CoV

In the SARS outbreak in 2002-03, patients with SARS frequently experienced GI symptoms. Diarrhoea was the most common GI symptom; occurring in 10.6%–73% of the patients with SARS³⁻¹⁴ (Table 1). The significant difference in the prevalence of diarrhoea has been postulated to be partly linked to the different modes of transmission.⁷ Among the first 138 patients with SARS in Hong Kong, 28 (20.3%) presented with watery diarrhoea, and up to 38.4% of the patients had symptoms of diarrhoea during the course of illness.⁷ In the clustered 75 cases of Amoy Gardens in Hong Kong, although only 1% of the patients had diarrhoea on admission, up to 73% of the patients eventually developed watery diarrhoea.⁶ The first 138 patients are believed to be infected by droplet transmission, whereas the community outbreak has been linked to the faulty sewage system in an apartment complex in which faecal-oral transmission might be a major route of transmission.⁷ The great variation in the prevalence of diarrhoea might also be attributed to differences in classifying the symptoms as diarrhoea and whether diarrhoea is reported on admission or during hospitalisation.^{4,6,7} Moreover, patients might receive different medications for SARS, such as lopinavir or antibiotics, some of which are likely to induce diarrhoea as an adverse event.⁷

Although diarrhoea on presentation is not associated with oxygen requirement and overall mortality of these patients, patients with diarrhoea have higher rates of admission to the intensive care unit and intubation.⁷ Further studies are necessary to determine whether the intestinal viral load is correlated with GI symptoms and, particularly, the clinical outcomes of these patients. Notably, GI symptoms may indicate the onset of infection in some patients with SARS. Among the first 138 patients in Hong Kong, 5.8% presented with fever and diarrhoea only before the onset of respiratory symptoms.⁷ Similarly, according to a single-centre case series study ($n = 267$), several patients have presented with high fever and frequent watery diarrhoea, with minimal cough and completely normal chest radiograph.¹²

2.2 | MERS-CoV

MERS-CoV was first reported in September 2012 in samples from a Saudi Arabian businessman who died from acute respiratory and renal failure.¹⁵ In the initial MERS-CoV outbreak in 2012, a quarter of patients with MERS-CoV reported GI symptoms such as diarrhoea or abdominal pain at presentation.¹⁶ Subsequent cohorts have consistently reported GI symptoms among patients¹⁷⁻²² (Table 1). The number of MERS-CoV-infected cases reported in children is low. In the case series of seven children, vomiting (28%) and diarrhoea (28%) have been reported as the most common GI symptoms.²²

Similarly, GI symptoms might serve as the indicator of onset in some patients with MERS.^{18,23} Patients with MERS have presented with diarrhoea, without any respiratory symptoms.^{19,21}

TABLE 1 Comparison of Severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-2 and common human coronaviruses (HCoVs) infection

Characteristics	Highly pathogenic HCoVs			Common HCoVs			
	SARS-CoV ^{6,11,14,120,121}	MERS-CoV ^{16,22,60,122-124}	SARS-CoV-2 ^{24,37,65,125-127}	HCoV-229E ^{40,128}	HCoV-OC43 ^{41,129}	HCoV-NL63 ^{42,130}	HCoV-HKU1 ^{43,131}
Epidemiology							
Location of origin	Guangdong, China	Saudi Arabia	NA	America	America	Netherlands	Hong Kong, China
Year	2002-2003	2012	2019-Present	1966	1967	2004	2005
Confirmed cases	8422	2494	183,198,019	NA	NA	NA	NA
Deaths	916	858	3,971,687	NA	NA	NA	NA
Mortality (%)	10.8	34.4	2.2%	NA	NA	NA	NA
Incubation period (days)	1-14, a mean of 4-6	2-14, 12, a mean of 5-8	2-14, a mean of 5	2-5, a mean of 3	2-5	2-4	2-4
Receptor	ACE2	DPP4	ACE2	hAPN	9-O-Ac-sia	ACE2	9-O-Ac-sia
Gastrointestinal characteristics							
Diarrhoea (%)	10.6-73.0	5.9%-28%	3.8-37.8	Vomiting:80%	Vomiting:5.7%	Gastrointestinal symptoms:33.3%	NA
Nausea and/or vomiting (%)	10.0-29.5	5.9-21.3	5.0-22.3				
Percentage of patients with positive faecal samples	100% on 12-14 days	14.6%	53.4%	0.2%-0.4%	NA	NA	NA

Abbreviation: NA, not applicable.

2.3 | SARS-CoV-2

Some patients with coronavirus disease 2019 (COVID-19) have reported GI symptoms during disease onset and subsequent hospitalisation. However, the overall proportion of GI symptoms reported were lower than that reported for SARS-CoV and MERS-CoV infections.²⁴

The first case of COVID-19 presented with nausea and vomiting upon hospital admission, followed by diarrhoea and abdominal discomfort.²⁵ Subsequent cohorts have consistently reported GI symptoms among patients with COVID-19²⁶⁻³³ (Table 1). The occurrence of GI symptoms can not only coexist but also precede the typical phenotype of COVID-19. A man with a 4-day history of diarrhoea, without typical symptoms of COVID-19, subsequently tested positive.³⁴ Similarly, among 138 patients with COVID-19, 14 first experienced diarrhoea and nausea, preceding fever.³⁵ Of note, GI symptoms can be the only presentation of SARS-CoV-2 infection.³⁶ Among the nine patients with only GI symptoms at admission, four had no respiratory symptoms or fever during hospitalisation.

A large study that collected data from 1099 patients from 552 hospitals in China reported nausea or vomiting in 5.0% and diarrhoea in 3.8% of patients.³⁷ In a cohort of 140 patients with COVID-19 in Wuhan, GI symptoms, including nausea (17.3%), diarrhoea (12.9%), and vomiting (5.0%) has been reported in up to 39.6% of the patients.²⁴ The incidence of GI symptoms in patients with COVID-19 in the Wuhan area has been found to be significantly higher than the overall incidence of GI symptoms in patients with COVID-19 across China, which may be related to the virulence of SARS-CoV-2.

Similar to adults, GI symptoms have been observed in a cohort of 171 paediatric patients with COVID-19.³⁸ Diarrhoea and vomiting have been observed in 8.8% and 6.4% children, respectively. Although different clinical features, such as a milder disease course³⁸ and fewer respiratory symptoms,³⁹ have been reported in children with COVID-19, GI symptoms appear to be similar. However, more clinical data are needed for further confirmation.

2.4 | Common HCoVs

Similar to the aforementioned HP-HCoVs, common circulating HCoVs can also cause GI symptoms (Table 1). HCoV-229E has caused vomiting in up to 80% of infected patients.⁴⁰ Of the 35 samples that tested positive for HCoV-OC43, two were associated with vomiting.⁴¹ Similarly, digestive problems have been noted in approximately one-third of patients infected with HCoV-NL63.⁴² HKU-1 is also commonly associated with intestinal illness.⁴³ It is also suggested that the frequency of GI symptoms does not differ between patients with OC43, NL63, and HKU-1.⁴⁴ GI symptoms have also been observed in children with common HCoVs and are more common in children than that in adults.⁴⁵

3 | EVIDENCE OF INTESTINAL INFECTION

3.1 | Intestinal expression of HCoVs receptors

3.1.1 | SARS-CoV and SARS-CoV-2

Entry of SARS-CoV and SARS-CoV-2 into the cells is dependent on the cellular surface protein angiotensin-converting enzyme 2 (ACE2). ACE2 is abundantly expressed in the brush border of enterocytes in all parts of the small intestine,^{46,47} which is consistent with previous findings that ACE2 mRNA is highly expressed in the GI tract.⁴⁸ The expression patterns of ACE2 indicate the potential for SARS-CoV and SARS-CoV-2 intestinal infections.

3.1.2 | MERS-CoV

MERS-CoV uses dipeptidyl peptidase-4 (DPP4) as its receptor.⁴⁹ The human small intestine expresses the highest level of DPP4 mRNA and protein among all human organs, including the lungs and bronchi.⁵⁰ The abundantly expressed DPP4 in the human intestine may account for the high susceptibility of these cells to MERS-CoV.

3.1.3 | Common HCoVs

HCoV-NL63 also uses ACE2 as its receptor.⁵¹ The expression patterns of ACE2 also indicate the potential of HCoV-NL63 intestinal infection. Human aminopeptidase N (hAPN) mediates the entry of HCoV-229E and is widely expressed in the intestinal epithelium, particularly in the apical regions.⁵² HCoV-OC43 and HCoV-HKU1 employ sialoglycan-based receptors with 9-O-acetylated sialic acid (9-O-Ac-Sia) as a key component, and 9-O-Ac-Sia is selectively expressed on the luminal surface of the intestinal epithelium.⁵³ In summary, the expression patterns of these receptors indicate the potential for common HCoV intestinal infections.

3.2 | Detection and isolation of HCoVs from faeces

3.2.1 | SARS-CoV

Viral presence in faeces is an important finding because it suggests the possibility of faecal-oral transmission. Multiple studies have reported the positive detection of viral RNA in faecal samples from patients with SARS-CoV.^{6,7,10,54} A retrospective cohort study indicated that viral RNA could be detected in the faeces of 28% of the patients.⁵⁵ Prolonged faecal shedding of viral RNA is common, and faecal samples remain positive even after the respiratory and/or sputum samples exhibit no detectable virus.⁵⁶ Viral RNA has been detected in a patient's faeces 73 days after symptom onset.⁷ Moreover, in certain cases, the viral load of the faecal specimens has been

found to be higher than that of nasopharyngeal aspiration specimens.⁵⁷ Notably, viable SARS-CoV has been isolated from faeces,⁵⁸ suggesting the possibility of GI tract infection and possible faecal-oral transmission.

3.2.2 | MERS-CoV

MERS-CoV has also been detected in the faeces of patients with MERS.⁵⁹ However, only 14.6% of faecal samples yielded viral RNA, and the duration of viral shedding in faecal samples has never been reported.⁶⁰ Interestingly, subgenomic RNA of the N gene, an intermediate in the replication cycle of MERS-CoV, has been detected in faecal samples,⁶⁰ which implies that the virus was probably replicated in the GI tract of the patient with MERS.

3.2.3 | SARS-CoV-2

Several studies have demonstrated the presence of viral RNA in faeces or anal/rectal swabs of patients with COVID-19.^{25,61–64} Rather, the faeces remained positive in 23% of patients even after respiratory specimens tested negative for viral RNA.⁶⁵ In some cases, the viral load in faeces was higher than that in pharyngeal swabs. Persistent faecal viral shedding is also prominent in paediatric patients.⁶² The presence and persistence of such amounts of viral RNA in faeces is unlikely to be explained by only the swallowing of virus particles replicated in the throat, but rather suggests the potential for enteric SARS-CoV-2 infection.

Given that extrapulmonary detection of viral RNA does not mean infectious virus is present, further positive viral culture suggests the possibility of GI tract infection and possible transmission. More recently, the isolation of infectious SARS-CoV-2 viruses from faecal samples of patients with COVID-19^{66–68} confirmed the release of infectious virions into the GI tract and directly proved that SARS-CoV-2 could spread via faeces.

3.2.4 | Common HCoVs

The viral RNA of all common HCoVs has been detected in faeces. HCoV-43 and HCoV-HKU1 have been found to be more abundant than HCoV-NL63 and HCoV-229E.^{26,27} These results suggest the possibility of GI tract infections in common HCoVs.

3.3 | Gastrointestinal pathological findings in patients with HCoV infections

3.3.1 | SARS-CoV

Recently, many groups have reported the enteric involvement of SARS infection. To et al. reported that positive cytoplasmic signals

of SARS-CoV had been detected in the surface enterocytes of the small intestine obtained from autopsies of fatal cases of SARS, demonstrating SARS-CoV tropism towards the small intestine.²⁸ Active SARS-CoV replication in the enterocytes of the small and large intestine from colonoscopy biopsy and autopsy has been reported by Leung et al.⁷ Small intestine biopsy specimens obtained from post-mortem examination, which is usually performed within a few days after the death of patients, still yielded viable SARS-CoV. Moreover, the culture yield from the small intestine has been found to be even higher than that from lung tissues, which is generally believed to be the primary target organ of this virus.⁷ In a study by Shi et al.,²⁹ scattered epithelial cells in the small and large intestines have been infected with SARS-CoV. Moreover, the viral particles are present in the dilated endoplasmic reticulum of the mucosal epithelial cells but not on the surface of the microvilli of superficial enterocytes, as reported by Leung et al.⁷ This might be attributed to better preservation of samples from biopsy than that from autopsy.

3.3.2 | MERS-CoV

Timely biopsy and autopsy studies on MERS victims were not performed, which limited the knowledge about the pathogenesis of MERS-CoV. In this scenario, it is difficult to determine whether there is enterocyte damage in the intestines of patients with MERS.

3.3.3 | SARS-CoV-2

The first autopsy report was of an 85-year-old man with COVID-19,³⁰ who showed segmental dilatation and stenosis of the small intestine. Similarly, histological examination of another patient who died of severe COVID-19 has shown degeneration, necrosis, and shedding of the GI mucosa at varying degrees.³¹

Further, a GI endoscopy and biopsy report of a 78-year-old patient with COVID-19 in Guangdong Province, China, has shown symptoms of GI bleeding.⁶⁵ The GI tract contained numerous infiltrating plasma cells and lymphocytes with interstitial oedema. Besides staining of the viral N in the cytoplasm of GI epithelial cells demonstrates that SARS-CoV-2 could infect these glandular epithelial cells.⁶⁵ In summary, although further histological assessments might be needed to determine enterocyte damage in the intestine, the endoscopic and histological examination in patients with COVID-19 provides direct evidence of active SARS-CoV-2 replication in the intestine.

3.3.4 | Common HCoVs

Studies including the pathological analysis of GI tissues are needed to determine enterocyte damage among patients infected with these common HCoVs.

4 | INTESTINAL INFECTION

4.1 | *In vitro* models for HCoV intestinal infection

4.1.1 | SARS-CoV

Several studies based on human cell lines have confirmed that SARS-CoV can infect intestinal cells *in vitro*. SARS-CoV has been reported to infect colon carcinoma-derived lines Caco-2 and CL14.³² The virus can grow and produce cytopathic effect (CPE) in Caco-2 cells.³³ Further studies are needed to determine whether enteroids are susceptible to SARS-CoV infection.

4.1.2 | MERS-CoV

MERS-CoV has been reported to infect the primary enterocytes. All the inoculated enterocytes have shown to highly express viral N, undergo significant membrane fusion, and form syncytia, with greatly increasing viral load.²⁰ The normal human small intestine is also susceptible to MERS-CoV and supports viral replication. In the infected intestine, N-positive enterocytes explicitly revealed that the infected enterocytes form syncytia, similar to those in the primary cells. Notably, although only patchy areas of the epithelium are infected, an increased viral load has been observed.²⁰ In summary, both human primary intestinal epithelial cells and the small intestine can be infected by MERS-CoV and support viral replication. Human enteroids are also highly susceptible to MERS-CoV and support robust viral replication. Normalised viral loads exhibit a constant increase in the infected enteroids, which is consistent with productive MERS-CoV infection in enteroids, as evidenced by the strong signal of viral N in the virus-inoculated enteroids. In addition, infected enteroids develop progressive CPE over time.²⁰ Notably, MERS-CoV replicates more robustly in human enteroids than that in primary epithelial cells and *ex vivo* human tissues.²⁰ In summary, these results strongly suggest that MERS-CoV can enter and replicate in the intestinal epithelial cells.

4.1.3 | SARS-CoV-2

Many studies based on human cell lines or organoids have confirmed that SARS-CoV-2 can infect the intestinal cells *in vitro*.⁶⁸⁻⁷⁰ The human colon carcinoma-derived cell line, Caco-2, could produce much higher amounts of infectious SARS-CoV-2 than that produced by the human lung adenocarcinoma cell line Calu-3.⁶⁹ In addition, SARS-CoV-2 readily infects human enteroids,^{71,72} which then release mature viral particles from the basolateral and apical cells of the lumen.⁷² The robust SARS-CoV-2 replication in human enteroids suggests that the human intestinal tract may be a transmission route for SARS-CoV-2. Additionally, bat enteroids, which are susceptible to human SARS-CoV-2 infection and sustain robust viral replication, have been cultured to explore SARS-CoV-2 replication in the bat

gut.⁶⁸ Moreover, bat enteroids might enable virus isolation with higher efficiency than that by Vero E6 cells, which is commonly used for virus isolation.⁶⁸ Thus, bat intestinal organoids can also be used for mechanistic studies of SARS-CoV-2 intestinal infection. In summary, these *in vitro* studies confirm that SARS-CoV-2 can enter and replicate in the intestinal epithelial cells.

4.1.4 | Common HCoVs

Several studies based on human cell lines have confirmed that common HCoVs can infect intestinal cells *in vitro*. HCoV-OC43 has been reported to infect the human intestinal cell line, HRT18,⁷³ while HCoV-NL63 can infect Caco-2 cells.⁷⁴ Whether common HCoVs can infect enteroids requires further studies.

4.2 | Animal models for HCoV intestinal infection

4.2.1 | SARS-CoV

The development of animal models to study HCoV biology and pathogenesis is of interest to the scientific community, particularly if the models appropriately mimic human infection.

Several inbred mouse strains have been evaluated as models of SARS-CoV infection.⁷⁵⁻⁷⁸ Although mice show evidence of infection and lung disease, inbred mouse strains do not accurately reproduce diffuse alveolar damage, oedema, pneumocyte necrosis, and hyaline membrane formation observed in humans.^{79,80} Transgenic mice expressing human ACE2 (hACE2) have been explored to mimic mild SARS-CoV infection. Upon intranasal inoculation of SARS-CoV in hACE2 transgenic mice, the mucosal layers of the GI tract show signs of oedema, small vessel dilation, and lymphocyte infiltration. Moreover, in the small intestine, some epithelial cells appear desquamative, and some lymph nodes show severe haemorrhage and necrosis.⁸¹

In addition to mouse models, evidence from several other animal models supports intestinal viral infection. SARS-CoV has been reported to infect masked palm civets. Viral RNA has been detected in the small intestine of virus-inoculated civets, and the positive signal is mainly localised to macrophages. Moreover, mild focal haemorrhages have been observed in the lamina propria of the small intestine.⁸² Furthermore, in the ferret models, intranasal infection with SARS-CoV shows intestinal infection, and viral RNA is detectable in the GI tract.⁸³ In addition, a few enterocytes in the ileum express SARS-CoV N.⁸⁴

SARS-CoV has been shown to infect non-human primates, providing the most genetically relevant infection model to mimic human infections. Upon intranasal inoculation, the viral genome has been detected in the faecal samples of all rhesus macaques.⁸⁵ Similarly, positive signals for N have been detected in the small intestines of all the four rhesus macaques infected with SARS-CoV via intranasal inoculation of SARS-CoV.⁸⁶

4.2.2 | MERS-CoV

In contrast to SARS-CoV, mice are not naturally susceptible to infection by MERS-CoV because the mouse DPP4 receptor differs from the human counterpart in crucial regions of interaction with the MERS-CoV S protein.⁸⁷

MERS-CoV intestinal infection has been suggested in an earlier study of intranasally inoculated human DPP4 mice,⁸⁸ the mouse intestines show an increasing viral load after MERS-CoV inoculation. Direct intragastric MERS-CoV inoculation initiates an infection in the intestinal mucosa, leading to progressive inflammation and epithelial degeneration. With the progression of intestinal MERS-CoV infection, a sequential respiratory infection occurs.²⁰ Attempts to experimentally infect hamsters and ferrets with MERS-CoV have not been successful.^{89,90} Thus, although MERS-CoV has been shown to infect non-human primates,^{91,92} GI pathology remains unaddressed.

4.2.3 | SARS-CoV-2

Because SARS-CoV-2 cannot infect wild-type mice, many efforts have been made to establish suitable animal models for mimicking specific aspects of SARS-CoV-2 infection in humans.⁹³⁻⁹⁶

Transgenic mice expressing hACE2 have been explored to mimic mild SARS-CoV-2 infection.⁹⁵ However, upon intranasal inoculation of SARS-CoV-2 in hACE2 transgenic mice, viral RNA is only transiently detected on the first day post infection (dpi), and no histopathological lesions of SARS-CoV-2 have been observed. The failure to establish intestinal infection in this model could be related to suboptimal viral replication in transgenic mice.⁹⁵

Knock-in mice expressing hACE2 have been designed as a more relevant infection model than transgenic mice.⁹³ Notably, aged hACE2 mice had high levels of viral RNA in the faeces.⁹³ Importantly, intragastric administration of SARS-CoV-2 could cause productive infection in the respiratory tracts of hACE2 mice, as demonstrated by the presence of high levels of viral RNAs and active viral protein expression.⁹³ This result also highlights the possibility of faecal-oral transmission of SARS-CoV-2.

In addition to mouse models, evidence from several other animal models also supports intestinal viral infection of SARS-CoV-2.⁹⁴ When naive ferrets are inoculated with faecal supernatants of infected specimens, infectious SARS-CoV-2 has been isolated from subsequent nasal washes, thereby providing direct evidence of faecal-oral transmission of SARS-CoV-2 in ferrets.⁹⁴ Furthermore, although diarrhoea is not clinically evident in challenged hamsters intranasally infected with SARS-CoV-2, viral RNA has been continuously detectable in the faecal samples of infected hamsters for 14 days and viral N has been detected in the enterocytes.⁹⁶ Severe enterocyte necrosis, damaged and deformed intestinal villi, and increased lamina propria mononuclear cell infiltration have been observed, thereby providing direct evidence for intestinal infection of SARS-CoV-2 in hamsters.⁹⁶

SARS-CoV-2 has been shown to infect non-human primates, such as rhesus macaques.^{97,98} Upon intranasal inoculation of SARS-CoV-2 to seven rhesus macaques, viral RNA-positive anal swabs are observed in all infected monkeys.⁹⁸ Virus-positive cells and inflammatory cell infiltration in the intestines of these animals has been observed. Similarly, on infection of rhesus macaques with SARS-CoV-2 via a combination of intranasal, intratracheal, oral, and ocular inoculation, two of the eight primates have shown viral RNA-positive anal swabs.⁹⁷ Small numbers of antigen-positive lymphocytes and macrophages were detected in the lamina propria of the intestinal tract of all four macaques. Notably, viral mRNA, which indicates active viral replication, could be detected in the GI tissue of a rhesus macaque. Similar to clinical studies in patients, prolonged rectal shedding of viral RNA has also been observed in rhesus macaques.⁹⁷ Notably, both intranasal and intragastric inoculation cause histopathological damage in the GI tract, including infiltration of inflammatory cells and exfoliation of the mucosal epithelium. Moreover, a decrease in Ki67 and the number of mucin-containing goblet cells and an increase in cleaved caspase 3, suggest impairment of the GI barrier due to inflammation induced by SARS-CoV-2 inoculation, causing severe infection.⁹⁹

Additionally, viral RNA has been found in the anal swabs of SARS-CoV-2-infected cats and dogs.¹⁰⁰ In summary, data from many animal models confirm the intestinal infection of SARS-CoV-2 and the potential for faecal-oral transmission.

4.2.4 | Common HCoVs

Transgenic mice expressing hAPN have been used to mimic HCoV-229E infection. Upon infection, large amounts of HCoV-229E have been found in the gut. In addition, the small intestine shows haemorrhagic areas along with coronavirus particles, which can be detected using electronic microscope.¹⁰¹ Attempts to experimentally infect animal models with other common HCoVs have not been successful.

5 | EVIDENCE OF FAECAL-ORAL TRANSMISSION

Three key issues should be addressed to determine whether HCoVs can establish faecal-oral transmission. First, whether HCoVs can tolerate gastric acid exposure to subsequently establish an intestinal infection. Second, whether infectious virus particles can tolerate intestinal fluid, which can then be shed through faeces. Finally, whether the virus particles outside the host are of sufficient concentration and infectivity for subsequent transmission needs to be determined.¹⁰²

5.1 | Can HCoVs tolerate gastric acid and survive passage into the gut?

The stomach environment varies over the course of the gastric residence of a meal, which might affect the tolerance of pathogenic

viruses in the gastric fluid.¹⁰³ Based on human clinical studies, fed-state gastric fluid (FeSGF) has a hyperosmolar content and a higher pH than that of fasting-state gastric fluid (FaSGF).¹⁰³ *In vitro*, FaSGF is often a salt solution, containing sodium taurocholate, lecithin, and pepsin, at a pH of 1.6, whereas the FeSGF is a pH 5.0, milk-based medium, to simulate the carbohydrate-to-protein-to-fat ratio observed in the stomach after the consumption of meals.¹⁰⁴ HP-HCoVs and common HCoVs are less tolerant to the high acidity of FaSGF; however, to some extent, they can resist the digestive enzymes in FeSGF, which suggests that eating might facilitate the invasion of these viruses.^{20,105}

5.2 | Can infectious HCoVs be shed in faeces?

Viral shedding through faeces is another essential characteristic of faecal-oral transmission. A study on human duodenal enteroids has found that SARS-CoV-2 is released predominantly from the apical side into the lumen, suggesting the possibility of viral shedding and accumulation in the faeces of patients with COVID-19.

Different coronaviruses have varying tolerance to small intestinal fluids. SARS-CoV and SARS-CoV-2 can retain viability and infectivity in fasting-state intestinal fluid (FaSIF); however, they poorly tolerate fed-state intestinal fluid (FeSIF).¹⁰⁵ However, MERS-CoV maintains some viability in the presence of FeSIF.²⁰ The matrix and N of MERS-CoV can form relatively hard inner and outer shells, increasing virus stability in the environment,¹⁰⁶ which may partly explain the increased tolerance of MERS-CoV to the small intestine fluid compared to that of SARS-CoV or SARS-CoV-2. Similar to SARS-CoV and SARS-CoV-2, HCoV-229E well tolerates FaSIF and poorly tolerates FeSIF.²⁰ Thus, HCoVs could plausibly remain infectious in the faeces, especially when the patient has diarrhoea symptoms.

Consistent with these findings, several reports have shown the successful isolation of HCoVs from human faeces.^{107,108} For example, live SARS-CoV-2 has been found in the faecal samples from two patients with COVID-19.¹⁰⁷ The isolation of infectious viruses from the patient's faeces suggests SARS-CoV-2 enteric infection.

5.3 | Can HCoVs maintain infectivity outside the host?

5.3.1 | SARS-CoV

These data confirm that SARS-CoV is viable under environmental conditions that could facilitate faecal-oral transmission. SARS-CoV RNA has been found in the sewage water of hospitals.¹⁰⁹ Although studies on isolating infectious viruses in sewage are lacking, the ability to maintain infectivity in liquids makes it possible for SARS-CoV to be transmitted through sewage. SARS-CoV can survive for up to 2 weeks after drying, remaining viable for up to 5 days at temperatures of 22–25°C and 40%–50% relative humidity.¹¹⁰ In

addition, at 20 and 4°C, SARS-CoV can persist in faeces for 3 and 17 days, respectively.¹¹¹

5.3.2 | MERS-CoV

MERS-CoV remains stable at low temperature and low humidity, and can be recovered after exposure to the environment for 48 h, thus supporting fomite transmission.¹¹² Accordingly, viral RNA can be extensively detected in the environmental surfaces of patients with MERS.¹¹³

5.3.3 | SARS-CoV-2

The possibility of faecal-oral transmission of SARS-CoV-2 has implications, particularly in areas with poor sanitation.¹¹⁴ Chan et al. demonstrated that SARS-CoV-2 could survive in faeces for up to one to 2 days.¹¹⁵ A recent environmental study suggested that SARS-CoV-2 could remain viable in aerosols for hours and could remain stable on plastic and stainless steel for at least 72 h.¹¹⁶ Thus, transmission via the faecal-oral route is theoretically possible, particularly in individuals with reduced gastric acidity due to medications such as proton pump inhibitors.

5.3.4 | Common HCoVs

HCoV-NL63 suspensions diluted with phosphate buffered saline and stored for up to 7 days at room temperature remained infective.¹¹⁷ Similar to HCoV-NL63, both HCoV-229E and HCoV-OC43 can survive for several days in suspensions.¹¹⁸ A study with HCoV-229E has demonstrated a 99.9% die-off of 10 days in tap water at 23°C and over 100 days at 4°C.¹¹⁹ The ability to maintain infectivity in liquids makes it possible for common HCoVs to be transmitted through sewage.

6 | CONCLUSIONS

The current study provides strong evidence for intestinal infection of HCoVs. However, further evidence is needed to determine the mechanism of intestinal infection and the possibility of faecal-oral transmission of HCoVs.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the discussion of the content. TTN wrote the article. STZ and YCW reviewed and edited the manuscript before submission.

ACKNOWLEDGEMENTS

This work was supported by the Young Scientists Fund of the National Natural Science Foundation of China (grant number

8210030093), the Research Foundation of Beijing Friendship Hospital, Capital Medical University (grant number yzzz202030), and Natural Science Foundation of Capital Medical University (grant number PYZ20142). We wish to express our appreciation to Mengran Zhao for her kind assistance in English editing of the manuscript.

CONFLICT OF INTEREST

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

The data support the findings of this study, which are openly available in the cited references.

ORCID

Youchun Wang  <https://orcid.org/0000-0001-9769-5141>

REFERENCES

- Weiss SR, Navas-Martin S. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. *MMBR (Microbiol Mol Biol Rev)*. 2005;69(4):635-664. <https://doi.org/10.1128/MMBR.69.4.635-664.2005>
- Su S, Wong G, Shi W, et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. *Trends Microbiol*. 2016;24(6):490-502. <https://doi.org/10.1016/j.tim.2016.03.003>
- Booth CM, Matukas LM, Tomlinson GA, et al. Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area. *Jama*. 2003;289(21):2801-2809. <https://doi.org/10.1001/jama.289.21.JOC30885>
- Lee N, Hui D, Wu A, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. *N. Engl J Med*. 2003;348(20):1986-1994. <https://doi.org/10.1056/NEJMoa030685>
- Hsu LY, Lee CC, Green JA, et al. Severe acute respiratory syndrome (SARS) in Singapore: clinical features of index patient and initial contacts. *Emerg Infect Dis*. 2003;9(6):713-717. <https://doi.org/10.3201/eid0906.030264>
- Peiris JS, Chu CM, Cheng VC, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet*. 2003;361(9371):1767-1772. [https://doi.org/10.1016/s0140-6736\(03\)13412-5](https://doi.org/10.1016/s0140-6736(03)13412-5)
- Leung WK, To KF, Chan PK, et al. Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection. *Gastroenterol*. 2003;125(4):1011-1017. [https://doi.org/10.1016/s0016-5085\(03\)01215-0](https://doi.org/10.1016/s0016-5085(03)01215-0)
- Zhao Z, Zhang F, Xu M, et al. Description and clinical treatment of an early outbreak of severe acute respiratory syndrome (SARS) in Guangzhou, PR China. *J Med Microbiol*. 2003;52(Pt 8):715-720. <https://doi.org/10.1099/jmm.0.05320-0>
- Choi KW, Chau TN, Tsang O, et al. Outcomes and prognostic factors in 267 patients with severe acute respiratory syndrome in Hong Kong. *Ann Intern Med*. 2003;139(9):715-723. <https://doi.org/10.7326/0003-4819-139-9-200311040-00005>
- Chan JW, Ng CK, Chan YH, et al. Short term outcome and risk factors for adverse clinical outcomes in adults with severe acute respiratory syndrome (SARS). *Thorax*. 2003;58(8):686-689. <https://doi.org/10.1136/thorax.58.8.686>
- Cheng VC, Hung IF, Tang BS, et al. Viral replication in the nasopharynx is associated with diarrhea in patients with severe acute respiratory syndrome. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2004;38(4):467-475. <https://doi.org/10.1086/382681>
- Kwan AC, Chau TN, Tong WL, et al. Severe acute respiratory syndrome-related diarrhea. *J Gastroenterol Hepatol*. 2005;20(4):606-610. <https://doi.org/10.1111/j.1440-1746.2005.03775.x>
- Tsang KW, Ho PL, Ooi GC, et al. A cluster of cases of severe acute respiratory syndrome in Hong Kong. *N. Engl J Med*. 2003;348(20):1977-1985. <https://doi.org/10.1056/NEJMoa030666>
- Poutanen SM, Low DE, Henry B, et al. Identification of severe acute respiratory syndrome in Canada. *N. Engl J Med*. 2003;348(20):1995-2005. <https://doi.org/10.1056/NEJMoa030634>
- Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N. Engl J Med*. 2012;367(19):1814-1820. <https://doi.org/10.1056/NEJMoa1211721>
- Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, et al. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *Lancet Infect Dis*. 2013;13(9):752-761. [https://doi.org/10.1016/S1473-3099\(13\)70204-4](https://doi.org/10.1016/S1473-3099(13)70204-4)
- Saad M, Omrani AS, Baig K, et al. Clinical aspects and outcomes of 70 patients with Middle East respiratory syndrome coronavirus infection: a single-center experience in Saudi Arabia. *Int J Infect Dis. IJID Off Pub Internat Soci Infect Dis*. 29. 2014;301-306. <https://doi.org/10.1016/j.ijid.2014.09.003>
- Assiri A, McGeer A, Perl TM, et al. Hospital outbreak of Middle East respiratory syndrome coronavirus. *N. Engl J Med*. 2013;369(5):407-416. <https://doi.org/10.1056/NEJMoa1306742>
- Mackay IM, Arden KE. MERS coronavirus: diagnostics, epidemiology and transmission. *Virology*. 2015;12(1):222. <https://doi.org/10.1186/s12985-015-0439-5>
- Zhou J, Li C, Zhao G, et al. Human intestinal tract serves as an alternative infection route for Middle East respiratory syndrome coronavirus. *Sci Adv*. 2017;3(11):eaao4966. <https://doi.org/10.1126/sciadv.aao4966>
- Mailles A, Blanckaert K, Chaud P, et al. First cases of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infections in France, investigations and implications for the prevention of human-to-human transmission, France. *Euro Surveill Bull Eur les Mal Transm Eur Commun Dis. Bull*. 2013;18(24). <https://doi.org/10.2807/ese.18.24.20502-en>
- Alfaraj SH, Al-Tawfiq JA, Altuwaijri TA, Memish ZA. Middle East respiratory syndrome coronavirus in pediatrics: a report of seven cases from Saudi Arabia. *Front Med*. 2019;13(1):126-130. <https://doi.org/10.1007/s11684-017-0603-y>
- Memish ZA, Zumla AI, Al-Hakeem RF, Al-Rabeeah AA, Stephens GM. Family cluster of Middle East respiratory syndrome coronavirus infections. *N. Engl J Med*. 2013;368(26):2487-2494. <https://doi.org/10.1056/NEJMoa1303729>
- Zhang JJ, Dong X, Cao YY, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy*. 2020;75(7):1730-1741. <https://doi.org/10.1111/all.14238>
- Holshue ML, DeBolt C, Lindquist S, et al. First case of 2019 novel coronavirus in the United States. *N. Engl J Med*. 2020;382(10):929-936. <https://doi.org/10.1056/NEJMoa2001191>
- Xiong LJ, Zhou MY, He XQ, Wu Y, Xie XL. The role of human coronavirus infection in pediatric acute gastroenteritis. *Pediatr Infect Dis J*. 2020;39(7):645-649. <https://doi.org/10.1097/INF.0000000000002752>
- Risku M, Lappalainen S, Rasanen S, Vesikari T. Detection of human coronaviruses in children with acute gastroenteritis. *J Clin Virol Off Publ Pan Am Soc Clin Virol*. 2010;48(1):27-30. <https://doi.org/10.1016/j.jcv.2010.02.013>
- To KF, Tong JH, Chan PK, et al. Tissue and cellular tropism of the coronavirus associated with severe acute respiratory syndrome: an

- in-situ hybridization study of fatal cases. *J Pathol.* 2004;202(2):157-163. <https://doi.org/10.1002/path.1510>
29. Shi X, Gong E, Gao D, et al. Severe acute respiratory syndrome associated coronavirus is detected in intestinal tissues of fatal cases. *Am J Gastroenterol.* 2005;100(1):169-176. <https://doi.org/10.1111/j.1572-0241.2005.40377.x>
 30. Liu Q, Wang RS, Qu GQ, et al. Gross examination report of a COVID-19 death autopsy. *Fa Yi Xue Za Zhi.* 2020;36(1):21-23. <https://doi.org/10.12116/j.issn.1004-5619.2020.01.005>
 31. Guidance on coronavirus disease 2019 (COVID-19). China (Vision 7) (in Chinese) <http://www.nhc.gov.cn/jkj/s3577/202009/318683c6faee4191aee29cd774b19d8d.shtml>
 32. Cinatl J, Jr., Hoever G, Morgenstern B, et al. Infection of cultured intestinal epithelial cells with severe acute respiratory syndrome coronavirus. *Cell Mol Life Sci CMLS.* 2004;61(16):2100-2112. <https://doi.org/10.1007/s00018-004-4222-9>
 33. Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Treatment of SARS with human interferons. *Lancet.* 2003;362(9380):293-294. [https://doi.org/10.1016/s0140-6736\(03\)13973-6](https://doi.org/10.1016/s0140-6736(03)13973-6)
 34. Song Y, Liu P, Shi XL, et al. SARS-CoV-2 induced diarrhoea as onset symptom in patient with COVID-19. *Gut.* 2020;69(6):1143-1144. <https://doi.org/10.1136/gutjnl-2020-320891>
 35. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in wuhan, China. *Jama.* 2020;323(11):1061. <https://doi.org/10.1001/jama.2020.1585>
 36. An P, Chen H, Jiang X, et al. *Clinical Features of 2019 Novel Coronavirus Pneumonia Presented Gastrointestinal Symptoms but without Fever Onset*; 2020.
 37. Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med.* 2020;382(18):1708-1720. <https://doi.org/10.1056/NEJMoa2002032>
 38. Lu X, Zhang L, Du H, et al. SARS-CoV-2 infection in children. *N Engl J Med.* 2020;382(17):1663-1665. <https://doi.org/10.1056/NEJMc2005073>
 39. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* 2020;395(10229):1054-1062. [https://doi.org/10.1016/S0140-6736\(20\)30566-3](https://doi.org/10.1016/S0140-6736(20)30566-3)
 40. Ren L, Gonzalez R, Xu J, et al. Prevalence of human coronaviruses in adults with acute respiratory tract infections in Beijing, China. *J Med Virol.* 2011;83(2):291-297. <https://doi.org/10.1002/jmv.21956>
 41. Gaunt ER, Hardie A, Claas EC, Simmonds P, Templeton KE. Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method. *J Clin Microbiol.* 2010;48(8):2940-2947. <https://doi.org/10.1128/JCM.00636-10>
 42. Vabret A, Mourez T, Dina J, et al. Human coronavirus NL63, France. *Emerg Infect Dis.* 2005;11(8):1225-1229. <https://doi.org/10.3201/eid1108.050110>
 43. Vabret A, Dina J, Gouarin S, Petitjean J, Corbet S, Freymuth F. Detection of the new human coronavirus HKU1: a report of 6 cases. *Clin Infect Dis Off Publ Infect Dis Soc Am.* 2006;42(5):634-639. <https://doi.org/10.1086/500136>
 44. Bouvier M, Chen WJ, Arnold JC, et al. Species-specific clinical characteristics of human coronavirus infection among otherwise healthy adolescents and adults. *Influenza Respir Viruses.* 2018;12(2):299-303. <https://doi.org/10.1111/irv.12538>
 45. Vabret A, Mourez T, Gouarin S, Petitjean J, Freymuth F. An outbreak of coronavirus OC43 respiratory infection in Normandy, France. *Clin Infect Dis Off Publ Infect Dis Soc Am.* 2003;36(8):985-989. <https://doi.org/10.1086/374222>
 46. To KF, Lo AW. Exploring the pathogenesis of severe acute respiratory syndrome (SARS): the tissue distribution of the coronavirus (SARS-CoV) and its putative receptor, angiotensin-converting enzyme 2 (ACE2). *J Pathol.* 2004;203(3):740-743. <https://doi.org/10.1002/path.1597>
 47. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol.* 2004;203(2):631-637. <https://doi.org/10.1002/path.1570>
 48. Harmer D, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. *FEBS Lett.* 2002;532(1-2):107-110. [https://doi.org/10.1016/s0014-5793\(02\)03640-2](https://doi.org/10.1016/s0014-5793(02)03640-2)
 49. Zhou J, Chu H, Chan JF, Yuen KY. Middle East respiratory syndrome coronavirus infection: virus-host cell interactions and implications on pathogenesis. *Viral J.* 2015;12(1):218. <https://doi.org/10.1186/s12985-015-0446-6>
 50. Qi F, Qian S, Zhang S, Zhang Z. Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses. *Biochem Biophys Res Commun.* 2020;526(1):135-140. <https://doi.org/10.1016/j.bbrc.2020.03.044>
 51. Hofmann H, Pyrc K, vander Hoek L, Geier M, Berkhout B, Pohlmann S. Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. *Proceedings of the National Academy of Sciences of the United States of America.* 2005;102(22):7988-7993. <https://doi.org/10.1073/pnas.0409465102>
 52. Quaroni A, Nichols BL, Quaroni E, et al. Expression and different polarity of aminopeptidase N in normal human colonic mucosa and colonic tumors. *Int J Cancer.* 1992;51(3):404-411. <https://doi.org/10.1002/ijc.2910510312>
 53. Klein A, Krishna M, Varki NM, Varki A. 9-O-acetylated sialic acids have widespread but selective expression: analysis using a chimeric dual-function probe derived from influenza C hemagglutinin-esterase. *Proceedings of the National Academy of Sciences of the United States of America.* 1994;91(16):7782-7786. <https://doi.org/10.1073/pnas.91.16.7782>
 54. Fouchier RA, Kuiken T, Schutten M, et al. Aetiology: Koch's postulates fulfilled for SARS virus. *Nature.* 2003;423(6937):240. <https://doi.org/10.1038/423240a>
 55. Cheng PK, Wong DA, Tong LK, et al. Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome. *Lancet.* 2004;363(9422):1699-1700. [https://doi.org/10.1016/S0140-6736\(04\)16255-7](https://doi.org/10.1016/S0140-6736(04)16255-7)
 56. Chan KH, Poon LL, Cheng VC, et al. Detection of SARS coronavirus in patients with suspected SARS. *Emerg Infect Dis.* 2004;10(2):294-299. <https://doi.org/10.3201/eid1002.030610>
 57. Hung IF, Cheng VC, Wu AK, et al. Viral loads in clinical specimens and SARS manifestations. *Emerg Infect Dis.* 2004;10(9):1550-1557. <https://doi.org/10.3201/eid1009.040058>
 58. Xu D, Zhang Z, Jin L, et al. Persistent shedding of viable SARS-CoV in urine and stool of SARS patients during the convalescent phase. *Eur J Clin Microbiol Infect Dis.* 2005;24(3):165-171. <https://doi.org/10.1007/s10096-005-1299-5>
 59. Drosten C, Seilmaier M, Corman VM, et al. Clinical features and virological analysis of a case of Middle East respiratory syndrome coronavirus infection. *Lancet Infect Dis.* 2013;13(9):745-751. [https://doi.org/10.1016/S1473-3099\(13\)70154-3](https://doi.org/10.1016/S1473-3099(13)70154-3)
 60. Corman VM, Albarrak AM, Omrani AS, et al. Viral shedding and antibody response in 37 patients with Middle East respiratory syndrome coronavirus infection. *Clin Infect Dis Off Publ Infect Dis Soc Am.* 2016;62(4):477-483. <https://doi.org/10.1093/cid/civ951>

61. Zhang W, Du RH, Li B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg Microb Infect.* 2020;9(1):386-389. <https://doi.org/10.1080/22221751.2020.1729071>
62. Xu Y, Li X, Zhu B, et al. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. *Nat Med.* 2020;26(4):502-505. <https://doi.org/10.1038/s41591-020-0817-4>
63. Tang A, Tong ZD, Wang HL, et al. Detection of novel coronavirus by RT-PCR in stool specimen from asymptomatic child, China. *Emerg Infect Dis.* 2020;26(6):1337-1339. <https://doi.org/10.3201/eid2606.200301>
64. Young BE, Ong SWX, Kalimuddin S, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. *Jama.* 2020;3(15):1488. <https://doi.org/10.1001/jama.2020.3204>
65. Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for gastrointestinal infection of SARS-CoV-2. *Gastroenterol.* 2020;158(6):1831-1833. e3. <https://doi.org/10.1053/j.gastro.2020.02.055>
66. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. *Jama.* 2020;11. <https://doi.org/10.1001/jama.2020.3786>
67. Wilder-Smith A, Freedman DO. Isolation, quarantine, social distancing and community containment: pivotal role for old-style public health measures in the novel coronavirus (2019-nCoV) outbreak. *J Trav Med.* 2020;27(2). <https://doi.org/10.1093/jtm/taaa020>
68. Zhou J, Li C, Liu X, et al. Infection of bat and human intestinal organoids by SARS-CoV-2. *Nat Med.* 2020;26(7):1077-1083. <https://doi.org/10.1038/s41591-020-0912-6>
69. Stanifer ML, Kee C, Cortese M, et al. Critical role of type III interferon in controlling SARS-CoV-2 infection in human intestinal epithelial cells. *Cell Rep* 2020;32(1):107863. <https://doi.org/10.1016/j.celrep.2020.107863>
70. Chu H, Chan JF, Yuen TT, et al. Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study. *Lancet Microbe.* 2020;1(1):e14-e23. [https://doi.org/10.1016/S2666-5247\(20\)30004-5](https://doi.org/10.1016/S2666-5247(20)30004-5)
71. Zang R, Gomez Castro MF, McCune BT, et al. TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. *Science Immunol.* 2020(47):5. <https://doi.org/10.1126/sciimmunol.abc3582>
72. Lamers MM, Beumer J, van der Vaart J, et al. SARS-CoV-2 productively infects human gut enterocytes. *Science.* 2020;369(6499):50-54. <https://doi.org/10.1126/science.abc1669>
73. Arbour N, Cote G, Lachance C, Tardieu M, Cashman NR, Talbot PJ. Acute and persistent infection of human neural cell lines by human coronavirus OC43. *J Virol.* 1999;73(4):3338-3350. <https://doi.org/10.1128/JVI.73.4.3338-3350.1999>
74. Herzog P, Drosten C, Muller MA. Plaque assay for human coronavirus NL63 using human colon carcinoma cells. *Viol J.* 2008;5(1):138. <https://doi.org/10.1186/1743-422X-5-138>
75. Subbarao K, McAuliffe J, Vogel L, et al. Prior infection and passive transfer of neutralizing antibody prevent replication of severe acute respiratory syndrome coronavirus in the respiratory tract of mice. *J Virol.* 2004;78(7):3572-3577. <https://doi.org/10.1128/jvi.78.7.3572-3577.2004>
76. Glass WG, Subbarao K, Murphy B, Murphy PM. Mechanisms of host defense following severe acute respiratory syndrome-coronavirus (SARS-CoV) pulmonary infection of mice. *J Immunol.* 2004;173(6):4030-4039. <https://doi.org/10.4049/jimmunol.173.6.4030>
77. Hogan RJ, Gao G, Rowe T, et al. Resolution of primary severe acute respiratory syndrome-associated coronavirus infection requires Stat1. *J Virol.* 2004;78(20):11416-11421. <https://doi.org/10.1128/JVI.78.20.11416-11421.2004>
78. Wentworth DE, Gillim-Ross L, Espina N, Bernard KA. Mice susceptible to SARS coronavirus. *Emerg Infect Dis.* 2004;10(7):1293-1296. <https://doi.org/10.3201/eid1007.031119>
79. Ding Y, Wang H, Shen H, et al. The clinical pathology of severe acute respiratory syndrome (SARS): a report from China. *J Pathol.* 2003;200(3):282-289. <https://doi.org/10.1002/path.1440>
80. Franks TJ, Chong PY, Chui P, et al. Lung pathology of severe acute respiratory syndrome (SARS): a study of 8 autopsy cases from Singapore. *Hum Pathol.* 2003;34(8):743-748. [https://doi.org/10.1016/s0046-8177\(03\)00367-8](https://doi.org/10.1016/s0046-8177(03)00367-8)
81. Yang XH, Deng W, Tong Z, et al. Mice transgenic for human angiotensin-converting enzyme 2 provide a model for SARS coronavirus infection. *Comp Med.* 2007;57(5):450-459.
82. Xiao Y, Meng Q, Yin X, et al. Pathological changes in masked palm civets experimentally infected by severe acute respiratory syndrome (SARS) coronavirus. *J Comp Pathol.* 2008;138(4):171-179. <https://doi.org/10.1016/j.jcpa.2007.12.005>
83. Martina BE, Haagmans BL, Kuiken T, et al. Virology: SARS virus infection of cats and ferrets. *Nature.* 2003;425(6961):915. <https://doi.org/10.1038/425915a>
84. van den Brand JM, Haagmans BL, Leijten L, et al. Pathology of experimental SARS coronavirus infection in cats and ferrets. *Vet Pathol.* 2008;45(4):551-562. <https://doi.org/10.1354/vp.45-4-551>
85. McAuliffe J, Vogel L, Roberts A, et al. Replication of SARS coronavirus administered into the respiratory tract of African Green, rhesus and cynomolgus monkeys. *Viol.* 2004;330(1):8-15. <https://doi.org/10.1016/j.viol.2004.09.030>
86. Liu L, Wei Q, Nishiura K, et al. Spatiotemporal interplay of severe acute respiratory syndrome coronavirus and respiratory mucosal cells drives viral dissemination in rhesus macaques. *Mucosal Immunol.* 2016;9(4):1089-1101. <https://doi.org/10.1038/mi.2015.127>
87. Cockrell AS, Peck KM, Yount BL, et al. Mouse dipeptidyl peptidase 4 is not a functional receptor for Middle East respiratory syndrome coronavirus infection. *J Virol.* 2014;88(9):5195-5199. <https://doi.org/10.1128/JVI.03764-13>
88. Tao X, Garron T, Agrawal AS, et al. Characterization and demonstration of the value of a lethal mouse model of Middle East respiratory syndrome coronavirus infection and disease. *J Virol.* 2016;90(1):57-67. <https://doi.org/10.1128/JVI.02009-15>
89. de Wit E, Prescott J, Baseler L, et al. The Middle East respiratory syndrome coronavirus (MERS-CoV) does not replicate in Syrian hamsters. *PLoS One.* 2013;8(7):e69127. <https://doi.org/10.1371/journal.pone.0069127>
90. Raj VS, Smits SL, Provac LB, et al. Adenosine deaminase acts as a natural antagonist for dipeptidyl peptidase 4-mediated entry of the Middle East respiratory syndrome coronavirus. *J Virol.* 2014;88(3):1834-1838. <https://doi.org/10.1128/JVI.02935-13>
91. de Wit E, Rasmussen AL, Falzarano D, et al. Middle East respiratory syndrome coronavirus (MERS-CoV) causes transient lower respiratory tract infection in rhesus macaques. *Proceedings of the National Academy of Sciences of the United States of America.* 2013;110(41):16598-16603. <https://doi.org/10.1073/pnas.1310744110>
92. Yao Y, Bao L, Deng W, et al. An animal model of MERS produced by infection of rhesus macaques with MERS coronavirus. *J Infect Dis.* 2014;209(2):236-242. <https://doi.org/10.1093/infdis/jit590>
93. Sun SH, Chen Q, Gu HJ, et al. A mouse model of SARS-CoV-2 infection and pathogenesis. *Cell Host Microbe.* 2020;28(1):124-133. <https://doi.org/10.1016/j.chom.2020.05.020>

94. Kim YI, Kim SG, Kim SM, et al. Infection and rapid transmission of SARS-CoV-2 in ferrets. *Cell Host Microbe*. 2020;27(5):704-709. <https://doi.org/10.1016/j.chom.2020.03.023>
95. Bao L, Deng W, Huang B, et al. The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. *Nature*. 2020;583(7818):830-833. <https://doi.org/10.1038/s41586-020-2312-y>
96. Chan JF, Zhang AJ, Yuan S, et al. Simulation of the clinical and pathological manifestations of coronavirus disease 2019 (COVID-19) in a golden Syrian hamster model: implications for disease pathogenesis and transmissibility. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2020;71(9):2428-2446. <https://doi.org/10.1093/cid/ciaa325>
97. Munster VJ, Feldmann F, Williamson BN, et al. Respiratory disease in rhesus macaques inoculated with SARS-CoV-2. *Nature*. 2020;585(7824):268-272. <https://doi.org/10.1038/s41586-020-2324-7>
98. Deng W, Bao L, Liu J, et al. Primary exposure to SARS-CoV-2 protects against reinfection in rhesus macaques. *Science*. 2020;369(6505):818-823. <https://doi.org/10.1126/science.abc5343>
99. Jiao L, Li H, Xu J, et al. The gastrointestinal tract is an alternative route for SARS-CoV-2 infection in a nonhuman primate model. *Gastroenterol*. 2021;160(5):1647-1661. <https://doi.org/10.1053/j.gastro.2020.12.001>
100. Shi J, Wen Z, Zhong G, et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science*. 2020;368(6494):1016-1020. <https://doi.org/10.1126/science.abb7015>
101. Lassnig C, Sanchez CM, Egerbacher M, et al. Development of a transgenic mouse model susceptible to human coronavirus 229E. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;102(23):8275-8280. <https://doi.org/10.1073/pnas.0408589102>
102. Guo M, Tao W, Flavell RA, Zhu S. Potential intestinal infection and faecal-oral transmission of SARS-CoV-2. *Nat Rev Gastroenterol Hepatol*. 2021;18(4):269-283. <https://doi.org/10.1038/s41575-021-00416-6>
103. Kalantzi L, Goumas K, Kalioras V, Abrahamsson B, Dressman JB, Reppas C. Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies. *Pharmaceut Res*. 2006;23(1):165-176. <https://doi.org/10.1007/s11095-005-8476-1>
104. Jantratid E, Janssen N, Reppas C, Dressman JB. Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update. *Pharmaceut Res*. 2008;25(7):1663-1676. <https://doi.org/10.1007/s11095-008-9569-4>
105. Lee AC, Zhang AJ, Chan JF, et al. Oral SARS-CoV-2 inoculation establishes subclinical respiratory infection with virus shedding in golden Syrian hamsters. *Cell Rep Med*. 2020;1(7):100121. <https://doi.org/10.1016/j.xcrim.2020.100121>
106. Goh GK, Dunker AK, Uversky V. Prediction of intrinsic disorder in MERS-CoV/HCoV-EMC supports a high oral-fecal transmission. *PLoS Curr*. 2013. <https://doi.org/10.1371/currents.outbreaks.22254b58675cdebc256dbe3c5aa6498b>
107. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. *Jama*. 2020;323(18):1843-1844. <https://doi.org/10.1001/jama.2020.3786>
108. Xiao F, Sun J, Xu Y, et al. Infectious SARS-CoV-2 in feces of patient with severe COVID-19. *Emerg Infect Dis*. 2020;26(8):1920-1922. <https://doi.org/10.3201/eid2608.200681>
109. Wang XW, Li J, Guo T, et al. Concentration and detection of SARS coronavirus in sewage from xiao tang Shan hospital and the 309th hospital of the Chinese people's liberation army. *Water Sci Technol J Int Assoc Water Pollut Res*. 2005;52(8):213-221. <https://doi.org/10.2166/wst.2005.0266>
110. Chan KH, Peiris JS, Lam SY, Poon LL, Yuen KY, Seto WH. The effects of temperature and relative humidity on the viability of the SARS coronavirus. *Adv Virol*. 2011;2011:734690-734697. <https://doi.org/10.1155/2011/734690>
111. Wang XW, Li JS, Jin M, et al. Study on the resistance of severe acute respiratory syndrome-associated coronavirus. *J Virol Methods*. 2005;126(1-2):171-177. <https://doi.org/10.1016/j.jviromet.2005.02.005>
112. van Doremalen N, Bushmaker T, Munster VJ. Stability of Middle East respiratory syndrome coronavirus (MERS-CoV) under different environmental conditions. *Euro Surveill Bull Eur les Mal Transm Eur Commun Dis Bull*. 2013;18(38). <https://doi.org/10.2807/1560-7917.es2013.18.38.20590>
113. Bin SY, Heo JY, Song MS, et al. Environmental contamination and viral shedding in MERS patients during MERS-CoV outbreak in South Korea. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2016;62(6):755-760. Mar 15. <https://doi.org/10.1093/cid/civ1020>
114. Geller C, Varbanov M, Duval RE. Human coronaviruses: insights into environmental resistance and its influence on the development of new antiseptic strategies. *Viruses*. 2012;4(11):3044-3068. <https://doi.org/10.3390/v4113044>
115. Chan KH, Sridhar S, Zhang RR, et al. Factors affecting stability and infectivity of SARS-CoV-2. *J Hosp Infect*. 2020;106(2):226-231. <https://doi.org/10.1016/j.jhin.2020.07.009>
116. van Doremalen N, Bushmaker T, Morris DH, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *N Engl J Med*. 2020;382(16):1564-1567. <https://doi.org/10.1056/NEJMc2004973>
117. Muller A, Tillmann RL, Muller A, Simon A, Schildgen O. Stability of human metapneumovirus and human coronavirus NL63 on medical instruments and in the patient environment. *J Hosp Infect*. 2008;69(4):406-408. <https://doi.org/10.1016/j.jhin.2008.04.017>
118. Sizun J, Yu MW, Talbot PJ. Survival of human coronaviruses 229E and OC43 in suspension and after drying on surfaces: a possible source of hospital-acquired infections. *J Hosp Infect*. 2000;46(1):55-60. <https://doi.org/10.1053/jhin.2000.0795>
119. Gundy PM, Gerba CP, Pepper IL. Survival of coronaviruses in water and wastewater. *Food Environ Virol*. 2008;1(1):10. <https://doi.org/10.1007/s12560-008-9001-6>
120. WHO. Consensus Document on the Epidemiology of Severe Acute Respiratory Syndrome (SARS). <https://apps.who.int/iris/handle/10665/70863>
121. Leung CW, Kwan YW, Ko PW, et al. Severe acute respiratory syndrome among children. *Pediatrics*. 2004;113(6):e535-43. <https://doi.org/10.1542/peds.113.6.e535>
122. Middle WHO. East respiratory syndrome coronavirus (MERS-CoV). <https://www.who.int/emergencies/mers-cov/en/>
123. Park JE, Jung S, Kim A, Park JE. MERS transmission and risk factors: a systematic review. *BMC Publ Health*. 2018;18(1):574. <https://doi.org/10.1186/s12889-018-5484-8>
124. Al-Tawfiq JA, Hinedi K, Ghandour J, et al. Middle East respiratory syndrome coronavirus: a case-control study of hospitalized patients. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2014;59(2):160-165. <https://doi.org/10.1093/cid/ciu226>
125. WHO. COVID-19 weekly epidemiological update, 47, 2021. <https://apps.who.int/iris/handle/10665/342524>
126. Zhang H, Liao YS, Gong J, Liu J, Xia X, Zhang H. Clinical characteristics of coronavirus disease (COVID-19) patients with gastrointestinal symptoms: a report of 164 cases. *Dig Liver Dis: Off J Ital Soci Gastroenterol Ital Assoc St Liver*. 2020. <https://doi.org/10.1016/j.dld.2020.04.034>
127. Lauer SA, Grantz KH, Bi Q, et al. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. *Ann Intern Med*. 2020;172(9):577-582. <https://doi.org/10.7326/M20-0504>
128. Suedee R, Intakong W, Dickert FL. Molecularly imprinted polymer-modified electrode for on-line conductometric monitoring of haloacetic acids in chlorinated water. *Anal Chim Acta*. 2006;569(1-2):66-75. <https://doi.org/10.1016/j.aca.2006.03.081>

129. McIntosh K, Becker WB, Chanock RM. Growth in suckling-mouse brain of "IBV-like" viruses from patients with upper respiratory tract disease. *Proceedings of the National Academy of Sciences of the United States of America*. 1967;58(6):2268-2273. <https://doi.org/10.1073/pnas.58.6.2268>
130. van der Hoek L, Pyrc K, Berkhout B. Human coronavirus NL63, a new respiratory virus. *FEMS Microbiol Rev*. 2006;30(5):760-773. <https://doi.org/10.1111/j.1574-6976.2006.00032.x>
131. Woo PC, Lau SK, Chu CM, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol*. 2005;79(2):884-895. <https://doi.org/10.1128/JVI.79.2.884-895.2005>

How to cite this article: Ning T, Liu S, Xu J, et al. Potential intestinal infection and faecal-oral transmission of human coronaviruses. *Rev Med Virol*. 2022;e2363. <https://doi.org/10.1002/rmv.2363>