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Can SARS-CoV-2 be transmitted via faeces?

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Abstract

Purpose of review: COVID-19 patients can present gastrointestinal symptoms, being diarrhoea one of the most frequent, suggesting intestinal health can be impacted by COVID-19. Here we will discuss whether there is a correlation between the presence of SARS-CoV-2 RNA in faeces and diarrhoea, the relevance of gastrointestinal symptoms is disease diagnosis and transmission, and how COVID-19 can impact the gut microbial balance.

Recent findings: SARS-CoV-2 RNA has been reported in faeces or rectal swabs of COVID-19 patients with and without diarrhoea, suggesting faecal shedding can occur independently to gastrointestinal symptoms. However, the presence of the virus in the intestine can persist beyond its presence in the respiratory tract, with some reports suggesting that SARS-CoV-2 in the faeces can be infectious. COVID-19 can impact in the gut microbiota causing an enhancement of biosynthesis pathways that favour the expansion of bacterial pathogens in the inflamed gut, and causing a decline in commensals involved in the human immune response.

Summary: Gastrointestinal symptoms may be the first indication of COVID-19. SARS-CoV-2 in faeces can potentiate routes of disease transmission, particularly as the high viral loads reported in patients with severe illness suggest virus replication in the intestine may be possible.

Introduction

The main pathway of transmission of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of coronavirus disease 2019 (COVID-19), is through exposure to respiratory droplets which can transfer more easily in closed environments and through human contact [1].

In addition to respiratory symptoms, COVID-19 patients can also present gastrointestinal manifestations, such as nausea, abdominal pain, vomiting, and diarrhoea, with the latter being the most frequent [2-4]. Incidence of diarrhoea as a symptom in COVID-19 patients can vary between 5% and 55% [2-10]. However, comparison between studies can be difficult as samples sizes and patient recruitment criteria vary between studies. Nonetheless, diarrhoea has been more commonly reported in patients of America and Europe, with one study observing a 31% higher risk of hospitalisation in patients with diarrhoea [7], whereas others suggested this symptom is not associated with higher disease severity or poorer outcome [2, 3, 8, 9].

COVID-19 occurs when the SARS-CoV-2 spike glycoproteins bind to the extracellular-facing N-terminal domain of the angiotensin-converting enzyme 2 (ACE2) receptors present in the lungs epithelial cells [11, 12], allowing the virus to enter the host cells [13]. These receptors are also expressed in epithelial cells of the large and small intestine, where ACE2 can act as a potential entry point for the virus [11, 14]. This has been confirmed by visualization of the viral nucleocapsid protein in the cytoplasm of gastric, duodenal, and rectum epithelial cells [14].

It is therefore important to understand the potential implications of SARS-CoV-2 faecal excretion in disease transmission and whether there is a correlation between the presence of virus in faeces and diarrhoea as a symptom of COVID-19. Here we will discuss the relevance of these manifestations in disease diagnosis and transmission, and how COVID-19 can impact the gut microbial balance.

SARS-CoV-2 can persist in the intestine of COVID-19 patients

Most studies focusing on SARS-CoV-2 detection in different tissues/samples use RT-PCR assays to target the presence of viral RNA [15, 16]. This is due to the speed, reliability and low limits of detection of molecular testing, but also due to the difficulties to isolate and culture the live virus [17]. In addition to the nose and throat swabs used for COVID-19 diagnosis, SARS-CoV-2 RNA has also been detected in faeces or rectal swabs of patients with COVID-19 [5-7, 10, 14, 15, 18-20]. In early 2020, faecal samples of 41 (out of 74) patients tested positive for the virus for an average of 28 days. The respiratory samples of the patients showing faecal excretion of SARS-CoV-2 remained positive for approximately 17 days, 11 days less than the faecal samples, but over a day longer than the observed for the COVID-19 patients with negative faecal samples [10]. Other studies have also suggested that SARS-CoV-2 shedding through the faeces continues to occur after the respiratory samples have converted to negative [14, 19, 21]. In two reports, over 40% of the patients investigated were positive for viral RNA in faeces for up to 14 days after pharyngeal swabs tested negative [10, 21]. In one other study, a patient with a clinical presentation consistent with COVID-19, including respiratory symptoms and lung damage, tested negative for SARS-CoV-2 on the pharyngeal swab but positive on the faecal sample [18].

The detection of SARS-CoV-2 RNA in faeces for such lengthy periods of time raises the question whether patients should be considered free of the virus based just on negative nasopharyngeal swabs [14, 19]. Furthermore, as intestinal cells express the ACE2 receptors required for SARS-CoV-2 infection [11], the possibility of a faecal-oral route of transmission cannot be excluded [14, 18]. These concerns gathered additional relevance as Wang et al. reported SARS-CoV-2 RNA in 44% of 153 faecal samples tested, 2 of which also shown presence of live virus [15]. Similarly, Xiao et al. cultured SARS-CoV-2 from stool samples of 2 patients, one of which showing higher viral loads in the faeces compared to nasopharyngeal samples [17]. In both these studies, cycle threshold values in stool were consistent with a high viral load, suggesting the virus can persist and potentially replicate in the digestive tract [15, 17]. These observations were further supported by the sequencing of

SARS-CoV-2 in stool samples of COVID-19 patients, showing a higher density and coverage of the virus genome at the of the 3' end region which is associated with viral activity, replication and infection of the host cells [22].

Relevance of gastrointestinal symptoms in disease transmission

As the presence of SARS-CoV-2 genetic material and live virus in patients faeces became apparent, apprehension increased as COVID-19 patients can also present gastrointestinal symptoms such as diarrhoea [3-9, 17]. The occurrence of loose stools in COVID-19 patients extends the risk of environmental contamination of toilets and contact surfaces through aerosols or faecal matter deposition [23]. Facilities in areas shared by individuals more susceptible to severe illness such as wards or care homes are of particular concern, since patients are deemed virus-free upon negative nasopharyngeal tests, even though the presence of virus in the intestine can outlast its presence in the respiratory tract [10, 14, 19, 21].

Despite the evidence of gastrointestinal symptoms in COVID-19, a direct association between SARS-CoV-2 in faeces and diarrhoea has not been clearly established. In a study involving 22 patients with mild COVID-19 in China, the virus was most frequently detected in faeces of patients with digestive symptoms, being diarrhoea the most prevalent [6]. These observations are consistent with other reports from China [4], South American [7] and Hong Kong [16], but while one study found similar viral loads in stools of patients with gastrointestinal symptoms and in those asymptomatic [7]; in another study patients with diarrhoea showed higher concentration of virus (5.1 $\log_{10}$ copies/ml) compared to those without lose stools (3.9 $\log_{10}$ copies/ml) [16]. However, a study involving 9 COVID-19 patients with diarrhoeal symptoms at time of stool collection, 13 patients in which diarrhoea had ceased prior to sample collection, and 22 patients without any diarrhoeal symptoms, SARS-CoV-2 RNA was only detected in stools of asymptomatic patients or in those in which symptoms had already ceased [5], suggesting patients without diarrhoea can also show SARS-CoV-2 shedding through the faeces.

Faecal excretion of SARS-CoV-2 can be a factor in disease transmission, particularly as gastrointestinal symptoms may be the only manifestation of COVID-19 at illness onset, increasing the risk of exposure to the virus. Song et al. reported the case of a patient experiencing 3 to 4 episodes of diarrhoea daily for 4 days, accompanied by low fever prior to receiving medical assistance. This clinical presentation suggested gastrointestinal illness. Further testing revealed the patient was negative for all common pathogens associated to digestive illness and was indeed positive for SARS-CoV-2 [24]. Similarly, a study by Pan et al. reported 103 patients with COVID-19 and showing digestive symptoms. Six of those patients did not present respiratory symptoms and one also did not present fever [3]. Although diarrhoea was present in 34% of the patients with gastrointestinal symptoms, the study did not specify if that was the case for the 6 patients without respiratory symptoms [3]. One other study identified 183 COVID-19 patients reporting only digestive symptoms, including 69 patients with diarrhoea [25]; whereas a study following mild disease in 67 patients, observed 13 patients that experienced diarrhoea as the first symptom prior to developing respiratory symptoms [6].

SARS-CoV-2 shedding can occur through the faeces and evidence suggests COVID-19 may present initially via digestive symptoms only, leading to longer times from disease onset to hospital admission [3], therefore the potential for virus transmission in the community through the faecal-oral route should not be disregarded.

Role of gut microbiota in COVID-19

The intestinal flora plays an important role in human immunity and physiological balance required for disease prevention. Infection by SARS-CoV-2 disrupts this equilibrium leading to gastrointestinal manifestations such as diarrhoea, which are potentially aggravated by antibiotic therapy during COVID-19 treatment [4]. Additionally, a biomarker of intestinal inflammatory response, faecal calprotectin, has be found elevated in patients with COVID-19 with diarrhoea [5]. As the virus binds to ACE2 receptors which are also present in human upper and lower intestinal cells [11, 14], and

have a role in regulation of intestinal inflammation and the ecology of the gut microbiome [20]; it has been hypothesised SARS-CoV-2 presence may also affect composition and diversity of the gut microbiota [3], which is naturally decreased in the elderly [26], those more affected by severe COVID-19.

Metagenomics analysis of faecal samples from 6 COVID-19 patients showed a depletion of bacterial commensals that are suspected to be involved in the human immune response, namely Faecalibacterium prausnitzii, *Eubacterium rectale*, *Ruminococcus obeum*, and *Dorea formicigenerans*. Independent of antibiotic use or gastrointestinal symptoms, samples of COVID-19 patients also showed a higher prevalence of opportunistic pathogens (*Clostridium hathewayi*, *Actinomyces viscosu*s, and *Bacteroides nordii*) compared to a pre-pandemic cohort of controls [20]. This agrees with a previous study from the same authors reporting an enhancement of amino acid biosynthesis pathways such as L-serine in patients with higher SARS-CoV-2 infectivity, which can favour the expansion of bacterial pathogens in the inflamed gut of COVID-19 patients [22]. While *F. prausnitzii*, *E. rectale* and some *Bacteroides* species that have been associated with the downregulation of ACE2 were underrepresented in COVID-19 patients; the opposite was observed for bacteria of the genus *Coprobacillus* like *C. hathewayi*, known to upregulate the expression of this receptor [20]. These results were largely confirmed in a subsequent study involving 87 COVID-19 patients where, in addition to the previously observed changes, bifidobacteria was also depleted in ill patients [27]. While no significant differences in diversity were observed between COVID-19 patients and controls [27], in both studies the gut dysbiosis persisted after the patients nasopharyngeal swabs tested negative for the virus [20, 27].

An increase in opportunistic pathogens seems to be generically observed in COVID-19 patients, even with less discriminatory analysis such as 16S sequencing. Gu and colleagues observed a predominance of genus *Streptococcus*, *Rothia*, *Veillonella*, and *Actinomyces* in 30 COVID-19 patients, and a reduction in the abundance of several genera from the Lachnospiraceae family. This analysis

also showed a decline in bacterial diversity compared to healthy individuals, which reflected the low relative abundance of beneficial commensals [28].

Microbial analysis of faecal samples suggests a long-term increase in opportunistic gut pathogens, an imbalance that may affect the human inflammatory responses in COVID-19 [20, 27]. Further studies will potentiate the clear identification of biomarkers of disease severity and create opportunities for modulation of the gut microbiota through replacement therapies.

Conclusion

The human gut plays a key role in immunity and disease prevention. Detection of SARS-CoV-2 RNA in the faeces of COVID-19 patients at high levels suggests virus replication in the intestine may be possible. The reports of patients showing only gastrointestinal symptoms as the first indication of COVID-19 further strengths this possibility. Infection by SARS-CoV-2 appears to impact the microbial balance of the gut population, causing a reduction of beneficial commensals and an increase of opportunistic pathogens associated with the upregulation of ACE2 receptors in the intestine. Although reports of viable virus in faeces are rare, this may be a reflection of the difficulties associated with culture of a highly infectious virus compared to use of molecular techniques, particularly during periods of heavy burden to healthcare systems. Therefore, the risk of disease transmission through the faecal-oral route cannot be excluded.

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