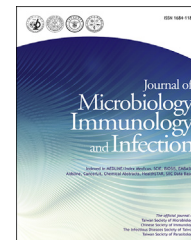


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Short Communication

Do children need a longer time to shed SARS-CoV-2 in stool than adults?

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Abstract SARS-CoV-2 can be shed in the stool of patients in the recovery phase. Children show a longer shedding time than adults. We analyzed the possible causes of this finding and recommend that a negative stool sample be included in a patient's discharge criteria. Copyright © 2020, Taiwan Society of Microbiology. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

In late 2019, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), caused an outbreak of coronavirus disease (COVID-19) in Wuhan, China. The infection spread very quickly throughout China and over

100 countries. To date, over 100,000 cases have been confirmed worldwide and at least 3000 patients have died. According to recent guidelines published in China,¹ most of the infected individuals were discharged after showing significant improvements in respiratory symptoms, a stable body temperature for more than 3 days, and two negative nucleic acid quantitative RT-PCR (qRT-PCR) nasal and throat swabs.

However, in Jinan, we found that 8 of 27 (29.6%) patients, all of whom were diagnosed with mild to moderate infection and discharged 1–2 weeks ago, showed positive PCR results in their stool but negative results in their respiratory specimens. Interestingly, six (75%) of these eight patients were children. In addition, the families of these children showed negative results in all types of

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specimens. More interestingly, besides these patients, a boy showed a positive result in his stool 3 weeks after close contact with his father, a confirmed patient. However, all other samples (i.e., nasal swab, throat swab, and stool) of the boy had been negative for the past 3 weeks. These findings indicate that the stool specimens of children remain positive for a longer time than those of adults. In the present work, we analyzed possible reasons behind this phenomenon.

Methods

RT-PCR

Real-time PCR was conducted by using the SARS-CoV-2 (ORF1ab/N) nucleic acid detection kit (Bio-germ, Shanghai, China). The following primers and probe were used: forward primer, 5'-TCAGAAATGCCAATCTCCCAAC-3'; reverse primer 5'-AAAGGTCCACCCGATACATTGA-3'; and probe, 5'-CY5-CTAGTTACTAGCCATCCTTACTGC-3' BHQ1. The amplification conditions were 50 °C for 15 min and

95 °C for 3 min followed by 45 cycles of 95 °C for 15 s and 60 °C for 30 s.

Results

Six children (cases 1–6) and two adults (cases 7–8) showed positive results in their stool after discharge (Table 1). Table 1 reveals that the respiratory specimens of all eight patients were positive; however, their stool samples were negative/not available in the first week after onset of symptoms. Three children and one adult showed positive results in their stool 4 weeks after onset.

Discussion

This study found viral shedding in the feces of eight patients with SARS-CoV-2 infection, six of whom were children. Thus, we first sought to determine whether patients shed SARS-CoV-2 in stool.

Coronaviruses are a large family of viruses. To date, six CoVs are known to cause human infection. Of these CoVs,

Table 1 General information of the eight patients included in this study.

Case	Gender	Age	Symptoms	Contact history	First admission date	Specimen	Onset of symptom day				
							1–7 days	8–14 days	15–21 days	22–28 days	29–35 days
1	F	8y1m	Fever for 1 day (38.5 °C)	Father took train several times (15–22 Jan)	30 Jan	NS					
						TS					
						SS					
2	M	3y7m	None	Father transferred from a flight from Wuhan on 14 Jan (stayed 1 day)	1 Feb	NS					
						TS					
						SS					
3	F	2y9m	None	Father went to Wuhan on 24 Jan	25 Jan	NS					
						TS					
						SS					
4	M	11m	None	Parents work in Wuhan and returned home on 22 Jan	6 Feb	NS					
						TS					
						SS					
5	F	9y	None	Parents work in Wuhan and returned home on 22 Jan	6 Feb	NS					
						TS					
						SS					
6	F	3y7m	Fever for 1 day (37–37.6 °C)	Mother went to Wuhan (11–16 Jan)	26 Jan	NS					
						TS					
						SS					
7	F	33y	Fever for 1 day (37.6 °C)	Returned from Wuhan (21 Jan)	30 Jan	NS					
						TS					
						SS					
8	F	39y	None	Traveled with a companion from Hubei (20–25 Jan)	4 Feb	NS					
						TS					
						SS					

Notes: NS, nasal swab; TS, throat swab; SS, stool sample. Sputum specimens were not compared because none of the children provided sputum samples. Red, positive; Green, negative; Yellow, not available.

two zoonotic viruses, SARS-CoV and MERS-CoV, were responsible for serious outbreaks in China in 2002–2003 (SARS-CoV) and in the Middle East in 2012 (MERS-CoV). The 2019-nCoV virus is closely related to SARS-CoV with over 85% identity.² Moreover, the epidemiological and biological characteristics of these viruses are relatively similar. When SARS broke out in China, researchers found that the virus could survive in feces for over 96 h.³ Similarly, studies on SARS-CoV-2 revealed that live viral strains could be isolated from feces, thus indicating the potential infectiousness of this body waste.⁴ Further research found that different specimens express different results at different stages of the disease. For example, a report indicated that specimens obtained from the respiratory tract are more likely to yield viral RNA in the early stages of illness than other specimens. However, this likelihood gradually decreased after the first week of onset. The same report further found that stool specimens are 100% positive on the third week after onset and 30% positive 29 days later.⁵ Recent data from China revealed that sputum shows the highest positive RT-PCR rates. Viral nucleic acids could be positively detected in 10% of the patients' blood samples at the acute period and in 50% of the patients' feces.⁴ These studies infer that live viruses and viral nucleic acids could be detected in stool. Moreover, compared with respiratory specimens, stool specimens may remain positive longer, similar to the findings of the present study.

Next, we sought to determine why the shedding time of the virus in feces is longer in children than in adults.

One reason that can explain why the shedding time of the virus in feces is longer in children than in adults is that the former often have poorer hand hygiene practices than the latter. Poor hygiene can cause contamination of the gastrointestinal tract by repeated touching with hands containing the virus or its fragments. For example, we described earlier the case of a boy who showed no symptoms after close contact with his father and had repeated negative results for all of his airway specimens. However, the stool results of this same boy were positive 3 weeks after contact.

Second, when SARS broke out in 2003, angiotensin-converting enzyme 2 (ACE2) was confirmed to be a functional receptor for SARS-CoV. Interestingly, subsequent findings indicated that 2019-nCoV also uses ACE2 as an entry receptor.⁶ Previous studies demonstrated that ACE2 is abundantly present in humans in the epithelia of the lungs and small intestine⁷ and, especially, in proximal and distal enterocytes. Animal studies have also shown that ACE2 has different distributions in the lung in different genders and ages and that ACE2 expression in rat lung decreases with age.⁸ Therefore, we believe that the expression of ACE2 in the intestine of children may differ from that of adults; this difference may explain why children have a longer viral shedding period than adults.

Third, children often cough weakly but they are prone to silent aspiration. Thus, virus in the sputum or saliva may enter the gastrointestinal tract through swallowing, which could explain why children shed virus in their stool.

Although a positive PCR result cannot confirm the presence of live viruses, because of the novel nature of this new virus and disease and the fact that studies have confirmed that SARS can be transmitted through aerosols,⁹ treating

nucleic acid-positive PCR results in stool as evidence of continued infection would be prudent. Because children show less susceptibility to SARS-CoV¹⁰ and have milder symptom than adults, evaluating the stool PCR results of children is an important step in controlling infection.

Our report presents a number of limitations. Because we only presented eight patients with mild to moderate infection, the data obtained may not be generalizable to all cases, especially severe ones. Second, lopinavir/ritonavir was used only in adult patients; as such, its role in children cannot be determined. Third, we cannot estimate the exact time at which the patients were exposed to the virus and when they began to shed the virus in their feces. Such data are urgently needed to understand the SARS-CoV-2 virus better and implement the necessary control strategies as early as possible.

The results of the present study revealed the novel finding that SARS-CoV-2 can be shed in the stool of some convalescent-phase patients. Thus, discharge criteria should be carefully evaluated, especially among children. Discharge of patients still infected with SARS-CoV-2 could increase the risk of infection to the public. Thus, patients should be released only after all their specimens are negative to reduce possible infection. Our results enhance the current knowledge on this emerging infectious disease and offer implications for clinical diagnostics and infection control.

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Declaration of Competing Interest

All authors report no conflicts of interest relevant to this article.

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