Review Article

Prolonged viral shedding in feces of pediatric patients with coronavirus disease 2019

Yu-Han Xing a,1, Wei Ni b,1, Qin Wu b, Wen-Jie Li b, Guo-Ju Li b, Wen-Di Wang b, Jian-Ning Tong b, Xiu-Feng Song b, Gary Wing-Kin Wong a,**, Quan-Sheng Xing b,*

a Department of Paediatrics, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China
b Qingdao Women and Children’s Hospital, Qingdao University, Qingdao, Shandong Province, China

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KEYWORDS
SARS-CoV-2; COVID-19; Pediatric patient; Fecal shedding

Abstract  Objective: To determine the dynamic changes of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in respiratory and fecal specimens in children with coronavirus disease 2019 (COVID-19).
Methods: From January 17, 2020 to February 23, 2020, three paediatric cases of COVID-19 were reported in Qingdao, Shandong Province, China. Epidemiological, clinical, laboratory, and radiological characteristics and treatment data were collected. Patients were followed up to March 10, 2020, and dynamic profiles of nucleic acid testing results in throat swabs and fecal specimens were closely monitored.
Results: Clearance of SARS-CoV-2 in respiratory tract occurred within two weeks after abatement of fever, whereas viral RNA remained detectable in stools of pediatric patients for longer than 4 weeks. Two children had fecal SARS-CoV-2 undetectable 20 days after throat swabs showing negative, while that of another child lagged behind for 8 days.
Conclusions: SARS-CoV-2 may exist in children’s gastrointestinal tract for a longer time than respiratory system. Persistent shedding of SARS-CoV-2 in stools of infected children raises the possibility that the virus might be transmitted through contaminated fomites. Massive efforts should be made at all levels to prevent spreading of the infection among children after reopening of kindergartens and schools.

* Corresponding author. No.6 Tongfu Road, 266000, Qingdao, China.
** Corresponding author. Lui Che Woo Clinical Sciences Building, Department of Paediatrics, Prince of Wales Hospital, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China.
E-mail addresses: wingkinwong@cuhk.edu.hk (G. Wing-Kin Wong), xinqs0532@163.com (Q.-S. Xing).
1 Contributed equally.

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Introduction

On December 12, 2019, 27 pneumonia cases of unknown cause emerged in Wuhan, Hubei Province, China. The etiological agent was identified as a novel pathogen and was later renamed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the world health organization (WHO). The pandemic of coronavirus disease 2019 (COVID-19) has wreaked havoc in China and spread rapidly to 188 countries and regions, constituting a global threat to public health if fitness for discharge is based on clinical recovery. Epidemiological evidence gained in China suggested that most individuals lack relevant immunity and are generally susceptible to the virus. The majority of published studies of COVID-19 focused on adult populations. While knowledge on SARS-CoV-2 infection in children are still yet to be fully developed and a limited number of studies on pediatric patients are currently available.

As of February 23, 2020, a cumulative total of 60 confirmed COVID-19 cases were reported in Qingdao, Shandong Province, China, of which three cases were children under 10 years of age. Notably, SARS-CoV-2 RNA was detected in the stool of these children 8–20 days after negative conversion of viral RNA in respiratory specimens. Whereas the majority of adult patients had negative results of nucleic acid testing in respiratory and fecal specimens synchronously. Prolonged presence of SARS-CoV-2 RNA in feces after showing negative in respiratory specimens may be an infectious source of COVID-19 in the community and a threat to public health if fitness for discharge is based on the current version of Diagnosis and Treatment Plan of Corona Virus Disease 2019 (“with normal body temperature for more than 3 days”, “with obvious features of absorption of inflammation shown in lung imaging” and “negative results of the nucleic acid tests of respiratory pathogens for consecutive two times [sampling interval at least 1 day]”). This study aimed to characterize the dynamic profiles of viral shedding in respiratory and fecal specimens in children with COVID-19.

Methods

Patients

From January 17, 2020 to February 23, 2020, a total of 60 patients were diagnosed with COVID-19 in Qingdao, Shandong Province, China. We recruited all three pediatric patients with laboratory confirmed SARS-CoV-2 infection who were reported by the local health authority. Diagnosis of COVID-19 was based on the WHO interim guidance. Patients were followed up on a regular basis after hospital discharge till March 10, 2020, the final date of follow-up.

This study was approved by the Ethics Commission of Qingdao Women and Children’s Hospital (QFFLL-KY-2020-11) and written informed consent was obtained from patients’ legal guardians prior to enrolment.

Data collection

Personal, clinical, laboratory, and radiological characteristics and treatment and outcomes information were obtained with standardized data collection forms from electronic medical records. Additionally, we directly contacted patients’ families to ascertain recent exposure history, epidemiological and symptom information. Data were entered into a computerized database and double-checked by two researchers independently.

Sample collection and detection of SARS-CoV-2

Throat swabs were obtained from patients on admission. Fecal specimens were first collected in two patients (case 1 and case 2) on day 4 after onset of the disease. While the stool sample from another patient (case 3) was obtained 9 days after hospital discharge. To monitor the dynamic changes of viral shedding, we obtained throat swabs from patients every day during hospitalization and every other day during follow-up after discharge. Fecal specimens were collected applying a similar rationale since the first day of sample collection (samples from case 3 were only collected during follow-up period). Presence of SARS-CoV-2 RNA was detected by real-time fluorescence reverse-transcriptase-polymerase-chain reaction (RT-PCR) using a commercial kit approved by the China Food and Drug Administration as described previously. The PCR assay simultaneously amplified two target genes of SARS-CoV-2 included open reading frame 1ab (ORF1ab) and nucleocapsid protein (N). A cycle threshold (Ct) value no more than 40 with evident amplification curve was considered as a positive test, and a value of 40 indicated the virus was molecularly undetectable. Ct values were used to approximately reflect the viral load (inversely related to Ct value) in respiratory tract and digestive system, a method suggested in recent studies on a similar topic.

Results

Transmission of SARS-CoV-2 within three familial clusters

Three pediatric patients were identified between January 17, 2020 and February 23, 2020. All the three pediatric cases had household contacts with adults whose symptoms developed earlier. Pedigree of the three familial clusters of COVID-19 is depicted in Fig. 1. None of these children had
travel history outside of Qingdao one month before onset of the disease and children’s infection occurred after the family members’ infection. There was no evidence showing the virus was transmitted from the children to others.

Clinical presentations, laboratory and radiological findings

Demographic information, clinical characteristics and treatment of the three pediatric patients are summarized in Table 1. Severity of disease was mild to moderate according to Diagnosis and Treatment Plan of Corona Virus Disease 2019 (tentative fifth/sixth edition).13,15 Fever (≥38.5 °C) was the most consistent and predominant symptom at onset of illness in all children. Case 2 had the highest body temperature of 40 °C; he also presented with symptoms of upper respiratory infection such as cough and runny nose. Case 2 was the only child who had gastrointestinal symptoms including abdominal pain and diarrhea. None of them developed severe complications nor required intensive care or mechanical ventilation. All children showed good response to anti-viral and supportive treatment including inhalation of interferon, oral Ribavirin and traditional Chinese medicine.

Laboratory findings of these children on admission to hospital are shown in Table 2. All children showed increased lymphocytes (>4.4 × 10^9/L) on admission. Only case 1 showed decreased neutrophil count (<1.7 × 10^9/L), whereas those of the other two children were within normal range. Elevation of platelets was observed in case 1 and case 2. Case 3 had increased levels of procalcitonin (0.73 ng/mL) and C-reaction protein (10.5 mg/L), while serum level of D-dimer (860.0 ng/mL) was found elevated in case 1. Reduced level of serum creatinine was detected in case 1 and case 2 (<41.0 μmol/L). Radiological changes were not typical for COVID-19 in these children (Fig. 2). No abnormality on chest radiograph was found in case 3 on admission or during hospitalization. On admission, transverse chest computed tomograms (CT) showed delicate patches of ground glass opacity of lower lobe of right lung in case 1 and consolidation changes of the left lower lobe near the pleura in case 2. Chest X-ray (CXR) taken up on admission showed increased bilateral lung markings in case 1 and patchy shadows over the left lower lung in case 3.

![Figure 1. Epidemiologic linkage of three pediatric COVID-19 cases.](image)

**Table 1** Baseline characteristics and treatment of pediatric patients with COVID-19.

| No    | Demographic Characteristics | Signs and Symptoms | Treatment       |
|-------|-----------------------------|--------------------|-----------------|
|       | Age (years) | Sex | Weight (kg) | Fever | Cough | Runny nose | Abdominal pain | Diarrhea | Interferon inhalation | Ribavirin | Traditional Chinese Medicine |
| Case 1 | 1.5     | Male | 11         | ●      | ●     | ●         | ○             | ○        | ●                  | ●          | ●                             |
| Case 2 | 5       | Male | 18         | ●      | ●     | ●         | ●             | ●        | ●                  | ●          | ●                             |
| Case 3 | 6       | Female | 25        | ●      | ○     | ●         | ○             | ○        | ●                  | ●          | ●                             |

●, yes; ○, no.
Persistent fecal shedding of SARS-CoV-2 in pediatric patients

Two pediatric patients (case 1 and case 2) and their family members with SARS-CoV-2 infection were admitted in Qingdao Women and Children’s Hospital. During clinical practice, we noticed the time for viral RNA in respiratory specimens turning negative was similar between pediatric patients and infected adults (these children’s family members). At this point, adult patients had negative results for nucleic acid testing in fecal specimens, whereas SARS-CoV-2 RNA remained detectable in stools of the two infected children. Therefore, we conducted a quarantine and surveillance protocol for the children and their family members who were already qualified for hospital discharge according to current standards (with two consecutively negative RT-PCR test results in throat swabs and sampling interval at least 1 day). Concerned about the possibility of fecal-oral transmission, we recorded the timeline of changes in nucleic acid testing results in both throat swabs and fecal samples collected from these patients (Fig. 3). All adult patients of the two families were negative for fecal SARS-CoV-2 RNA detection. Strikingly, RT-PCR results remained positive in stools of the two children for 8 and 20 days, respectively, after nucleic acid turning negative in respiratory samples. Consistently, Ct values of the serial fecal and respiratory specimen test suggested the gastrointestinal tract might shed virus for a longer duration than the respiratory system with a greater load in case 1 (Fig. 4). While viral RNA measurements were not available for the other two children as they were either transferred or admitted in another hospital where the laboratory only reported positive or negative results for RT-PCR testing of SARS-CoV-2 without additional information.

We suspected whether this phenomenon also existed in the third pediatric patient (case 3, 6-year-old female) who was admitted in another designated hospital in Qingdao. Unfortunately, by the time we reached the patient, this child had already been “cured” and discharged. Medical professionals of this hospital did not collect fecal specimens from the patient and they only performed RT-PCR testing for throat swabs plus CT according to Diagnosis and Treatment Plan of Corona Virus Disease 2019 (tentative fifth edition). Municipal Centre of Disease Control and Prevention of Qingdao conducted nucleic acid testing in stool samples collected from the girl and her family members 9 days after hospital discharge. This 6-year-old girl showed positive results for RT-PCR analysis in feces. During quarantine and follow-up period, clearance of SARS-CoV-2 in stool samples occurred 20 days after viral RNA in respiratory specimens turning negative. Detailed information was not available as no fecal sample was examined during hospitalization.

Discussion

The newly issued report of the WHO-China Joint Mission on COVID-19 summarized the evidence so far on SARS-CoV-2 and pointed out 2.4% of those infected were individuals below 18 years of age. According to data released by the China Centers for Disease Control and Prevention, only 0.9% of COVID-19 patients were children under the age of 10 years. Among the 60 patients confirmed with SARS-CoV-2 in Qingdao, three cases were children younger than 10 years of age.

In general, pediatric patients of COVID-19 had mild disease with good treatment response and a relatively short time to resolution. Consistent with previous findings, the three infected children in our study only presented with fever and mild cough or with no obvious symptom but non-typical radiological abnormalities. From available data, children appeared to be slightly affected by SARS-CoV-2, a feature resembling that of SARS-CoV emerged 17 years ago. However, the relatively low attack rate of COVID-19 in children could be explained by the stringent implementation of home confinement and prolonged school closure during the outbreak as required by the Chinese governments. Whether it is also the case during school year is hard to tell. One distinct feature of pediatric cases is that the majority of them are infected through household contact with adult patients. As with all new diseases, many characteristics of COVID-19 still remain unknown. There is a paucity of data to support the notion that children are less susceptible to SARS-CoV-2 infection or virus transmission is less effective among them.
Viral RNA has been constantly detected in stool samples
and anal swabs collected from confirmed cases of COVID-
19. In one study, fecal specimens from 9 (53%) of
17 patients were positive for nucleic acid testing, although
viral loads of the stool were less than those of respiratory
samples. Presence of SARS-CoV-2 was even detected in
environmental samples taken from the surface of toilet
bowl and sink in infection isolation rooms. Moreover,
SARS-CoV-2 remained viable in stools of infected patients as
demonstrated by some case reports. Evidence so far indi-
cates the potential for SARS-CoV-2 to be transmitted
through contaminated fomites. Although the role of fecal
shedding in viral transmission has not been systematically
determined, a cautious approach should be considered
when conducting aerosol-generating procedures.

While we were closely monitoring the dynamic changes
of fecal SARS-CoV-2 in our patients, a study conducted in
Guangzhou, Guangdong Province, China, reported eight out
of ten pediatric patients of COVID-19 had prolonged pres-
ence of viral RNA in rectal swabs after showing negative in
nasopharyngeal swabs. The researchers also found that
viral load of SARS-CoV-2 in the gastrointestinal tract might
be greater and last longer than that in the respiratory
system, a finding also in line with our study. Xiao and col-
leagues demonstrated that about a quarter of COVID-19
patients had SARS-CoV-2 RNA detectable in feces after

Figure 2. Transverse chest computed tomographic images.

Figure 3. Dynamic profile of SARS-CoV-2 RNA in respiratory and fecal specimens.
viral clearance in respiratory tract. However, these researchers did not address whether persistent shedding of SARS-CoV-2 was more common in certain age group than the others (patient’s ages ranged from 10 months to 78 years in this study). Therefore, patients who are discharged on the basis of current guidelines depending on respiratory tract test might be a potential source of viral transmission and follow-up of recovered patients during convalescent phase is advisable.

In the context of emerging infectious disease and limited data on pediatric patients, it is unclear what role children play in SARS-CoV-2 transmission or to what extent children are affected by the virus. More attention should be drawn to children, especially for young children who cannot handle their own excretions. Caregivers of these children should take precautionary measures and avoid direct contact with children’s waste. During the SARS outbreak of 2002–2003, viral RNA was detected in the sewage water from hospitals receiving SARS patients. In some rural areas and underdeveloped regions with poor sanitation in China, stools are often used as natural fertilizer in agriculture. As sewage cannot be properly disinfected in these places, it might be possible that SARS-CoV-2 polluted water causes infection in wild animals which bring the virus back to humans, creating a vicious cycle. Strict hygiene measures are needed after reopening of kindergartens and schools to prevent spreading of the infection among preschool and schoolchildren.

The limitations of this study should also be noted. In response to the emerging disease, only respiratory specimens were required for the detection of SARS-CoV-2 according to clinical guidelines in the early stage of COVID-19 outbreak. We therefore failed to obtain stool samples from the patients during their first few days of hospitalization and could not determine whether throat swabs and fecal samples showed positive on RT-PCR analysis simultaneously. Moreover, we did not culture the virus isolated from the feces to test the viability nor accurately measure viral loads in the samples due to limited conditions. We only used the Ct values to approximately reflect the viral loads in case 1 who were initially admitted to Qingdao Women and Children’s Hospital. While viral RNA measurements were absent for the other two children as case 2 was a transferred case and case 3 was admitted in another hospital. Laboratories of other hospitals in Qingdao and Qingdao Municipal Centre for Disease Control and Prevention only reported positive or negative results for RT-PCR testing of SARS-CoV-2 without additional information.

Conclusions

Taken together, SARS-CoV-2 may exist in gastrointestinal tract for a longer time than respiratory system. Detection of SARS-CoV-2 in fecal specimens should be considered as one of the routine diagnostic tests to guide decision-making on hospital discharge and release of isolation. Although we urge to re-evaluate the current discharge guideline based on three cases, multi-center studies with larger sample size would be warranted to verify our findings and further the available knowledge on pediatric patients.

Postscript

We reported to the local health authority immediately once we noticed the persistent shedding of SARS-CoV-2 in feces of our pediatric patients about three weeks ago. When this paper was finalized, the seventh edition of *Diagnosis and Treatment Plan of Corona Virus Disease* 2019 had just been issued and indicated the possibility of fecal-oral transmission of SARS-CoV-2. Currently, capable hospitals in some parts of China (such as Shanghai, Guangdong Province, and Shandong Province) have already included negative nucleic
acid testing result of fecal specimens as one of the standards for hospital discharge and release of isolation.

Author contributions

The corresponding authors have full access to all data in this study and attest that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. Q-SX and G-W designed the study and conceptualized the paper. WN, QW, G-JL, W-DW, J-NT and X-FS collected the epidemiological, clinical, laboratory and radiological data. WL contributed to laboratory testing. WN collected the epidemiological, clinical, laboratory and radiological data. Y-HX, WN and QW wrote the initial draft of the manuscript. All authors provided critical feedback and approved the final version.

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Patient consent for publication

Obtained.

Data sharing

After publication, the data that support the findings of this study will be made available from the corresponding author on reasonable request. It will be necessary to provide a proposal with detailed description of study objectives and statistical analysis plan for evaluation of the reasonability of requests. Additional materials might also be needed during the process of evaluation. Deidentified participant data will be provided after approval from the corresponding author Quansheng Xing, Qingdao Women and Children’s Hospital, Qingdao University, Qingdao, Shandong Province, China, No.6 Tongfu Road, 266000, Qingdao, China. xingqs0532@163.com.

Ethical approval

This study was approved by the Ethics Commission of Qingdao Women and Children’s Hospital (QFFLL-KY-2020-11).

Declaration of Competing Interest

None reported.

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