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SHORT COMMUNICATION

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# SARS-CoV-2 RNA detection in stool samples from acute gastroenteritis cases, Brazil

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## Abstract

We described the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in stool samples from patients presenting only acute gastroenteritis (AGE) symptoms. From January to July 2020, 121 AGE stool samples were screened by quantitative reverse-transcription polymerase chain reaction. We detected SARS-CoV-2 in 27.5% of samples received during the epidemic period. No infectious viruses were observed in Vero E6 cells.

**KEYWORDS**

acute gastroenteritis, Brazil, children, SARS-CoV-2, stool samples, young adults

## 1 | INTRODUCTION

Several countries around the world are facing one of the worst public health crises since the 1918 Influenza (H1N1) and AIDS (HIV-1) pandemics. In late 2019, the discovery of a novel coronavirus in China, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes the coronavirus disease 2019 (COVID-19), triggered an unprecedented pandemic that reached the 1-million milestone of confirmed deaths on September 28, 2020, according to the Coronavirus Resources Center, Johns Hopkins University & Medicine in Maryland, USA.[1] In addition, the ongoing pandemic has hugely impacted countries health system and economy. In Brazil, over 145,000 COVID-19-related deaths have been officially confirmed as of October 5, and the country figures as the world's second worst in number of lost lives.[2]

SARS-CoV-2 is a positive-sense single-stranded RNA virus, within the family *Coronaviridae*, genus *Betacoronavirus*.[3] Coronaviruses are usually related with respiratory and intestinal infections in animals and humans.[4] Specifically, SARS-CoV-2-infected patients can hugely vary regarding disease symptoms. Most people who develop COVID-19 will show only moderate disease. However, a small fraction of patients will develop serious complications, progressing from severe pneumonia to acute respiratory distress syndrome, septic shock, and/or multiple organ failure.[5,6] Whilst most of cases present as a classical respiratory disease, COVID-19 can include gastrointestinal, neurological, renal, and cardiovascular symptoms, as well as other complications. SARS-CoV-2 has been detected in organs other than the lungs, including the kidneys, liver, heart, brain, and blood.[7] Several studies have demonstrated the presence of the SARS-CoV-2 in stool

  

samples from patients with or without diarrhea-associated symptoms.[8,9]

## 2 | MATERIAL AND METHODS

Brazil's first COVID-19 case and death was respectively confirmed on February 25th and March 17th, 2020. From April onwards, it was observed a rapidly increase in the number of confirmed cases and deaths (Figure 1). In the present study, we sought to investigated SARS-CoV-2 RNA in stool samples from acute gastroenteritis (AGE) cases before and during the COVID-19 epidemic in Brazil. For this purpose, we screened SARS-CoV-2 RNA in a total of 121 stool samples received between January and July 2020, previously tested negative for rotaviruses A (RVA) and noroviruses. Stool samples were retrospectively tested from the AGE biospecimen repository obtained from the Regional Rotavirus Reference Laboratory–Laboratory of Comparative and Environmental Virology. The laboratory is part of the ongoing national network for viral AGE surveillance and receives stool samples from eleven Brazilian states, coordinated by General Coordination of Public Health

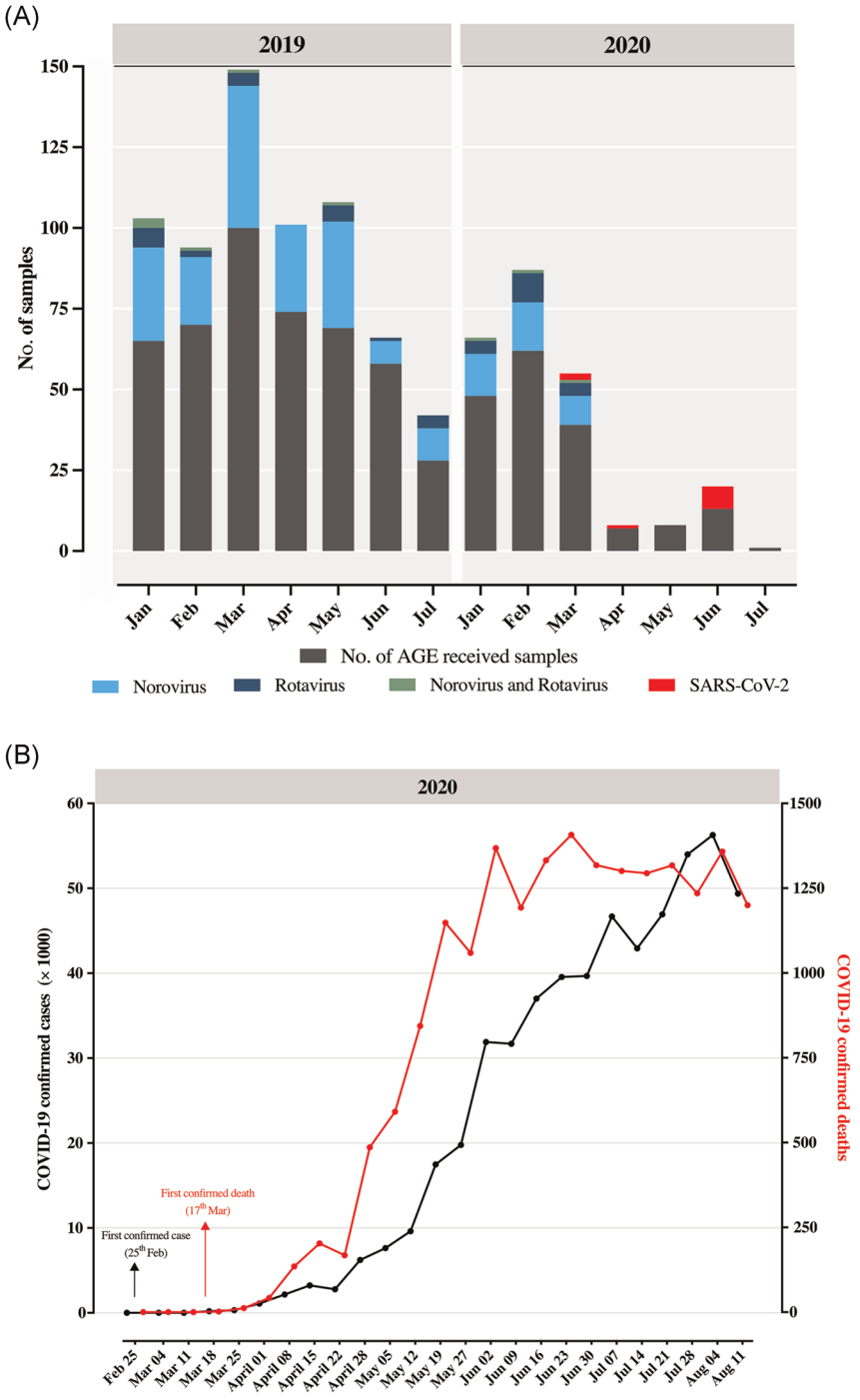


**FIGURE 1** Detection of norovirus, rotavirus and SARS-CoV-2 among acute gastroenteritis cases in 11 states in Brazil in the first semester of 2019 and 2020 (A). Number of COVID-19 confirmed cases and deaths in Brazil from late February to August 2020 (B). COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

Laboratories (CGLAB), Brazilian Ministry of Health. AGE surveillance is performed through a hierarchical network in which samples are provided by medical request in hospitals and health centers, monitored by the Brazilian Unified Health System. Patients' data were maintained anonymously and securely. This study is approved by the Ethics Committee of the Oswaldo Cruz Foundation (FIOCRUZ), approval no. CAAE: 94144918.3.0000.5248.

SARS-CoV-2 RNA was detected and quantified using a quantitative reverse-transcription polymerase chain reaction (RT-qPCR) with US CDC 2019-nCoV_N1 and N2 primers and probe sets synthesized by Integrated DNA Technologies (https://www.idtdna.com), as previously described.[10] Viral RNA was extracted from 140 μl of clarified stool suspension (10% w/v) using a QIAamp® Viral RNA Mini kit (QIAGEN, https://www.qiagen.com) and a QIAcube® automated system (QIAGEN), and eluted in a final volume of 60 μl. Quantitative one step PCR (RT-qPCR) was performed in a final volume of 20 μl with the SuperScript™ III Platinum™ One-Step RT-qPCR Kit (Thermo Fisher Scientific, https://www.thermofisher.com) in the Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific) using 5 μl of purified RNA. Samples that crossed the threshold line under 38 cycles (threshold [$C_t$]) < 38 were considered as positive. RVA and noroviruses were detected by using a TaqMan®-based RT-qPCR assays, with primers and probe targeting the conserved NSP3 segment and ORF1/2 junction region, respectively, as previously described.[11,12]

Positive stool samples, stored at −80°C, were processed for viral isolation in cell culture inside a biosafety Level 3 facility. Briefly, stool suspensions were filtered (0.22 μm, Millex-GP Syringe Filter Unit, Millipore) and treated with 1% penicillin/streptomycin (P/S) and 0.5 μg/ml Amphotericin B before inoculation. Vero E6 cells, maintained in Modified Eagle's Medium supplemented with 5% fetal bovine serum, 1% penicillin/streptomycin (P/S), 0.5 μg/ml Amphotericin B and 1% L-glutamine, were inoculated and incubated at 37°C with 5% $CO_2$ for 72 h. Following incubation of 3 days and up to the third passage, cytopathic effect (CPE) was evaluated under a microscope. Additionally, to check for SARS-CoV-2 replication, supernatant from the three passages were collected, RNA extracted and tested using RT-qPCR, as described above.

## 3 | RESULTS AND DISCUSSION

From the 121 stool samples, we detected SARS-CoV-2 RNA in samples from 10 patients. Positive samples were retested (RNA extraction and RT-qPCR detection), and SARS-CoV-2 RNA positivity was confirmed in all samples with minor variations of Ct values. Positive samples were collected in March ($n = 2$), April ($n = 1$), and June ($n = 7$; Figure 1). If we consider the AGE stool samples received from April to July 2020 ($n = 29$), when the number of COVID-19 cases took off in Brazil, SARS-CoV-2 RNA was detected in 27.5% of samples. Interesting, the number of AGE cases reported in 2020 was lower compared with 2019 (Figure 1). Especially from April onwards, we observed a sharp decrease in the number of AGE reported cases compared with the same period of the previous year. Also, we did not detect neither RVA or noroviruses amongst the AGE samples received in this period. The low number of AGE cases recorded from April to July coincides with the period when each state in Brazil has introduced strict measures to prevent further spread of SARS-CoV-2, such as cities lockdowns and shutdown of public and private schools of all levels of education. As a major proportion of viral AGE cases and outbreaks are related with children in childcare facilities and schools, the shutdown of these "hotspots" settings certainly has contributed to the decrease in the number of reported cases. Nevertheless, as the health system was drowned having patients with COVID-19, even in case of children with diarrhea symptoms, it is less likely that parents would visit health units to report and get support, especially in mild cases. Song et al.[13] reported a clinical case of a young

**TABLE 1** Clinical and virological data of all SARS-CoV-2 infected patients analyzed from January to July 2020 in Brazil

| | Epidemiological features | | | | | SARS-CoV-2 detection | | | |
|---|---|---|---|---|---|---|---|---|---|
| Patient ID | Symptom onset | Collection date | Gender | Age | State | N1 Ct value | Viral load | N2 Ct value | Viral load[a] |
| 30759 | 03/01/2020 | 03/04/2020 | Female | 06 m | Rio Grande do Sul | 36.8 | $4.9 \times 10^3$ | ND | ND |
| 30753 | 03/20/2020 | 03/26/2020 | Female | 13 m | Rio Grande do Sul | 37.0 | $4.3 \times 10^3$ | ND | ND |
| 30746 | 04/24/2020 | 04/28/2020 | Female | 08 m | Rio de Janeiro | 32.4 | $8.7 \times 10^4$ | 35.1 | $1.5 \times 10^4$ |
| 31222 | 06/23/2020 | 06/29/2020 | Female | 13 m | Bahia | 36.6 | $5.6 \times 10^3$ | ND | ND |
| 31224 | 06/15/2020 | 06/18/2020 | Male | 31 y | Espírito Santo | 35.1 | $1.5 \times 10^4$ | ND | ND |
| 31225 | 06/15/2020 | 06/18/2020 | Male | 30 y | Espírito Santo | 34.3 | $2.5 \times 10^4$ | 37.8 | $2.6 \times 10^3$ |
| 31226 | 06/17/2020 | 06/18/2020 | Male | 24 y | Espírito Santo | 30.9 | $2.3 \times 10^5$ | 35.6 | $1.1 \times 10^4$ |
| 31227 | 06/15/2020 | 06/18/2020 | Male | 35 y | Espírito Santo | 29.8 | $4.7 \times 10^5$ | 34.9 | $1.7 \times 10^4$ |
| 31228 | 06/17/2020 | 06/18/2020 | Male | 37 y | Espírito Santo | 36.9 | $4.6 \times 10^3$ | ND | ND |
| 31230 | 06/15/2020 | 06/18/2020 | Male | 33 y | Espírito Santo | 31.5 | $1.5 \times 10^5$ | 34.3 | $2.5 \times 10^4$ |

Abbreviations: Ct, cycle threshold; date, month/day/year; m, month; ND, not detected; SARS-CoV-2, severe acute respiratory syndrome; y, year.
[a]Viral load expressed as genome copies per gram of stool.

adult male patient showing diarrhea as the only onset symptom that was later confirmed positive for SARS-CoV-2. In this same line, Chan et al.[14] investigating a family cluster of pneumonia associated with SARS-CoV-2, demonstrated that the two younger adults initially had diarrhea. Previous studies showed that up to 30% of patients infected with Middle East respiratory syndrome coronavirus and 10.6% with SARS coronavirus (SARS-CoV) also had diarrhea.[15,16]

Among the 10 SARS-CoV-2 positive stool samples, four were children under 2 year old and six were from young adults (Table 1). The $C_t$ values varied from 29.8 to 37 and from 34.3 to 37.8 for N1 and N2 assays, respectively, but N2 set failed to detect five of N1-positive samples with $C_t > 35$. Unfortunately, no information regarding respiratory clinical signs, or molecular diagnostic of SARS-CoV-2 in other clinical specimens was available. In attempt to investigate the presence of infectious SARS-CoV-2 in the stool samples, four positive samples that presented low $C_t$ values (from 29.8 to 32.4) were submitted to virus isolation in Vero E6 cell cultures. No CPE was observed after three passages for any of the tested samples, and all the supernatant samples collected from the passages tested negative, indicating no viral replication and all samples were considered negative for SARS-CoV-2 in vitro infectiousness. Bullard et al.[17] demonstrated that SARS-CoV-2 Vero cell infectivity was only observed for nasopharyngeal swab samples showing Ct < 24. One study reported the detection of infectious SARS-CoV-2 from feces of an intubated patient who died of COVID-19.[18] The Ct value of the fecal sample was 20.82 (nucleoprotein gene) and visible CPE was observed in Vero E6 cells after 48 h after a second-round passage. So, low viral titers observed in the SARS-CoV-2 positive samples from our study may explain the in vitro noninfectiousness results.

## 4 | CONCLUSIONS

We describe the investigation of SARS-CoV-2 in patients showing symptoms of AGE in Brazil. We observed that among the AGE samples received from April to July, 27.5% were positive for SARS-CoV-2 by RT-qPCR. No evidence of infectious viruses was found. All SARS-CoV-2 positive samples were from children or young adults, the age group characterized to develop mild COVID-19 symptoms or asymptomatic infection. It is worth mentioning that we did not have access to information if patients develop respiratory symptoms after AGE disease. Increase testing, contact tracing and quarantine are essential to prevent the spread of SARS-CoV-2. Our findings reinforce the need to increase attention to gastrointestinal symptoms as an early sign of SARS-CoV-2 infection, particularly among children and young adults highly exposed, as for example attending school classes. In addition, efforts on virus isolation from stool samples should be encouraged to investigate the possibility of fecal–oral transmission.

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## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

## AUTHOR CONTRIBUTIONS

**Tulio M. Fumian**: Design of study and conceptualization; data analysis; funding acquisition and writing – original draft. **Fábio C. Malta**: Laboratory experiments; data analysis and writing – review & editing. **Débora R. L. dos Santos** and **Alex Pauvolid-Corrêa**: cell culture NB3 experiments; data analysis and writing – review & editing. **Alexandre M. Fialho**: Laboratory experiments; database organization & analysis and writing – review & editing. **José P. G. Leite** and **Marize P. Miagostovich**: Funding acquisition and writing – review & editing.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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