Journal of INDEPENDENT MEDICINE

Detection of SARS-CoV-2 from Patient Fecal Samples by Whole Genome Sequencing

Andreas Papoutsis[1], Thomas Borody[2], Siba Dolai[2], Jordan Daniels[1], Skylar Steinberg[1], Brad Barrows[1], Sabine Hazan[1,3]

Abstract

Background: SARS-CoV-2 has been detected not only in respiratory secretions, but also in stool collections, by RT-PCR, thus herein, we sought to identify faecal SARS-CoV-2, and its variants, via next-generation sequencing (NGS).

Methods: Fecal samples from COVID patients were collected and whole genome analysis was used to detect and characterize SARS-CoV-2 mutational variations. Of note, NGS detects genomic RNA sequences, not infectious agents.

Results: Study participants underwent testing for SARS-CoV-2 from fecal samples by whole genome enrichment NGS (n = 14), and RT-PCR nasopharyngeal swab analysis (n = 12). The concordance of SARS-CoV-2 detection by enrichment NGS from stools with RT-PCR nasopharyngeal analysis was 100%. Unique variants were identified in four patients, with a total of 33 different mutations among those in which SARS-CoV-2 was detected by whole genome enrichment NGS.

Conclusion: These results highlight the presence of SARS-CoV-2 in faeces, that is, the presence of SARS-CoV-2 particles in the GI tract leads to their shedding in faeces. Further studies on virus viability are needed to attest its possible role in fecal-oral transmission. These findings are exploratory as they are based on a small sample size.

Keywords: Coronavirus, SARS-CoV-2, COVID-19, NGS, fecal-oral transmission, stools

Introduction

In December 2019, the virus, which would cause a worldwide pandemic, was first identified in the city of Wuhan, China. (1) In January 2020, it was implicated in various pneumonia cases, and was rapidly isolated from a bronchoalveolar lavage sample, analyzed via next-generation sequencing (NGS), and identified to be a novel betacoronavirus, (2,3) the same family of viruses responsible for severe acute respiratory syndrome (SARS) and middle east respiratory syndrome (MERS). (4–6) This new virus, named the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on February 11, 2020, by the International Committee on the Taxonomy of Viruses (ICTV), has now been implicated in over 13.4 million cases worldwide, and over 580,000 deaths. (7,8) Over 138,000 of these deaths have been in the United States alone.

Early diagnosis of SARS-CoV-2 has been challenging and may contribute to its high mortality rates. Due to the similarities between SARS-CoV-2 and SARS-CoV, a similar diagnostic approach utilizing real time polymerase chain reaction (RT-PCR) from nasopharyngeal swabs has been adopted as the standard of care. (9) Contributing to the spread of

[1.] ProgenaBiome, Ventura, CA, USA
[2.] Centre for Digestive Diseases, Sydney, Australia
[3.] Ventura Clinical Trials, Ventura, CA, USA

Corresponding author:
Sabine Hazan, MD
ProgenaBiome and Ventura Clinical Trials, Ventura, CA, USA
Email: drhazan@progenabiome.com

the disease, has been the viral load being below the threshold of detection in some individuals. (10) Additionally, acquired mutations may assist in its evasion of detection from specifically targeted PCR primers. (11) Further, samples collected soon after infection, or after symptoms have resolved, have resulted in high false negative rates. Samples collected in these timeframes have been shown to have false-negative rates as high as 33%. (12) The exact rate of false positives for SARS-CoV-2 varies depending on many factors, as reviewed in Braunstein et al., 2021. (13)

Symptoms of SARS-CoV-2 infection cover a broad range including fever, malaise, body aches, chills, headaches, cough, and shortness of breath, as well as gastrointestinal symptoms such as diarrhea, nausea, and vomiting. (14,15) Research from SARS and MERS has shown that coronaviruses can be present in stools of infected patients. Wang et al in 2005 demonstrated that SARS-CoV was not only present in stool samples collected from patients, but also in the wastewater of two hospitals. (16) In a review of these data, with inclusion of SARS-CoV-2, a meta-analysis by Parasa et al. found that 40% of patients with positive RT-PCR for SARS-CoV-2 from nasopharyngeal swabs or respiratory secretions were also found to be positive from stool samples. (17) These data were supported by two recent studies documenting positive SARS-CoV-2 fecal samples from "recovered" COVID-19 patients with negative nasopharyngeal swabs. (18,19)

In this study, we sought to 1. Identify the presence of the SARS-CoV-2 by next-generation sequencing (NGS) in faecal samples of symptomatic study participants positive for SARS-CoV-2 by nasopharyngeal sample RT-PCR, in addition to asymptomatic individuals (with or without prior nasopharyngeal sample RT-PCR); and 2.To execute whole genome analysis to characterize SARS-CoV-2 mutational variations to identify potentially significant nucleotide changes.

Methods

Study participants

Study subjects were recruited from a GI clinic, where they attended either for regular check-ups or for treatment of GI symptoms. Inclusion criteria were no symptoms reported, the presence of GI symptoms and/or COVID symptoms, and aged 18 yrs. and older. Participants were excluded if younger than 18 yrs. old and if symptoms were severe enough to require hospitalization. A total of n = 14 consecutive participants who attended the GI clinic underwent testing for SARS-CoV-2 from fecal samples by whole genome enrichment NGS. Of the 14 study participants, ten were symptomatic. Participants self-reported their symptoms had been present for an average of 5 days prior to the GI clinic visit.

Stool samples and NGS

Stool samples were collected on the first day of the GI clinic visit, except for 3 patients who received treatment *prior* to stool sample collection. Following fecal collection (Zymo Research Shield Fecal Collection Tubes), RNA was extracted (Qiagen Allprep Power Viral Kit), reverse transcribed (New England Biolabs NEBNext 1st and 2nd Strand Synthesis Modules), library prepped (Illumina Nextera Flex for Enrichment), enriched (Ilumina Respiratory Virus Oligo Panel), and sequenced on Illumina's NextSeq 550 System. Sequences were then mapped to the SARS-CoV-2 Wuhan-Hu-1 (MN90847.3) complete genome utilizing One Codex's SARS-CoV-2 bioinformatics analysis pipeline. SARS-CoV-2 positive samples were further analyzed for mutational variants that differed from the reference genome. Of the 14 study participants, 12 also had their nasopharyngeal swabs tested for SARS-CoV-2 by RT-PCR.

Results

We evaluated the results from patients that had their stool samples tested by whole genome enrichment NGS, and their nasopharyngeal swabs tested by RT-PCR for the presence of SARS-CoV-2. Of the 14 study participants, ten were symptomatic and tested positive for SARS-CoV-2 by RT-PCR, two asymptomatic individuals tested negative, and two other asymptomatic individuals did not undergo RT-PCR testing (**Table 1**). Patients 5 and 7, that tested positive by RT-PCR from nasopharyngeal swabs, were treated with Hydroxychloroquine (HCQ), Azithromycin, vitamin C, vitamin D, and zinc for 10 days prior to fecal collection. Similarly, after positive nasopharyngeal swab, patient 13 was treated with vitamin C, vitamin D, and zinc for 10 days before fecal collection. The concordance of SARS-CoV-2 detection by enrichment NGS from stools among positive non-treated patients tested by RT-PCR nasopharyngeal analysis was 100% (7/7), though only applicable to untreated symptomatic positives. Patient 8, who did not undergo nasopharyngeal analysis, tested positive for SARS-CoV-2 by NGS. The three patients (5, 7, 13) that received treatment prior to providing fecal samples, all tested negative by NGS.

Asymptomatic patients 2 and 9, who tested negative by nasopharyngeal swab, were also negative by NGS, as was asymptomatic patient 14. However, these results are anecdotal and cannot imply treatment efficacy.

All fecal samples analyzed by enrichment NGS from positive patients by RT-PCR, achieved 100% genome coverage of SARS-CoV-2 (though only applicable to untreated symptomatic positives, n=7), except for patient 3 which had 45%, and patient 10 which had 93% coverage (**Table 2**). The total number of SARS-CoV-2 mapped reads for patients 1, 3, 4, 6, 8, 10, 11, and 12 were 465645, 5984, 131582, 793603, 496852, 5929, 1270734, and 38256 respectively. The mean read depths of SARS-CoV-2 for patients 1, 3, 4, 6, 8, 10, 11, and 12 were 1129.8x, 31.7x, 318.6x, 1924.6x, 1206.7x, 15.5x, 3075.3x, and 92.7x respectively. The read depths at specific coordinates along the SARS-CoV-2 genome for each patient are captured in **Figure 1**.

Following alignment and mapping of SARS-CoV-2, patient genomes were compared to the Wuhan-Hu-1 (MN90847.3) SARS-CoV-2 reference genome via One Codex's bioinformatics pipeline to identify mutational variations. This analysis identified nucleotide variants at positions nt241 (C → T) and nt23403 (A → G) across all positive patients, and variants at positions nt3037 (C → T) and nt25563 (G → T) in seven of the eight patients (**Table 3**). Interestingly, patients 8, 11, and 12 harbored the same set of variants, as did patients 4 and 6 (who were kindreds). Unique variants not identified in any of the other individuals were detected in patients 1, 3, 6, and 10, with patient 3 harboring the most distinct SARS-CoV-2 genome with eight unique variants, followed by patient 1 with seven. However, Patient 3 had only 45% genome coverage, thus the number of variants could have been due to artifact interpretation, including possible false positives. Collectively, the thirty-three different mutations among the patients in which SARS-CoV-2 was detected by whole genome enrichment NGS, may suggest the molecular evolution of SARS-CoV-2, however, these data remain preliminary, as sequencing limitations were encountered.

Discussion

Coronaviridae is a family of enveloped, single-stranded, positive-sense RNA viruses. (20,21) The total length of the genome is 30 Kb, consisting of a 5'-terminal noncoding region, an open reading frame (ORF) 1a/b-coding region, an S region encoding the spike glycoprotein (S protein), an E region encoding the envelope protein (E protein), an M region encoding the membrane protein (M protein), an N region encoding the nucleocapsid protein (N protein), and a -3'-terminal noncoding region. (22–25) Among them, the poly protein encoded in the ORF1a/b region of the nonstructural protein can be cut by 3CLpro and PLpro of the virus to form RNA-dependent RNA polymerase and helicase, which guides the replication, transcription, and translation of the virus genome. The M and E proteins are involved in the formation of the envelope, while the N protein is involved in assembly. The spike protein binds to the receptor of the host cell and confers specificity for viral invasion into susceptible cells.

Once decoded, the SARS-CoV-2 genome was found to share high sequence identity with the bat coronavirus, BatCoV RaTG13 (96.2%). (2) Upon further investigation, it was discovered that SARS-CoV-2 harbored significant sequence homology with the viruses responsible for SARS and MERS, with a notable exception found in the receptor binding domain (RBD). (26) Shang et al. elucidated the RBD structure of the human ACE2 receptor (angiotensin-converting enzyme 2), (27) demonstrating that the replacement of several residues within the protein caused it to have a much more compact hydrophobic pocket. This change increased the binding affinity of SARS-CoV-2 to ACE2 as compared to SARS-CoV. While this has contributed to its greater virulence, it also represents a potential therapeutic target (28). This concept was detailed in the mini-review by Yang and Shen, wherein they proposed that SARS-CoV-2 may be susceptible to the inhibitory effect of chloroquine (CQ), a lysosomotropic agent, via its accumulation in the acidic organelles. (29) The therapeutic effect of CQ may be a result of its ability to neutralize the endosome-lysosomal acidic pH and block the protease activity necessary for viral entry. (30) However, the efficacy of HCQ to prevent and/or treat COVID remains controversial. (31) Adding to the complexity of COVID-19 treatment and prevention, is that SARS-CoV-2 appears to be mutating at an alarming rate, as reported in the Icelandic study which identified the presence of 291 sequence variants that were not present in the Global Initiative on Sharing All Influenza Data (GISAID) reference database as of March 22. (11)

SARS-CoV-2 can enter the gut via respiratory droplets and the gastrointestinal (GI) tract, where the virus infects cells in the gut lining. Once in the GI tract, the virus uses the angiotensin-converting enzyme 2 (ACE2) receptor, which is present on the surface of enterocytes, to enter and infect them. Tis-

sues of both the respiratory and the gastrointestinal (GI) tracts have the highest expression of ACE2, and therefore a high sensitivity to infection. (32) One study found that ACE2 expression is indeed higher in the GI tract compared to the respiratory tract of humans. (33) Thus, the presence of SARS-CoV-2 particles in the GI tract leads to their shedding in faeces.

Although previous studies have identified SARS-CoV-2 in fecal collections by RT-PCR, (34,35) this is the first, to our knowledge, to report whole genome sequencing (WGS) of SARS-CoV-2 from stool samples. Herein, we were able to identify SARS-CoV-2 in patients that tested positive by nasopharyngeal swab RT-PCR analysis and observed unique genomes in 62.5% of the NGS positive patients. The overall homology among the genomes was high (99.97%), with variations identified in the ORF regions 1a, 1b, S, 3a, 8, and N. Of particular interest, was the adenine to guanine change in the S protein at position nt23403 which converts aspartic acid to glycine. Although the significance of this variation is unclear, it warrants further investigation to understand its effect on spike glycoprotein ACE2 binding and virulence. The conversions of glycine to arginine (nt28883) and proline to arginine (nt29364) in the nucleoprotein also necessitate further examination. However, viral viability was not tested, nor confirmed, thus, this study cannot distinguish between detection and infectivity. Other limitations include the small sample size, the genome coverage was inconsistent, and the sampling was non-longitudinal.

Conclusion

Next generation sequencing identified the SARS-CoV-2 whole genome sequence in patients with positive nasopharyngeal RT-PCR and did not detect it in treated patients, or those with negative RT-PCR. Further research is needed to confirm these findings, with larger future studies to include viral culture and longitudinal testing.

Ethics approval and consent to participate

This study was approved by the institutional review board of Advarra. Consent to participate in this study from each patient was agreed via signed informed consent.

Consent for publication

Agreed to by patients on the signed informed consent, demonstrating that the patient understood the procedures and the purpose of the study.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from

the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by ProgenaBiome.

Authors' contributions

Conceptualization, AP and SH; Methodology, AP and SH; Investigation, AP and SH; Writing-original draft, AP; Writing-review and editing, AP, TB, SD, JD, SS, BB, and SH; Supervision, SH; Funding acquisition, SH. All authors read and approved the final manuscript.

Acknowledgements

We thank Dr. Alan Miller at Alta Pharmaceuticals for contributing to providing patient samples.

Table 1. Symptoms and SARS-CoV-2 testing results

Sample ID	Symptoms	Nasopharyngeal Swab (RT-PCR) Ct≤38	Treated	Fecal (NGS)	Patient Location
Patient 1	febrile, diarrhea, anosmia, O2 sat. <90%	+	no	+	PA
Patient 3	febrile, diarrhea, O2 sat. <90%	+	no	+	CA
Patient 4	febrile, diarrhea, anosmia, O2 sat. <90%	+	no	+	AZ
Patient 6	febrile, cough, anosmia	+	no	+	AZ
Patient 8	none	n/a	no	+	CA
Patient 10	febrile, cough, headache	+	no	+	GA
Patient 11	febrile, cough, headache	+	no	+	GA
Patient 12	febrile, cough	+	no	+	GA
Patient 5	febrile, cough	+	yes	–	CA
Patient 7	febrile, cough	+	yes	–	GA
Patient 13	febrile, cough	+	yes	–	GA
Patient 2	none	–	no	–	CA
Patient 9	none	–	no	–	CA
Patient 14	none	n/a	no	–	CA

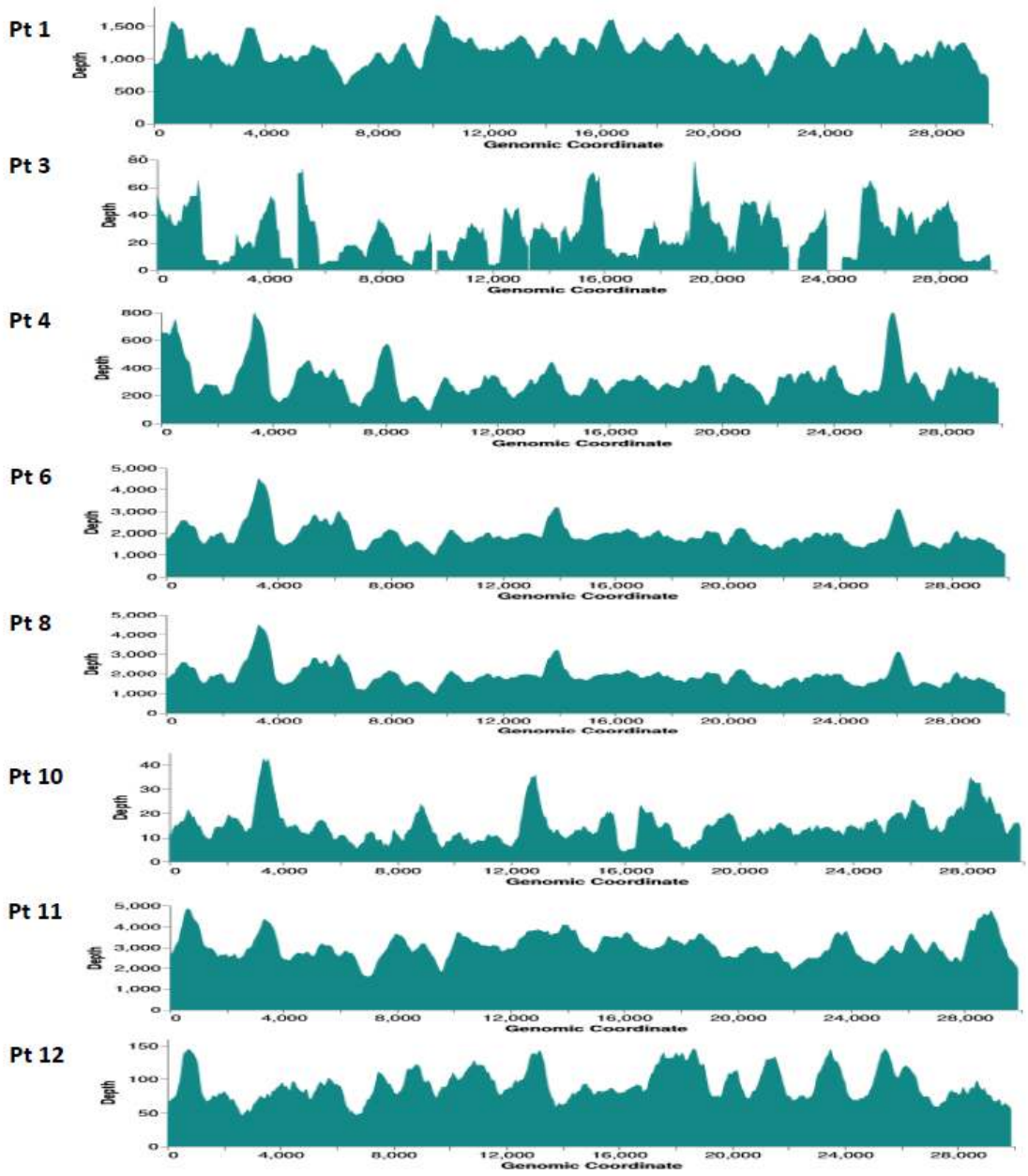
Table 2. Enrichment NGS metrics

Sample ID	Genome Coverage	Number of Variants	Mapped Reads	Mean Depth
Patient 1	100%	11	465645	1129.8x
Patient 4	100%	9	131582	318.6x
Patient 6	100%	10	793603	1924.6x
Patient 8	100%	10	496852	1206.7
Patient 11	100%	10	1270734	3075.3x
Patient 12	100%	10	38256	92.7x
Patient 10	93%	9	5929	15.6x
Patient 3	45%	11	5984	31.7x

Table 3. SARS-CoV-2 genomic positions, variant changes, and frequencies across the positive patient cohort

Region (ORF)	Position	Variant	Patient 1	Patient 3	Patient 4	Patient 6	Patient 8	Patient 10	Patient 11	Patient 12
5'-UTR	241	C → T	100%	100%	100%	100%	100%	100%	100%	100%
1a	833	T → C	x	x	x	x	100%	x	100%	100%
1a	1059	C → T	x	x	100%	100%	99%	100%	100%	100%
1a	1758	C → T	x	x	100%	100%	x	x	x	x
1a	1973	C → T	x	x	x	87%	x	x	x	x
1a	3037	C → T	100%	x	100%	100%	100%	100%	100%	100%
1a	3078	C → T	x	89%	x	x	x	x	x	x
1a	4866	G → T	75%	x	x	x	x	x	x	x
1a	6720	C → T	93%	x	x	x	x	x	x	x
1a	8102	G → T	x	100%	x	x	x	x	x	x
1a	9401	T → C	x	x	x	x	x	64%	x	x
1a	9403	T → A	x	x	x	x	x	64%	x	x
1a	10870	G → T	x	x	100%	100%	x	x	x	x
1a	11123	G → A	x	x	100%	100%	x	x	x	x
1b	14408	C → T	100%	x	100%	100%	100%	x	100%	100%
1b	14877	C → T	x	100%	x	x	x	x	x	x
1b	16616	C → T	x	x	x	x	100%	x	100%	100%
1b	16848	C → T	100%	x	x	x	x	x	x	x
1b	18652	C → A	x	x	x	x	x	83%	x	x
1b	19989	T → G	x	100%	x	x	x	x	x	x
Spike	21576	T → G	x	83%	x	x	x	x	x	x
Spike	23264	G → A	x	75%	x	x	x	x	x	x
Spike	23403	A → G	100%	100%	100%	100%	100%	100%	100%	100%
Spike	23603	C → T	82%	x	x	x	x	x	x	x
3a	25563	G → T	x	100%	100%	100%	100%	100%	100%	100%
3a	25976	C → A	x	x	x	x	100%	x	100%	100%
8	27964	C → T	x	x	x	x	100%	x	100%	100%
Nucleoprotein	28881	G → A	100%	x	x	x	x	x	x	x
Nucleoprotein	28882	G → A	100%	x	x	x	x	x	x	x
Nucleoprotein	28883	G → C	100%	x	x	x	x	x	x	x
Nucleoprotein	28997	C → T	x	100%	x	x	x	x	x	x
Nucleoprotein	29019	A → T	x	100%	x	x	x	x	x	x
Nucleoprotein	29364	C → G	x	x	x	x	x	85%	x	x

Figure 1. Whole genome alignment of SARS-CoV-2 in patients (Pt)



The x-axis depicts the genomic coordinates as aligned to the MN908947.3 reference genome, and the y-axis represents the read depth at specific loci.

References

1. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395: 497–506.
2. Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020; 579: 270–273.
3. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020; 382:727–733.
4. Drosten C, Günther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 2003; 348:1967–1976.
5. Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003; 348:1953–1966.
6. Zaki AM, van Boheemen S, Bestebroer TM, et al. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 2012; 367:1814–1820.
7. "Coronavirus." World Health Organization, World Health Organization, 15 July 2020, http://www.who.int/emergencies/diseases/novel-coronavirus-2019
8. Johns Hopkins University. Coronavirus Resource Center. COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU). 15 July 2020, www.coronavirus.jhu.edu/map.html.
9. 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; 17:323:1061–1069.
10. Tahamtan A, Ardebili A. "Real-Time RT-PCR in COVID-19 Detection: Issues Affecting the Results." Expert Review of Molecular Diagnostics, 2020; 20:453–454.
11. Gudbjartsson D, Helgason A, Jonsson H, et al. "Spread of SARS-CoV-2 in the Icelandic Population." New England Journal of Medicine 2020;382:2302–2315.
12. Wikramaratna P, Paton RS, Ghafari M, et al. "Estimating False-Negative Detection Rate of SARS-CoV-2 by RT-PCR." Euro Surveill 2020;25:2000568.
13. Braunstein GD, Schwartz L, Hymel P, et al. False Positive Results With SARS-CoV-2 RT-PCR Tests and How to Evaluate a RT-PCR-Positive Test for the Possibility of a False Positive Result. J Occup Environ Med. 2021; 63:e159-e162.
14. Report of the WHO-China joint mission on coronavirus disease 2019 (COVID-19); https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf
15. Kakodkar, Pramath, Baig MN. "A Comprehensive Literature Review on the Clinical Presentation, and Management of the Pandemic Coronavirus Disease 2019 (COVID-19)." Cureus 2020;12:e7560.
16. Wang, Xin-Wei. "Excretion and Detection of SARS Coronavirus and Its Nucleic Acid from Digestive System." World Journal of Gastroenterology 2005;11:4390–4395.
17. Parasa S, Desai M, Thoguluva Chandrasekar V, et al. "Prevalence of Gastrointestinal Symptoms and Fecal Viral Shedding in Patients With Coronavirus Disease 2019." JAMA Network Open 2020;3:e2011335.
18. Zhang B, Liu S, Dong Y, et al. Positive rectal swabs in young patients recovered from coronavirus disease 2019 (COVID-19). Journal of Infection 2020;81: e49-e52.
19. Chen L, Lou J, Bai Y, et al. COVID-19 Disease With Positive Fecal and Negative Pharyngeal and Sputum Viral Tests. Am J Gastroenterol 2020;115:790.
20. Weiss SR, Navas-Martin S. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol Mol Biol Rev 2005;69:635–64.
21. Scoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. Virol J 2019;16:69.
22. Zumla A, Chan J, Azhar, E., et al. Coronaviruses — drug discovery and therapeutic options. Nat Rev Drug Discov 2016;15:327–347.
23. Zhao L, Jha BK, Wu A, et al. Antagonism of the Interferon-Induced OAS-RNase L Pathway by Murine Coronavirus ns2 Protein Is Required for Virus Replication and Liver Pathology. Cell Host & Microbe 2012;11:607–616.
24. Belshaw R, Pybus OG, Rambaut A. The Evolution of Genome Compression and Genomic Novelty in RNA Viruses. Genome Research 2007;17:1496–1504.
25. Fehr A, Perlman S. Coronaviruses: An Overview of Their Replication and Pathogenesis. Methods in Molecular Biology 2015;1282:1-23.
26. Li F. Structure, Function, and Evolution of

Coronavirus Spike Proteins. Annual Review of Virology 2016;3:237–261.
27. Shang J, Ye G, Shi K, et al. Structural Basis of Receptor Recognition by SARS-CoV-2. Nature 2020;581:221–224.
28. Yang N, Shen H. Targeting the Endocytic Pathway and Autophagy Process as a Novel Therapeutic Strategy in COVID-19. Int J. Biological Sciences 2020; 16:1724-1731.
29. Degtyarev M, De Maziere A, Orr C, et al. Akt inhibition promotes autophagy and sensitizes PTEN-null tumors to lysosomotropic agents. J Cell Biol 2008;183:101-16.
30. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 2020.
31. Das S, Ramachandran AK, Birangal SR, et al. The controversial therapeutic journey of chloroquine and hydroxychloroquine in the battle against SARS-CoV-2: A comprehensive review. Med Drug Discov 2021;10:100085.
32. Troisi J, Venutolo G, Pujolassos Tanyà M, et al. COVID-19 and the gastrointestinal tract: Source of infection or merely a target of the inflammatory process following SARS-CoV-2 infection? World J Gastroenterol 2021;27:1406–1418.
33. Xu J, Chu M, Zhong F, et al. Digestive symptoms of COVID-19 and expression of ACE2 in digestive tract organs. Cell Death Discov 2020;6:76.
34. Chen Y, Chen L, Deng Q, et al. The Presence of SARS-CoV-2 RNA in the Feces of COVID-19 Patients. Journal of Medical Virology 2020;92: 833–840.
35. Wu Y, Guo C, Tang L, et al. Prolonged Presence of SARS-CoV-2 Viral RNA in Fecal Samples. The Lancet Gastroenterology & Hepatology 2020;5:434–435.

This page is intentionally left blank