Annals of Case Reports
Sabine H. Ann Case Rep: 9: 101599
www.doi.org/10.29011/2574-7754.101599
www.gavinpublishers.com

Case Report

Presence of SARS-CoV-2 in Feces of Vaccinated, PCR Negative Tested Patient: A Case Report

Hazan Sabine*

Progenabiome, Ventura Clinical Trials, Topelia Therapeutics, Microbiome Research Foundation, Ventura, California, USA.

***Corresponding author:** Hazan Sabine, Progenabiome, Ventura Clinical Trials, Topelia Therapeutics, Microbiome Research Foundation, Ventura, California, USA.

Citation: Sabine H (2024) Presence of SARS-CoV-2 in Feces of Vaccinated, PCR Negative Tested Patient: A Case Report. Ann Case Report 9: 1599. DOI: 10.29011/2574-7754.101599

Received: 12 January 2024; **Accepted:** 17 January 2024; **Published:** 19 January 2024

Abstract

The use of real-time reverse transcription-PCR (RT-PCR) testing to determine the presence or absence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may be inadequate and inaccurate for individuals who have been vaccinated against the virus. This case demonstrates that individuals vaccinated against SARS-CoV-2 may later produce viral particles and viral variants that exist systemically and may be undetected solely by RT-PCR testing. Regarding fecal transplantation, further testing, including NGS, is required for individuals who serve as fecal donors to avoid cross-contamination and viral spread.

Keywords: SARS-CoV-2; COVID-19; RT-PCR; Vaccine; Fecal Transplant; Microbiome

Introduction

Coronaviruses (CoVs) are a family of enveloped viruses with a single-strand, positive-sense RNA genome approximately 26–32 kilobases in size, which is the largest known genome for an RNA virus [1]. In humans, coronavirus infections primarily involve the upper respiratory tract and/or the gastrointestinal tract, and symptoms vary from mild, self-limiting disease (e.g., the common cold, diarrhea, nausea, and vomiting) to more severe manifestations (e.g., bronchitis and pneumonia with renal involvement) [2].

Polymerase chain reaction (PCR) testing is performed to detect genetic material and/or fragments of genetic material from a specific organism (e.g., virus). Real-time reverse transcription polymerase chain reaction (RT-PCR) is a variation of PCR. RT-PCR from nasopharyngeal swabs has been adopted as the "gold standard" test and remains the most common method used to identify severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19) [3]. Although the test was designed to diagnose individuals actively infected, testing results may remain positive after an individual is no longer infected, and individuals may test negative when the virus is present in other systems of the body (e.g., the digestive system). Acquired mutations of the virus may contribute to the evasion of detection from specifically targeted PCR primers, and samples collected soon after infection, or after symptoms have resolved, have resulted in high false negative rates [4]. PCR testing solely from nasopharyngeal swabs may not be the most reliable and accurate test to determine the presence or absence of SARS-CoV-2.

Fecal Microbiota Transplantation (FMT) involves the transplantation of an extremely heterogeneous biological sample (stool) from a healthy donor to the recipient with the goal of restoring the normal composition of gut microbiota in the recipient [5]. Although institutions have recommended interim precautions to screen new donors including the donor's history of travel to areas of outbreak, cohabitation with infected individuals, or diagnosis or suspicion of COVID-19 within the 28 days before recovery of donor tissue, the primary screening test remains RT-PCR [6]. Research surrounding severe acute respiratory syndrome (SARS) and middle east respiratory syndrome (MERS) demonstrated that the fecal–oral route may be a mode of transmission for coronaviruses, and Wang et al. showed that SARS-CoV was present in stool samples collected from patients as well as in the wastewater of two hospitals [7]. Since then, studies have demonstrated that asymptomatic patients tested positive for SARS-CoV-2 via next-generation sequencing (NGS) from stool, 38 days after positive nasopharyngeal RT-PCR test, and up to 45 days in symptomatic,

untreated patients [8,9]. This information suggests that the virus may linger for longer than anticipated in the gastrointestinal (GI) tract and warrants further investigation to understand if the virus is viable and/or transmissible via fecal material, and if so, how long is the virus contagious in this capacity.

This case demonstrates that vaccination against SARS-CoV-2 has the potential to produce viral particles and viral variants that exist systemically and may be undetected by conventional recommended PCR testing.

Case Presentation

Here we report a case involving a 52-year-old female who served as a long-time healthy fecal microbiota transplantation donor that tested negative for SARS-CoV-2 via PCR after vaccination yet tested positive for variants of SARS-CoV-2 via NGS. The patient has given written consent regarding the publication of this case report.

On 28 April 2021, the patient and a family member received the second dose of the BNT162b2 mRNA COVID-19 vaccine. Other than a very mild fever, she reported no adverse effects from the second vaccine. She remained isolated and denied exposure to anyone with COVID-19.

Throughout May 2021, the patient was screened for SARS-CoV-2 via RT-PCR on three different occasions. All three RT-PCR results were negative, (Figure 1).

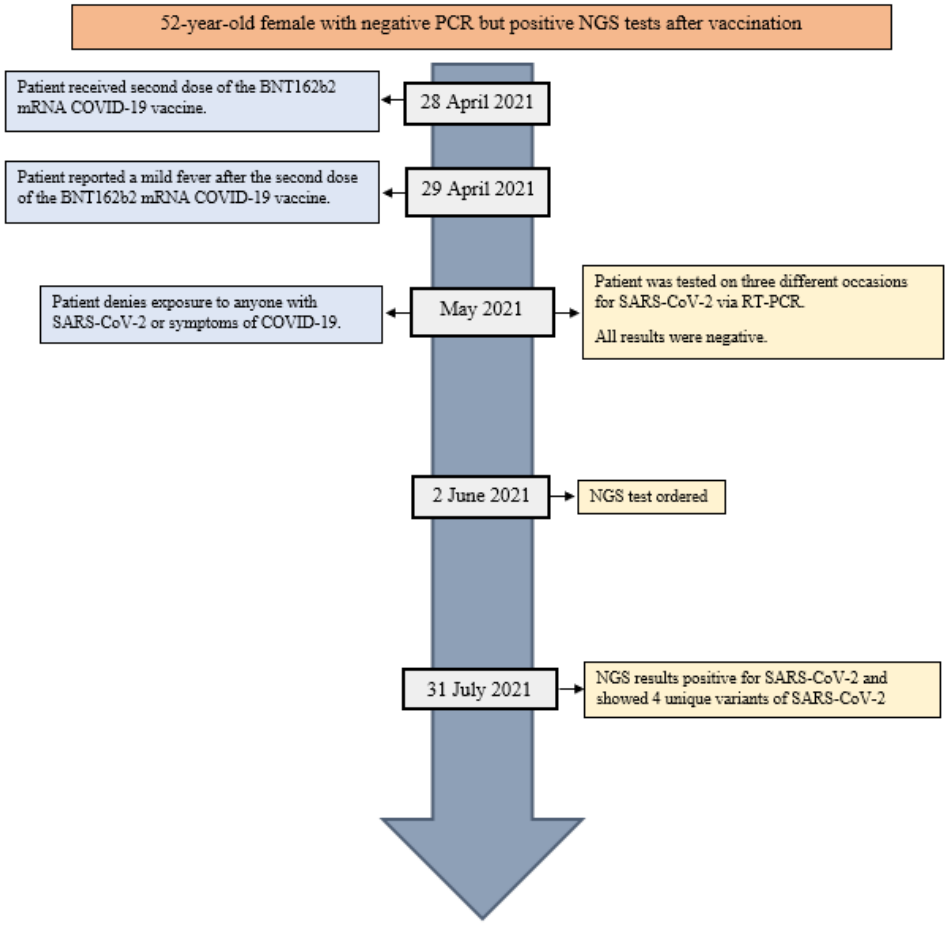
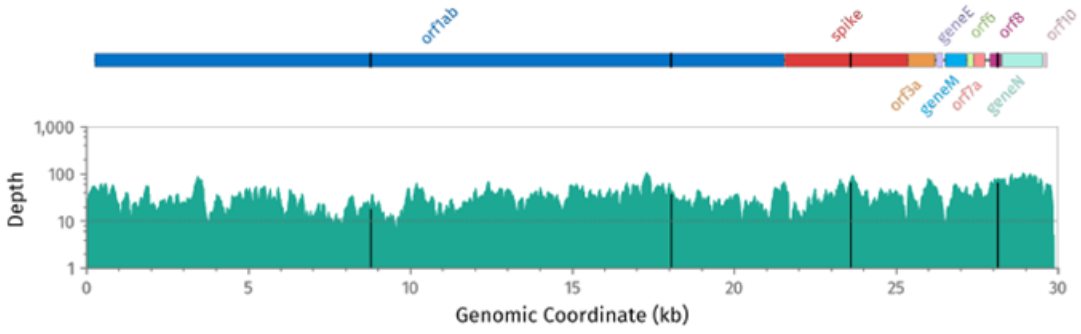


Figure 1: Timeline. The patient received the second dose of the BNT162b2 mRNA COVID-19 vaccine, 28 April 2021. She remained isolated and denied exposure to anyone with COVID-19. Throughout May 2021, the patient was screened for SARS-CoV-2 via RT-PCR on three different occasions.

In June 2021, the patient underwent testing for SARS-CoV-2 from a fecal sample by enrichment next-generation sequencing (NGS), and results were received on July 31, 2021. Following stool sample collection into a Zymo Research Shield Fecal Collection tube, RNA was extracted and purified, then reverse-transcribed, library prepped, enriched, and sequenced on Illumina's NextSeq 550 System. The sample contained 41,177,876 reads, with 0.0% mapping to the Wuhan-Hu-1 reference [10]. A total of 4 unique variants were detected at depths >10x, the minimum depth chosen for confident variant detection using Illumina sequencing data, (Figure 2). Vertical black lines on the coverage plot show the depth of high-quality reads (may be less than total reads) for each variant. NGS analysis identified nucleotide variants at positions nt8782 (C→T), nt18060 (C→T), nt23607 (G→T), and nt28144 (T→C). This genome is classified as Pangolin lineage A using PangoLEARN version 2021-06-15 and Nextclade lineage 19B with 2 private mutations.



Position (first ref nt)	Ref	Alt	Alt depth (high quality reads)	Total depth (high quality reads)	Alt frequency (%)	Gene	Variant type	Missense mutation
8782	C	T	17	17	100	nsp4 in orf1ab	synonymous variant	
18060	C	T	31	31	100	nsp14 in orf1ab	synonymous variant	
23607	G	T	35	52	67.31	spike	missense variant	R682L
28144	T	C	49	49	100	orf8	missense variant	L84S

SARS-CoV-2 variants. A variants TSV and consensus FASTA is available here.

This report was generated with commit number "2ad1a4a42d83042f9192b49e2077e65462eb8475".

Figure 2: Genomic coordinates and SARS-CoV-2 variants. NGS analysis identified nucleotide variants at positions nt8782 (C→T), nt18060 (C→T), nt23607 (G→T), and nt28144 (T→C). This genome is classified as Pangolin lineage A using PangoLEARN version 2021-06-15 and Nextclade lineage 19B with 2 private mutations.

Discussion

This case demonstrates that vaccination has the potential to produce viral particles and viral variants that exist systemically, in this case in the GI tract, which may be undetected by conventional PCR testing. A variety of hypotheses arise regarding why an individual who received an mRNA vaccine, never had SARS-CoV-2 or symptoms of COVID-19, and tested negative on three different occasions via PCR, would have SARS-CoV-2 present in their stools.

Hypothesis 1: The patient was exposed to SARS-CoV-2 and infected post-vaccination. Preclinical studies of adenovirus and mRNA candidate vaccines demonstrated persistent virus in nasal swabs despite preventing COVID-19 suggesting that systemically vaccinated patients, while asymptomatic, may still be become infected and transmit live virus [11].

Hypothesis 2: mRNA traveled through the blood stream and/or lymph nodes and encountered a previous coronavirus allowing the spike protein (S protein) of SARS-CoV-2 to penetrate host cells. The spike protein of all coronaviruses, which enables the viruses to infect cells, is present on the ectodomain and shares the same organization in two domains: a N-terminal domain named S1 that is responsible for receptor binding and a C-terminal S2 domain responsible for fusion [12]. Upon interaction with a potential host cell, the S1 subunit recognizes and binds to receptors on the host cell, whereas the S2 subunit, which is the most conserved region of the protein, is responsible for fusing the envelope of the virus with the host cell membrane. Without the S protein, coronaviruses including SARS-CoV-2 would not be able to interact with the cells of potential hosts. Although most of the mRNA vaccine stays in the injection site muscle, animal studies demonstrate that biodistribution also includes proximal and distal lymph nodes, liver, plasma, colon, ileum, rectum, spleen, kidneys, liver, lungs, and bone marrow [13].

Hypothesis 3: The batch of vaccine that the patient received was contaminated. Vaccines are subject to contamination by micro-organisms because their preparation involves materials of biological origin. Vaccine contamination, for example, can be found in the early days of development of the smallpox vaccine as well as contamination of human vaccines against poliomyelitis by SV40 virus from the use of monkey primary renal cells [14].

This interesting case raises the question of how many more patients carry SARS-CoV-2 in their stools and may unknowingly transmit the virus to others. Previous case studies surrounding COVID-19 have demonstrated RT-PCR positive SARS-CoV-2 fecal samples from “recovered” COVID-19 patients and negative results on multiple nasopharyngeal and sputum samples [15,16]. This case also raises the question of the impacts of SARS-CoV-2 and COVID-19 on the microbiome. Is SARS-CoV-2 inhabiting areas that would normally be colonized by other normal commensals such as Bifidobacteria? Further research is required to find solutions to these important questions.

One of the limitations of this study is that the definition of a healthy donor is not straightforward as donors are primarily selected to exclude known pathogens and mitigate the risk of transferring infectious diseases while ensuring recipient safety [17].

Conclusion

The use of RT-PCR testing to determine the presence or absence of SARS-CoV-2 may be inadequate and inaccurate for individuals who have been vaccinated against SARS-CoV-2. This case demonstrates that vaccination against SARS-CoV-2 produces viral particles and viral variants that exist systemically (e.g., in the stool) and may be undetected by the sole use of nasopharyngeal RT-PCR testing. Only with enhanced donor screening and validated stool tests for SARS-CoV-2 can we ensure safe and effective delivery of FMT to critically ill patients. The use of enrichment next-generation sequencing to identify the presence of SARS-CoV-2 and characterize mutational variations of SARS-CoV-2 should be required for individuals who serve as fecal donors to avoid cross-contamination and viral spread. NGS may also aid in determining the complete eradication of the virus for all COVID-19 patients.

Conflict of Interest: The author of this paper is the founder of Progena Biome, a research sequencing lab and Ventura Clinical trials.

References

1. Weiss SR, Navas-Martin S (2005) Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol Mol Biol Rev. 69: 635-664.
2. Wevers BA, van der Hoek L (2009) Recently discovered human coronaviruses. Clin Lab Med. 29:715-24.
3. LeBlanc JJ, Gubbay JB, Li Y, Needle R, Arneson SR, et al. (2020) Real-time PCR-based SARS-CoV-2 detection in Canadian laboratories. J Clin Virol. 128: 104433.
4. Gudbjartsson DF, Helgason A, Jonsson H, Magnusson OT, Melsted P, et al. (2020) Spread of SARS-CoV-2 in the Icelandic Population. N Engl J Med. 382: 2302-2315.
5. Dolai S, Ng J, Hazan S, Dawson V, Borody TJ (2021) Faecal Microbiota Transplantation as Primary Treatment for Clostridioidoes difficile Infection-evidence for Change. J Exp Pathol. 2: 90-101.
6. OpenBiome updates on COVID-19. https://www.openbiome.org/covid19 [Accessed 7 Aug 2021].
7. Wang XW, Li JS, Guo TK, Zhen B, Kong Q-X, et al. (2005) Excretion

and detection of SARS coronavirus and its nucleic acid from digestive system. World J Gastroenterol. 11: 4390-4395.

8. Parasa S, Desai M, Thoguluva Chandrasekar V, Patel HK, Kennedy KF, et al. (2020) Prevalence of Gastrointestinal Symptoms and Fecal Viral Shedding in Patients With Coronavirus Disease 2019: A Systematic Review and Meta-analysis. JAMA Netw Open. 3: e2011335.

9. Papoutsis A, Borody T, Dolai S, Daniels J, Steinberg S, et al. (2020) "Detection of SARS-COV-2 from Patient Fecal Samples by Whole Genome Sequencing.

10. Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome GenBank: MN908947.3.

11. Bleier BS, Ramanathan M Jr, Lane AP (2021) COVID-19 Vaccines May Not Prevent Nasal SARS-CoV-2 Infection and Asymptomatic Transmission. Otolaryngol Head Neck Surg. 164: 305-307.

12. Belouzard S, Millet JK, Licitra BN, Whittaker GR (2012) Mechanisms of Coronavirus Cell Entry Mediated by the Viral Spike Protein. Viruses. 4: 1011-1033.

13. Bahl K, Senn JJ, Yuzhakov O, Bulychev A, Brito LA, et al. (2017) Preclinical and Clinical Demonstration of Immunogenicity by mRNA Vaccines against H10N8 and H7N9 Influenza Viruses. Mol Ther. 25: 1316-1327.

14. Pastoret PP (2010) Human and animal vaccine contaminations. Biologicals. 38: 332-334.

15. Chen L, Lou J, Bai Y, Wang M (2020) COVID-19 Disease with Positive Fecal and Negative Pharyngeal and Sputum Viral Tests. Am J Gastroenterol. 115: 790.

16. Zhang B, Liu S, Dong Y, Zhang L, Zhong Q, et al. (2020) Positive rectal swabs in young patients recovered from coronavirus disease 2019 (COVID-19). J Infect. 81: e49-e52.

17. Duvallet C, Zellmer C, Panchal P, Budree S, Osman M, et al. (2019) Framework for rational donor selection in fecal microbiota transplant clinical trials. PLoS One. 14: e0222881.