



## Case Report

# SARS-CoV-2 Wuhan Strain Found by NGS in Feces of a Vaccinated Female Despite Repeat Negative Nasopharyngeal Swabs by PCR: A Case Report

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**Citation:** Hazan S, Vidal AC, Speicher DJ, Sfera A. (2024) SARS-CoV-2 Wuhan Strain Found by NGS in Feces of a Vaccinated Female Despite Repeat Negative Nasopharyngeal Swabs by PCR: 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 and lower respiratory tract as well as 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]. Detection of SARS-CoV-2 from nasopharyngeal swabs by PCR is the “gold standard” test to diagnose COVID-19 [3]. Although the test was designed to diagnose individuals actively infected with SARS-CoV-2, test samples may remain positive after an individual is no longer infectious, as the body is either shedding viral debris, or become negative when the viral infection moves to other systems (e.g., digestive system) and establishes a reservoir or a subclinical persistent infection. 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]. Therefore, PCR testing solely from

nasopharyngeal swabs may not be the most reliable test to screen fecal donors.

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 sample, 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 [7]. Moreover, a recent review on fecal-oral transmission of SARS-CoV-2 concluded that although with low certainty, SARS-CoV-2 has a potential for fecal-oral transmission between humans [8]. Wang et al. showed that SARS-CoV-2 was present in stool samples collected from patients as well as in the wastewater of two hospitals [9]. Since then, studies have demonstrated that asymptomatic patients can test positive for SARS-CoV-2 in their stools 38 days after an initial positive nasopharyngeal test, and up to 45 days in symptomatic, untreated patients [10,11]. This information suggests that the virus may establish a reservoir in

the gastrointestinal (GI) tract and warrants further investigation to understand if the virus is viable and/or transmissible during FMT.

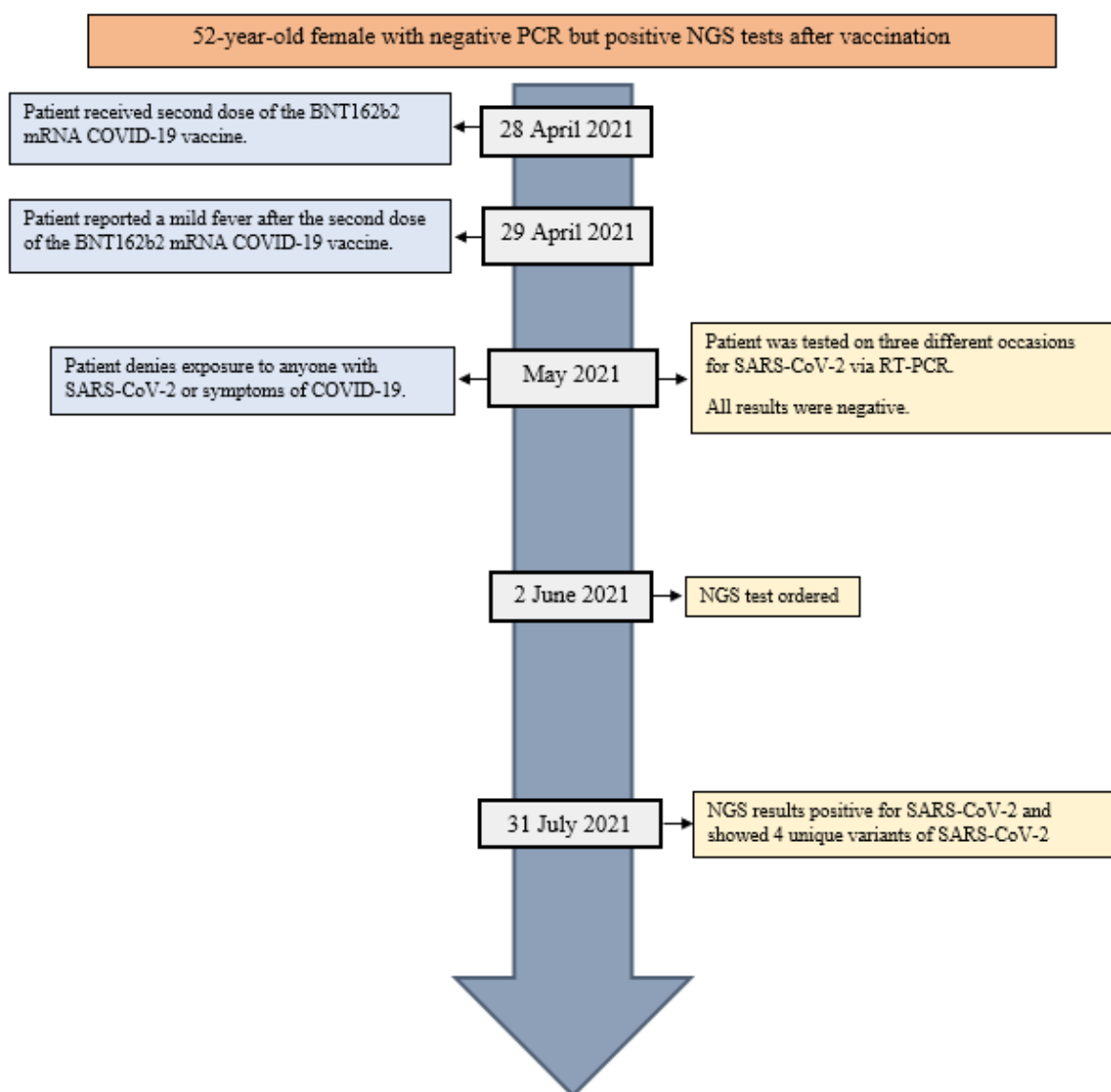
This case demonstrates that even in vaccinated individuals SARS-CoV-2 could establish a reservoir in the GI track regardless of symptoms that remains undetected by routine diagnostic testing and has the potential for replication-competent virion to be transplanted during FMT.

### Case Presentation

We report a case involving a vaccinated 52-year-old Californian Caucasian female who served as a long-time healthy FMT donor that tested negative in NPS for SARS-CoV-2 via PCR after vaccination yet tested positive for variants of SARS-CoV-2 via NGS. The patient provided informed written consent regarding the publication of this case report.

On April 28, 2021, the patient and a family member received the second dose of the Pfizer-BioNTech BNT162b2 COVID-19 mRNA vaccine. Other than a very mild fever, she reported no adverse events from the second vaccine. She remained at home in rural California isolated and with minimal chances of being exposed to anyone with COVID-19.

Throughout May 2021, the patient tested negative for SARS-CoV-2 on three consecutive NPS tests (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 [11]. The sample contained 41,177,876 reads, with 0.0% mapping to the Wuhan-Hu-1 reference [12]. 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.



SARS-CoV-2 variants. A variants TSV and consensus FASTA is available [here](#).

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**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 a need to test for SARS-CoV-2 in stool samples of asymptomatic donors prior to fecal microbiome transplantation as the GI tract may be a reservoir for the virus even when nasal and oral samples do not test positive by PCR. A variety of hypotheses arise regarding why an individual who received a COVID-19 mRNA vaccine, tested negative on three different occasions via PCR on NPS, plus never had a known SARS-CoV-2 infection or COVID-19 symptoms, 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 vaccines candidates 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 [13].

**Hypothesis 2:** mRNA-Lipid Nanoparticles (LNP) from the vaccine 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.<sup>13</sup> 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. Human and animal studies have shown that COVID-19 mRNA vaccines' biodistribution after injection includes proximal and distal lymph nodes, breast milk, liver, plasma, colon, ileum, rectum, spleen, kidneys, liver, lungs, and bone marrow [14,15]. Moreover, human coronaviruses, such as  $\alpha$ -coronaviruses HCoV-229E, NL63, HCoV-NL63, and  $\beta$ -coronaviruses HKU1, SARS-CoV-1, OC43, are known to cause gastrointestinal symptoms, [16] which raises the question of whether they could inhabit the human gut in a dormant state after the acute infection phase subsides. If this is the case, may be the vaccine mRNA-LNP may encounter these other coronaviruses and become active viruses.

**Hypothesis 3:** Vaccines are subject to contamination by microorganisms 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 [17].

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 [8]. 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 [18,19]. This case also raises the question of the impacts of SARS-CoV-2 and COVID-19 on the microbiome, as shown in other studies [20]. 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 [21]. An interesting observation in this case was the fact that the patient had the original Wuhan strain in her stools in 2021.

## Conclusion

The use of RT-PCR testing to determine the presence or absence of SARS-CoV-2 may be inadequate and inaccurate for individuals that 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 that serve as fecal donors to avoid cross-contamination and viral spread. NGS may also aid in determining complete eradication of the virus for all COVID-19 patients.

**Conflict of Interest:** SH is the founder of ProgenaBiome, a research sequencing lab, and Ventura Clinical trials.

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