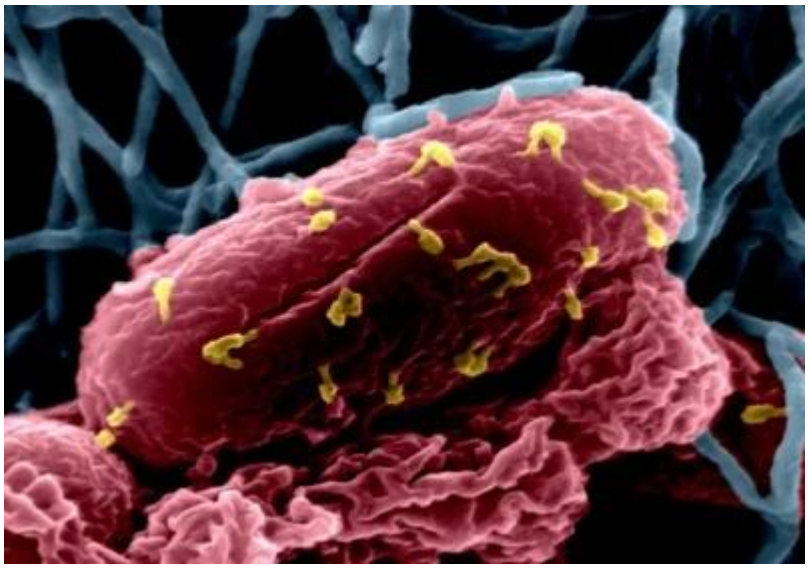
After recovering from COVID-19, a large proportion of patients suffer from long-lasting post-COVID symptoms, even after mild or asymptomatic forms of the disease. Based on recent findings, numerous scientists hypothesized not only that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) persists in the gastrointestinal tract of individuals diagnosed with long COVID, but also that the gut could be a viral reservoir. In this study, the Italian authors Petrillo M *et al*. conducted a series of experiments to determine the presence of SARS-CoV-2 in the cultivated fecal microbiota from individuals infected with SARS-CoV-2. They also investigated whether SARS-CoV-2 exhibits a”bacteriophage-like” behavior, as a virus that infects bacterial cells.

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus. Its genome encodes four structural proteins, namely the spike (S), envelope (E), nucleocapsid (N), and membrane (M) protein. It was assumed that the only possible host for SARS-CoV-2 is mammalian eukaryotic cells. However, the analysis of cultures of the human microbiome and SARS-CoV-2, using electron and fluorescence microscopy, the nitrogen isotope 15N assay, and proteomic analysis revealed virus-like particles within and near the bacteria. Labeling SARS-CoV-2 proteins with nitrogen 15N isotopes showed that bacteria can replicate, transcribe, and translate viral RNA. https://doi.org/10.3390/vaccines10050708

A recent study that investigated the presence of SARS-CoV-2 in rectosigmoid cells from participants diagnosed with post-COVID syndrome who underwent colorectal biopsy up to 2.5 years after initial infection, revealed that SARS-CoV-2 S protein RNA was detected in rectosigmoid cells in all participants who underwent biopsy. Almost all S protein RNA was found in cells located in the *lamina propria*, without any epithelial signal. https://discovermednews.com/elevated-t-cell-activation-vaccinated-covid-convalescents-2-years-after-infection/ Similarly, SARS-CoV-2 RNA and viral-specific antigens were detected in the gastrointestinal (GI) tract and non-GI tissues (appendix, skin, and breast tissues) of two post-COVID patients, 426 and 175 days after acute SARS-CoV-2 infection, respectively. https://discovermednews.com/sars-cov-2-rna-and-antigens-appendix-skin-breast-patients-long-covid/



About the study

This study analyzed two fecal samples, sample A from a person positive for SARS-CoV-2 and sample B from a healthy person. Sample B was inoculated with the supernatant of sample A, obtained through centrifugation (called sample $B_{(A+)}$). All specimens were incubated for 30 days under the same conditions. The viral RNA was measured in all specimens on days 1, 2, 3, 7, 14, 21, and 30, following the inoculation day (day 0). Three additional pairs of fecal specimens from different infected individuals (referred to as A1, A2, and A3) and healthy individuals (referred to as B1, B2, and B3) in all combinations of specimens were treated with the same experimental procedure.

On day 21, eighteen antibiotics were added to 18 aliquots derived from sample B(A+): metronidazole, clindamycin, lincomycin, piperacillin+tazobactam, vancomycin, amoxicillin, ampicillin, cefixime, ceftriaxone, meropenem, rifaximin, azithromycin, erythromycin, gentamicin, ciprofloxacin, colistin, levofloxacin, and teicoplanin. The RNA load of SARS-CoV-2 was measured before and three days after the administration of antibiotics.

Results

The findings confirmed the extra-corporal multiplication of SARS-CoV-2 RNA. As expected, the viral RNA load in sample B was constantly negative. However, there was a significant increase in the viral RNA load in sample B(A+) and a slight increase in the viral RNA load in sample A over time.

The entire experiment was repeated three times with the same samples to ensure reproducibility of the results. The initial results were confirmed. The viral RNA load in samples A and $B_{(A+)}$ increased over time, while the viral RNA load in sample B remained negative.

Transmission electron microscopy and scanning electron microscopy were used to determine the presence of eukaryotic cells in samples A, B, and $B_{(A+)}$ collected at various times. In more than 30 different preparations, only bacterial cells were found without any structure that resembled cells with nuclei. The images of samples A and $B_{(A+)}$ showed the virus-like particles interacting with bacterial cells.

Three additional pairs of fecal specimens from different infected individuals (referred to as A1, A2, and A3) and healthy individuals (referred to as B1, B2, and B3) in all combinations of specimens were treated with the same experimental procedure. Despite certain differences, the results confirmed that the viral RNA load increased over time in samples A and $B_{(A+)}$. The SARS-CoV-2 RNA load was particularly high in the A2×B2 combination.

This experiment showed that samples A and B(A+) contained some bacterial genera that were particularly abundant and metabolically active.

Importantly, the SARS-CoV-2 RNA load in aliquots derived from sample B(A+) has changed depending on the antibiotic added. The viral RNA load decreased to an undetectable level in four aliquots treated with metronidazole, vancomycin, amoxicillin, and azithromycin. Similarly, the viral RNA load decreased from 20% to 85% in aliquots treated with piperallicin+tazobactam, ampicillin, cefixime, ceftriaxone, meropenem, gentamicin,

ciprofloxacin, and teicoplanin. Cefixime reduced the viral RNA load by 85%, ciprofloxacin by 61%, and teicoplanin by 56%.

The viral load was not altered in aliquots treated with clindamycin, lincomycin, rifaximin, erythromycin, colistin, and levofloxacin.

Conclusion

These findings suggest that the SARS-CoV-2 genome can replicate outside the human body. The authors concluded that these results suggest a “bacteriophage-like” behavior of SARS-CoV-2, which, to their knowledge, has not been observed or described before. Further research is needed to investigate which bacterial species are targets of SARS-CoV-2.

This article was published in F1000Research.

Journal Reference

Petrillo M. et al. Increase of SARS-CoV-2 RNA load in faecal samples prompts for a rethinking of SARS-CoV-2 biology and COVID-19 epidemiology. F1000Research 2021, 10:370. (Open Access) https://doi.org/10.12688/f1000research.52540.3