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## Viewpoint

## Longitudinal sampling sheds light on SARS-CoV-2 fecal shedding dynamics

Michael P. Coryell<sup>1</sup> and Paul E. Carlson, Jr.<sup>1,\*</sup>

**Persistent fecal shedding of SARS-CoV-2 viral RNA has remained a clinical feature of interest throughout the COVID-19 pandemic. In this issue of *Med*, Natarajan et al. report fecal shedding dynamics of individuals diagnosed with mild-to-moderate COVID-19 disease and sampled longitudinally for up to 10 months<sup>1</sup>**

Systemic and respiratory symptoms of COVID-19 disease are often accompanied by gastrointestinal (GI) symptoms and fecal shedding of viral RNA, sometimes lasting long after resolution of symptoms and negative conversion of respiratory tests. Additionally, infectious virus has been isolated from the stool of infected individuals, indicating the potential for SARS-CoV-2 to infect and replicate in GI tissues.<sup>2</sup> Early reports of fecal shedding led the US Food and Drug Administration to issue safety alerts regarding the potential risk of transmission of SARS-CoV-2 in fecal microbiota transplant (FMT) products and recommendations for screening donors.<sup>3</sup> SARS-CoV-2 shedding in stool has led to an interest in understanding and utilizing stool shedding for research and public health purposes, catalyzing early efforts toward development and optimization of standardized testing methods for detection of SARS-CoV-2 RNA in stool,<sup>4</sup> with later efforts to further refine and benchmark these protocols,<sup>5</sup> as well as the development of methods for detection of viral RNA in wastewater.

Fecal shedding may have important epidemiological implications at the clinical level and for community surveillance tools such as wastewater monitoring for SARS-CoV-2 RNA. A more precise understanding of fecal shed-

ding dynamics during natural infection can help inform improvements in the use of wastewater testing as an epidemiological tool for surveillance and monitoring of local and regional COVID-19 outbreaks.<sup>6</sup> In order to efficiently implement both well-established and *ad hoc* public health strategies, it is important to understand the relationship between SARS-CoV-2 fecal shedding and both transmission and COVID-19 disease severity and to carefully define the prevalence, load, and duration of fecal shedding among infected individuals. The study conducted by Natarajan et al. published in this issue of *Med* begins to address these questions by describing paired fecal and oropharyngeal (OP) shedding dynamics of SARS-CoV-2 RNA.

The study population included a total of 113 individuals with mild-to-moderate COVID-19 symptoms sampled longitudinally for up to 10 months, making it one of the largest datasets to include longitudinal testing for fecal SARS-CoV-2 RNA shedding.<sup>1</sup> Using optimized PCR-based detection methods,<sup>5</sup> they found that 49% of patient fecal specimens tested positive during the week following diagnosis, with this positivity rate gradually declining to 4% positivity 7 months following diagnosis. Interestingly, when comparing OP and fecal positivity rates, positivity rates of

fecal samples surpassed those of OP specimens by 14 days post-infection, and no virus was detected in OP samples after 4 months.

Using demographic-adjusted logistic regression models, the authors identified self-reported symptoms that were significantly associated with fecal shedding, including GI-related symptoms like abdominal pain, nausea, and vomiting as well as systemic symptoms like joint ache, body ache, and chills. Several such associations were significant independently of continued positivity in paired OP specimens, suggesting that fecal shedding may be associated with extrapulmonary infection sites. This finding was bolstered by testing of stool samples for viral subgenomic RNA (sgRNA), which is generated only during active viral replication. Up to 23% of stool specimens tested positive for sgRNA during the week following diagnosis, with the latest detection after diagnosis observed at the 4-month time point. While there is some debate over the specificity of sgRNA positivity as an indicator of active infection, taken together with robust associations of stool shedding with GI symptoms, these findings support the hypothesis that the GI tract is a site of continuing infection persisting after respiratory clearance.

Notably, the paired nasal and stool samples in this study were collected as part of a randomized placebo-controlled trial studying peginterferon lambda 1a (peg-INF- $\lambda$ ) for treatment of uncomplicated COVID-19.<sup>7</sup> This provided for a unique opportunity to

<sup>1</sup>Laboratory of Mucosal Pathogens and Cellular Immunology, Division of Bacterial, Parasitic, and Allergenic Products, Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD, USA

\*Correspondence: [paul.carlson@fda.hhs.gov](mailto:paul.carlson@fda.hhs.gov)  
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conduct an exploratory analysis on the effects of this anti-viral intervention on stool shedding. While the study did find the peg-INF- $\lambda$  intervention was associated with reduced fecal shedding at the first timepoint (3 days post enrollment), this association failed to replicate when subjected to sensitivity analysis and did not persist past this early timepoint. Nonetheless, the inclusion of this intervention group may have introduced a small amount of bias into reported shedding dynamics.

Despite early recognition of and testing for SARS-CoV-2 shedding in stool, many questions remain regarding the possibility for SARS-CoV-2 to infect and replicate in the GI tract. In general, the findings in this study show that fecal positivity for SARS-CoV-2 RNA peaks later (~14 days post infection) and persists longer (~7 months after enrollment) than nasal positivity.<sup>1</sup> These findings are generally in agreement with other empirical and model-based studies of longitudinal fecal shedding dynamics.<sup>8</sup> While there have been conflicting reports on the associations between fecal shedding and clinical symptoms, the prospective recruitment design and robust associations identified in this study give it a strong weight of evidence.

One particular strength of this study was the enrollment of subjects with mild symptoms. To date, comparable longitudinal studies of fecal viral RNA shedding dynamics have mostly been limited to hospitalized study populations suffering from severe COVID-19 disease and/or other severe co-morbidities.<sup>9</sup> Understanding the temporal shedding dynamics of fecal shedding in individuals with mild or even asymptomatic infections is an important parameter in the implementation of

wastewater-based epidemiology,<sup>10</sup> as a substantial majority of current COVID-19 infections are likely to be mild or asymptomatic cases. Such an understanding may also prove useful in determining the optimal strategies for screening and testing of FMT donors, as detection of asymptomatic or mild cases of disease can be vital to the safety of FMT recipients.

To summarize, this study provides a unique analysis of fecal shedding dynamics among people with mild to moderate COVID-19.<sup>1</sup> These findings have potential implications for clinical interventions, community surveillance, and modeling predictions of future outbreaks. Further studies of this kind are warranted as it remains unclear how the emergence and spread of new variants will influence fecal shedding dynamics at a population level and how these changes may impact public health efforts moving forward.

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### DECLARATION OF INTERESTS

This article reflects the views of the authors and should not be construed to represent FDA's views or policies. The authors declare no competing financial interests.

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