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SARS-CoV-2 is detected in the gastrointestinal tract of asymptomatic endoscopy patients but is unlikely to pose a significant risk to healthcare personnel

Short title: SARS-CoV-2 prevalence in endoscopy patients

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Abbreviations:

COVID-19, coronavirus disease 2019; CT, cycle threshold; E gene, envelope gene; GI, gastrointestinal; N gene, nucleocapsid gene; NP, nucleocapsid protein; PFU, plaque-forming units; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

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The authors disclose no conflicts.

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The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Data Availability Statement:
The data that support the findings of this study are available from the corresponding author, D.B., upon reasonable request.
Abstract

**Background and Aims:** Recent evidence suggests that the gut is an additional target for SARS-CoV-2 infection. However, whether SARS-CoV-2 spreads via gastrointestinal secretions remains unclear. To determine the prevalence of gastrointestinal SARS-CoV-2 infection in asymptomatic subjects, we analyzed gastrointestinal biopsy and liquid samples from endoscopy patients for the presence of SARS-CoV-2.

**Methods:** We enrolled 100 endoscopic patients without known SARS-CoV-2 infection (cohort A) and 12 patients with a previous COVID-19 diagnosis (cohort B) in a cohort study performed at a regional hospital. Gastrointestinal biopsies and fluids were screened for SARS-CoV-2 by PCR, immunohistochemistry and virus isolation assay, and the stability of SARS-CoV-2 in gastrointestinal liquids *in vitro* was analyzed.

**Results:** SARS-CoV-2 RNA was detected by PCR in the colonic tissue of 1/100 patients in cohort A. In cohort B, three colonic liquid samples tested positive for SARS-CoV-2 by PCR, and viral nucleocapsid protein was detected in the epithelium of the respective biopsy samples. However, no infectious virions were recovered from any samples. *In vitro* exposure of SARS-CoV-2 to colonic liquid led to a 4-log fold reduction of infectious SARS-CoV-2 within 1 hour (*P* ≤ 0.05).

**Conclusions:** Overall, the persistent detection of SARS-CoV-2 in endoscopy samples after resolution of COVID-19 points to the gut as a long-term reservoir for SARS-CoV-2. Since no infectious virions were recovered and SARS-CoV-2 was rapidly inactivated in the presence of colon liquids, it is unlikely that performing endoscopic procedures is associated with a significant infection risk due to undiagnosed asymptomatic or persistent gastrointestinal SARS-CoV-2 infections.
Keywords: Endoscopy; SARS-CoV-2; transmission risk; colonic liquid

Introduction

Since December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected more than 500 million people worldwide, and the resulting disease, coronavirus disease 2019 (COVID-19), has killed more than 6.2 million individuals. Hospitalizations due to the COVID-19 pandemic have stressed hospitals for personnel and resources. Subsequently, many routine medical procedures including preventive cancer screenings have been delayed. Specifically, colorectal cancer screenings were reduced 85-90% in the US in 2020 compared to pre-pandemic levels, and a study from the UK predicted a 15-17% increase in deaths from colorectal cancer over five years due to deferred preventive screening during the pandemic. To avoid such an increase in cancer-related mortality, colonoscopy screenings must continue, but adapt to the challenges of the SARS-CoV-2 pandemic. One major challenge is to prevent transmission of SARS-CoV-2 to healthcare personnel, which can exacerbate staffing shortages due to staff illness or required quarantines.

Here, we sought to determine the prevalence of intestinal SARS-CoV-2 infection in gastrointestinal endoscopy patients without active COVID-19 so that we could gain initial insights about the likelihood of SARS-CoV-2 transmission to healthcare personnel during endoscopic procedures. Current guidelines from the American Gastroenterological Association state that routine SARS-CoV-2 testing prior to endoscopy is not needed to perform endoscopy safely. However, it is presently still unclear whether infectious SARS-CoV-2 is shed from the GI tract. Acute SARS-CoV-2 infections are frequently associated with gastrointestinal (GI)
symptoms including abdominal pain, diarrhea, and vomiting\textsuperscript{5,6}, and SARS-CoV-2 RNA is routinely detected in stool samples and in wastewater\textsuperscript{5-8}. Several groups have also reported replication of SARS-CoV-2 in human gut epithelium \textit{in vitro} using organoid models\textsuperscript{9-13}, and viral RNA, proteins and virions have been detected in small and large intestinal samples of COVID-19 patients\textsuperscript{8,14,15}. Importantly, prolonged shedding of SARS-CoV-2 from the GI tract for >6 weeks after clinical recovery in some patients has been demonstrated, suggesting that the GI epithelium may serve as a long-term viral reservoir\textsuperscript{16-19}.

Endoscopic procedures produce copious amounts of droplets and aerosols, which are major modes of transmission for SARS-CoV-2\textsuperscript{20,21}. Coughlan, et al.\textsuperscript{22} measured droplets produced by upper and lower endoscopies and determined that endoscopic aerosols would be sufficient for SARS-CoV-2 transmission. However, only one study describes the recovery of infectious SARS-CoV-2 virus from a GI sample\textsuperscript{23}.

We analyzed whether infectious SARS-CoV-2 was present in GI samples of asymptomatic male and female endoscopy patients as a basis for assessing the potential exposure risk for healthcare personnel performing endoscopy procedures. Our study revealed a low prevalence of SARS-CoV-2 infection in the gut of asymptomatic patients with no prior history of COVID-19, but a higher prevalence in patients with a previous COVID-19 diagnosis. SARS-CoV-2 protein was identified in the colon tissue in the majority of these patients, but no infectious virus was recovered from any SARS-CoV-2 positive GI samples. In addition, we demonstrate the rapid inactivation of SARS-CoV-2 in the presence of colonic liquids.
Methods

Study Design and Participants

For cohort A, we enrolled 100 consecutive patients who were undergoing upper or lower GI tract endoscopies for diagnostic purposes between April 2020 and October 2020 at Bozeman Health Deaconess Hospital, the major regional healthcare provider in Bozeman, MT. A sample size of 100 asymptomatic endoscopy patients was selected, using a simple Bayesian estimation procedure that assumed that the number of positives is binomially distributed with an uninformative prior on the \( p \) parameter (no prior beliefs regarding reasonable values of \( p \)). Male and female patients of any ethnicity aged between 18 and 70 years, weighing at least 50 kg and presenting for gastrointestinal endoscopy were included. Exclusion criteria were a known infection with HIV or hepatitis A, B or C virus, ongoing pregnancy or lactation, blood clotting disorders or current medication with a blood thinner, and presence of acute diarrhea or respiratory symptoms. Prior COVID-19 infections were not considered for this cohort, since testing was not widely available at the time of recruitment.

For cohort B, we enrolled 12 additional patients between November 2020 and May 2021 who had a prior history of COVID-19. Consecutive endoscopy patients presenting at the Bozeman Health Gastroenterology Clinic were recruited who (1) fulfilled the inclusion and exclusion criteria listed above, (2) had tested positive for COVID-19 in a PCR test between 2 weeks and 9 months prior to the endoscopy procedure, and (3) had recovered from any clinical symptoms at the time of the endoscopy.
Community Prevalence of SARS-CoV-2 Infection

Community spread of SARS-CoV-2 during the sample collection period was assessed based on wastewater surveillance data, as described previously, and on confirmed COVID-19 case numbers reported by the Gallatin City-County Health Department (Gallatin County, MT; https://www.healthygallatin.org/coronavirus-covid-19/). Healthcare personnel involved in the endoscopy procedures were tested for SARS-CoV-2 by PCR when they displayed any COVID-19 symptoms based on CDC guidelines or when they were identified as a close contact of a person with COVID-19. Throughout this study, none of the healthcare personnel tested positive for SARS-CoV-2.

Collection of Gastrointestinal Tissues and Fluids

Biopsies were collected from random sites in the stomach, duodenum, ileum, and/or colon with cold forceps during routine outpatient endoscopic procedures by a board-certified gastroenterologist (A.B.G.). All healthcare personnel involved in the endoscopy procedures were equipped with N95 respirators and face shields to prevent potential transmission of infectious agents. From each patient, 6-10 biopsies were recovered per site and were either fixed in formalin for paraffin embedding or were minced, aliquoted and frozen. Colon liquids were collected using a polyp trap during lower endoscopy procedures and then aliquoted and frozen.

Isolation of RNA

For isolation of viral RNA from colon liquids, the liquids were first clarified by centrifugation at 5,000 g for 5 min, then the QiAmp Viral RNA mini kit (QUIAGEN Sciences,
Germantown, MD) was used as instructed, with 280 μL of clarified colon liquid. For isolation of viral RNA from tissues, minced tissue was homogenized in 750 μL of ice-cold PBS using an OMNI THQ digital tissue homogenizer (OMNI International), then further broken up using a QIAShredder column, as directed. Next, genomic DNA was removed from the tissue lysate using a genomic DNA exclusion column. RNA was then extracted from the tissue lysate using the QiAmp Viral RNA mini kit following the manufacturer’s protocol, using 140 μL of lysate. To confirm that the sample processing protocol did not interfere with downstream SARS-CoV-2 detection, 5 μL of viral transport media from a confirmed SARS-CoV-2 positive nasal swab with N1 detected at cycle 19 was added to the tissue homogenate (n=3) or a colon liquid sample (n=1). These “spiked” samples were then immediately processed for SARS-CoV-2 detection as described above (Suppl. Fig. 2). SARS-CoV-2 N1 RNA was successfully recovered from both samples.

Quantitative RT-PCR Detection of SARS-CoV-2

RNA isolated from tissues or colon liquids was screened for presence of SARS-CoV-2 using quantitative RT-PCR and the CDC primers (N1, N2, and RP) and probes from the 2019-nCoV RUO Kit (IDT# 10006713). All primer and probe sequences are provided in Suppl. Table 1. SARS-CoV-2 RNA was quantified using one-step RT-qPCR in ABI 7500 Fast Real-Time PCR System according to the CDC protocol. In brief, 20 μL reactions included 8.5 μL of nuclease-free water, 1.5 μL of primer and probe mix (IDT, 10006713), 5 μL of TaqPath 1-Step RT-qPCR Master Mix (ThermoFisher, A15299) and 5 μL of the extracted RNA. Nuclease-free water was used as no template control (NTC). The 2019-nCoV_N positive control and Hs_RPP30 control plasmids (IDT# 10006625, 10006626) diluted to 200 copies/μL were used as
positive controls. Amplification was performed using the following program: 25°C for 2 min, 50°C for 15 min, 95 °C for 2 min followed by 45 cycles of 95 °C for 3 s and 55 °C for 30 s. Data were analyzed in SDS software v1.4 (Applied Biosystems). The NTC showed no amplification throughout the 40 cycles of qPCR. Samples positive for N1, N2, and human RNaseP were considered positive for SARS-CoV-2. Samples positive for RNaseP and either N1 or N2, but not both, were termed inconclusive, and samples negative for RNaseP were considered invalid. SARS-CoV-2-positive samples were re-analyzed for the presence of the N1 and envelope (E) genes in a second laboratory to confirm the results. For detection of the SARS-CoV-2 E gene, amplification was performed using UltraPlex 1-step ToughMix polymerase (QuantaBio, #95166) and the following program: 50°C for 10 min, 95°C for 3 min followed by 40 cycles of 95 °C for 10 s and 60°C for 60 s.

Viral Isolation Assay of Infected Liquids and Tissues

To detect infectious virus in positive samples, a viral isolation assay was performed using VeroE6 cells plated in 96-well tissue culture treated plates, as previously described. Colon liquids were centrifuged at 5000 g for 5 minutes to sediment particulates, then the clarified colon liquids, colon liquid sediments, and homogenized colon tissues were plated onto VeroE6 cells in 2-fold serial dilutions, starting with a 1:2 dilution, and monitored daily for cytopathic effects.

Inactivation of SARS-CoV-2 with Colon Liquids

Six colon liquids from cohort B were centrifuged at 5000 g for 5 minutes to remove particulates, then 250 µL of the clarified liquid was mixed with 1 x 10^6 PFU/mL of SARS-CoV-2 (strain USA-WA1/2020, BEI Resources, Manassas, VA). Colon liquids with virus or a
PBS control were incubated at 37°C for 10-minutes, 1 hour, or 24 hours, then serially diluted from $10^{-1}$ to $10^{-6}$ and plated onto VeroE6 cells in 6-well plates. After 1 hour incubation at 37°C, cell layers were coated with methylcellulose and antibiotic rich media to prevent bacterial or fungal growth from colon liquids. After four days, cell layers were stained with methylene blue, and plaques were quantified.

**Histology and Immunohistochemistry**

Formalin fixed, paraffin embedded tissues from four SARS-CoV-2 positive samples and three negative samples were sectioned and mounted on slides, then stained with hematoxylin and eosin. Each tissue was assessed for histopathological alterations by a blinded investigator. Tissue samples also were analyzed for the presence of SARS-CoV-2 viral nucleoprotein by immunohistochemistry, using a mouse monoclonal antibody (RRID Number: AB_2827977, #40143-MM05, Sino Biological, Wayne, PA). Briefly, samples were de-paraffinized, blocked, incubated with the primary antibody, followed by a secondary goat anti-mouse antibody conjugated to horseradish peroxidase (HRP; #1030-05; Southern Biotechnology, Birmingham, AL). ImmPact DAB (Vector Laboratories, Burlingame, CA) was used as a chromogen. Slides were counterstained with Hematoxylin (#7211; Richard-Allan Scientific, Kalamazoo, MI) and coverslipped. For quantification of nucleocapsid-positive cells, stained cells in the crypt epithelium of colon tissue samples from all donors that tested positive for SARS-CoV-2 by PCR were counted by two blinded observers. Three SARS-CoV-2-negative control tissues were analyzed in parallel.
Statistics

Statistical analysis of SARS-CoV-2 inactivation was performed with a non-parametric 2-way ANOVA and Friedman test for multiple comparisons. Prevalence of asymptomatic SARS-CoV-2 infection in the gut was determined by Bayesian analysis.

Study Approval

The study was approved by the Institutional Review Board of Montana State University, protocol #DB050718-FC, and was performed under a collaboration agreement between Montana State University and Bozeman Health Deaconess Hospital. Written informed consent was obtained from all participants.

Results

Patient Demographics and Community Prevalence of SARS-CoV-2

We enrolled 100 consecutive patients undergoing routine upper or lower endoscopies (cohort A) and collected 223 biopsies from the stomach, duodenum, ileum, and colon, and 77 colon liquids (Suppl. Table 2). Patient demographics are shown in Table 1. Sample collection for cohort A was performed between April 2020 and October 2020, while moderate community spread of SARS-CoV-2 occurred (Fig. 1A). We also studied the risk of SARS-CoV-2 transmission during endoscopies in a separate smaller cohort of patients who tested positive for SARS-CoV-2 less than nine months prior to an endoscopy procedure (cohort B). Detailed patient
demographics are shown in Table 2. Sample collection for cohort B was performed between November 2020 and May 2021 (Fig. 1A).

Detection of SARS-CoV-2 in colonic biopsy tissue obtained from a colonoscopy patient with no history of COVID-19 (cohort A)

Out of 100 sampled patients, one colon tissue biopsy collected from a 61-year-old female patient after a peak in local community transmission (Fig. 1A) tested positive for SARS-CoV-2 RNA by qRT-PCR (1% of patients/0.45% of tissues; Table 3, Suppl. Table 2). SARS-CoV-2 detection was independently confirmed in a second laboratory using a backup sample. The biopsy also tested positive for the SARS-CoV-2 envelope (E) gene. Tissue samples from other regions of the GI tract were not available from this patient, and no SARS-CoV-2 RNA was detected in the corresponding colon liquid. Based on these results, the actual prevalence of SARS-CoV-2 infection in the GI tract of individuals without a history of COVID-19 was estimated as 0.0118 with a 95% credible interval of [0.0018 and 0.0384].

Detection of SARS-CoV-2 in Colon Liquids of Patients Previously Diagnosed with COVID-19

Out of twelve patients in cohort B, three colon liquids out of ten (30%) tested positive for SARS-CoV-2 RNA, based on the presence of RNA for genes N1 and N2 (Fig. 1B, Tables 2 and 3 Suppl. Table 3). SARS-CoV-2 detection was independently confirmed in a second laboratory using backup samples. Expression of the E gene was detected in two of the three colon liquid samples that tested positive for genes N1 and N2. Based on these results, the prevalence of
SARS-CoV-2 in asymptomatic individuals with a history of COVID-19 is estimated as 0.2571 with a 95% credible interval of [0.0964, 0.4838].

All SARS-CoV-2 positive colon liquids were obtained from male patients undergoing routine screening colonoscopy who had either had an asymptomatic SARS-CoV-2 infection (2/3) or mild respiratory disease without any GI symptoms (1/3; Table 2). Other cohort B patients whose gastrointestinal samples tested negative for SARS-CoV-2 also only had mild to moderate COVID-19 symptoms, with no hospitalizations reported (Table 2). Surprisingly, one patient with SARS-CoV-2 detection in colonic liquid had received a COVID-19 diagnosis more than 5 months prior to the colonoscopy. In the three SARS-CoV-2-positive patients, a shorter time between COVID-19 diagnosis and sample collection corresponded to a higher recovery of SARS-CoV-2 RNA (Fig. 1B).

Long term detection of SARS-CoV-2 protein in the colonic epithelium of patients following a previous COVID-19 diagnosis

To determine whether detection of SARS-CoV-2 RNA in colonic liquids and tissue was associated with viral replication in the GI mucosa, we performed immunohistochemistry for SARS-CoV-2 nucleocapsid protein (NP). As shown in Fig. 2, viral NP was present in a small number of epithelial cells in the colonic crypts of all three SARS-CoV-2 RNA positive Cohort B patients, but not in control tissues from SARS-CoV-2 negative patients. In the three positive tissues, 2.5±0.4 NP-positive cells per 10 crypts were identified (Table 3). Interestingly, NP-positive material was generally confined to a circumscribed area in the basolateral portion of crypt epithelial cells. NP staining was not associated with goblet cells or the surface epithelium.
We did not detect any SARS-CoV-2 nucleocapsid protein in the corresponding ileal tissue sections of these patients. No NP-positive cells were detected in the colon of the cohort A patient who tested positive for the virus.

Histopathological analysis revealed that all three colon samples obtained from control patients and two of the SARS-CoV-2 positive donor tissues (BDH156 and BDH160) were histologically normal (Suppl. Fig. 1A). In contrast, colon tissues from two patients with positive SARS-CoV-2 PCRs (BDH121 and BDH154) showed increased plasma cell density in the lamina propria (Suppl. Fig. 1B,C).

Colonic tissues and liquid samples that tested positive for SARS-CoV-2 by PCR did not contain detectable levels of infectious virus.

We next used a viral isolation assay to detect infection-competent virions in SARS-CoV-2 RNA positive samples. However, no infectious virus was recovered from any of the SARS-CoV-2 positive colon liquid or tissue samples (Table 3). Thus, our study confirms multiple previous reports that had detected SARS-CoV-2 RNA or protein in GI tissues from COVID-19 patients but were unable to isolate infectious virus.¹⁵,²⁶

Colonic liquids consistently inactivate SARS-CoV-2 in vitro

Zang, et al.²⁷ previously reported that intestinal liquids may inactivate SARS-CoV-2 in the lumen of the GI tract, based on in vitro experiments with simulated GI liquids. Therefore, we studied the inactivation of SARS-CoV-2 (USA-WA1/2020, 10⁶ PFU/mL) in the presence of colon liquids that we had collected from six donors from cohort B, including samples from the
three patients that were positive for SARS-CoV-2. As soon as 10 minutes post incubation, one of the colon liquids from a SARS-CoV-2 negative patient had fully inactivated the virus. After 1 hour incubation, 50% of colon liquids had fully inactivated SARS-CoV-2, and by 24 hours post incubation no infectious virus was detected in any colon liquid (Fig. 3A). No significant difference was seen between colon liquids with and without SARS-CoV-2 detection (Fig. 3B).

Discussion

We here analyzed the prevalence of gastrointestinal SARS-CoV-2 infection in gastrointestinal endoscopy patients without active COVID-19 to better understand potential transmission risks arising from endoscopy procedures. Our study confirms previous reports that the GI tract is a site of active SARS-CoV-2 replication and serves as a potential long-term reservoir for the virus. We detected SARS-CoV-2 RNA in 30% of colon liquids collected from previously infected patients who had asymptomatic infections or who had recovered from mild infection, with one sample testing positive 5 months after the patient’s COVID-19 diagnosis. We also detected SARS-CoV-2 nucleoprotein in the colonic epithelium of all three patients from this cohort who had positive PCR results. All three of these patients were male, but no conclusions about sex-related differences could be drawn given the small group size. The presence of viral protein within GI tissue cells indicates active infection of the GI tract rather than carryover contamination of the GI tract from the upper respiratory tract, supporting previous reports of SARS-CoV-2 replication in GI epithelial cells. Similar to Cheung, et al. and Livanos, et al., we detected SARS-CoV-2 nucleocapsid in epithelial cells at the base of the crypts. However, we did not see any evidence of SARS-CoV-2 infection in goblet cells in our colonic samples, whereas Livanos, et al. demonstrated that goblet cells were a major target for
SARS-CoV-2 in the small intestine. Intriguingly, only 1/12 of the recovered COVID-19 patients in our study had reported any GI symptoms during the course of their illness.

Since viral RNA was detected as late as 151 days after acute SARS-CoV-2 infection, our data support the hypothesis that the GI tract can be a long-term viral reservoir that sheds virus or viral RNA after respiratory infection has ceased. While the number of patients with previous SARS-CoV-2 infections (cohort B) included in our study was low, the probability for SARS-CoV-2 detection in this group (30%) was much higher than in patients with no known prior SARS-CoV-2 infection (1%). Given the worldwide surges in SARS-CoV-2 infection due to the highly transmissible Delta and Omicron variants in summer/fall 2021 and in early 2022, the percentage of the population with a previous infection and thus potential virus persisting in the gut now is likely considerable. However, we recovered no infectious virus from any positive GI tissue or liquid sample. Notably, no infectious virus was detected from samples with a CT value above 30 in the SARS-CoV-2 RT-PCR assay in several previous studies \(^ {32,33}\), and all positive gastrointestinal samples in our study had CT values of >30 for all genes that were analyzed.

The detection of SARS-CoV-2 RNA in the colon liquid of patients is indicative of active GI infection with either shedding of SARS-CoV-2 virions or dying infected cells into the gut lumen. However, the harsh environment of the GI tract is expected to inactivate virus or viral RNA rapidly \(^ {34}\). In a previous study Zang, et al. \(^ {27}\) demonstrated that simulated colon liquid led to almost complete inactivation of SARS-CoV-2 \textit{in vitro} within 24 hours. We showed that incubation of infectious SARS-CoV-2 with colon liquids collected from endoscopy patients reduced the amount of infectious virus by approximately 4 log-fold within 1 h and led to complete inactivation of SARS-CoV-2 within 24 hours. Considering this rapid inactivation and
the relatively low amount of SARS-CoV-2 detected in the colon liquids, any virions shed into the GI lumen and aerosolized during endoscopy would likely pose minimal transmission risk.

We found no evidence of significant colonic pathology in SARS-CoV-2 positive subjects. However, some signs of increased inflammation were present, with two of the positive samples having an overabundance of plasma cells. These observations are consistent with findings from Qian, et al. 28, who reported abundant lymphoplasmacytic infiltrates in the rectal lamina propria upon intestinal SARS-CoV-2 infection. Likewise, Livanos, et al. 15 described significant changes in small intestinal immune cell subsets and functions upon intestinal SARS-CoV-2 infection. The clinical relevance and mechanism of these changes remains a subject for further investigation.

Our study has several limitations that should be taken into consideration. First, all samples analyzed in our study were collected before major viral variants of concern (Delta/B.1.617.2 and Omicron/B.1.1.529) emerged 29, 30. Differential cell and tissue tropism has been reported for certain viral variants 31, but it is currently unknown whether then Delta and Omicron variants have an altered tropism for the GI tract. Second, while our analyses clearly demonstrated that persistence of SARS-CoV-2 in the GI tract is relatively common, our overall sample size was small, none of the patients in our study had severe COVID-19 symptoms, and subjects from only one healthcare facility were recruited. Therefore, our results may not be entirely representative and will require further investigations.

In summary, our results highlight the multisystem pathogenesis of COVID-19. We uncovered asymptomatic SARS-CoV-2 infection of the gut in the absence of respiratory symptoms, though prevalence was low. Detection of SARS-CoV-2 in patients previously diagnosed with COVID-19 was considerably more common, suggesting extra precautions for endoscopy procedures on recovered COVID-19 patients could be necessary. Of importance, we
predict these asymptomatic infections pose minimal risk to healthcare workers performing endoscopies, since no infectious virus could be recovered from SARS-CoV-2 positive GI samples, since none of the healthcare personnel involved in our study contracted SARS-CoV-2 throughout the course of our study, and since we demonstrate the rapid inactivation of infectious SARS-CoV-2 in colon liquids, which are the main source of endoscopy aerosols and droplets produced during the procedures. The long-term detection of SARS-CoV-2 in GI tissue and liquids warrants further study of SARS-CoV-2 infection in the GI tract and its potential as a viral reservoir.
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Tables

Table 1. Demographics of the endoscopy patients in cohort A with no known history of COVID-19

|                | Total (n) | Average Age (years) | Median Age (years) | Range (years) |
|----------------|-----------|---------------------|--------------------|---------------|
| All Patients   | 100       | 51.9                | 55                 | 22-69         |
| Female         | 52        | 51.7                | 54.5               | 24-69         |
| Male           | 48        | 52.0                | 55.5               | 22-69         |
Table 2. Demographics of the endoscopy patients in cohort B with prior SARS-CoV-2 infections

| Patient ID | Sample Types† | Sex‡ | Age (years) | BMI | D.M.∗ | Hypertension | Immuno-suppr.♯ | Clinical signs of COVID-19 | Days since SARS-CoV-2 diagnosis | SARS-CoV-2 detection |
|------------|----------------|------|-------------|-----|-------|-------------|----------------|---------------------------|--------------------------------|---------------------|
| BDH 154    | I, C, CL       | M    | 54          | 25.4| N     | N           | N              | Mild, cough               | 13                             | Y                   |
| BDH 155    | C, CL          | M    | 56          | 23.6| N     | N           | N              | Loss of taste and smell  | 24                             | N                   |
| BDH 156    | I, C, CL       | M    | 55          | 28.4| N     | N           | N              | Asymptomatic, close contact | 40                             | Y                   |
| BDH 157    | S, D           | M    | 44          | 25.3| N     | Y           | N              | Asymptomatic, close contact | 113                            | N                   |
| BDH 158    | I, C, CL       | F    | 67          | 30.8| N     | Y           | N              | Cough, loss of taste and smell | 69                             | N                   |
| BDH 159    | C, CL          | F    | 59          | 22.0| N     | Y           | N              | Sore throat               | 108                            | N                   |
| BDH 160    | I, C, CL       | M    | 60          | 27.2| N     | N           | N              | Asymptomatic, close contact | 151                            | Y                   |
| BDH 161    | I, C, CL       | F    | 57          | 41.7| N     | N           | N              | Body aches, chills, headache, rhinorrhea | 76                             | N                   |
| BDH 162    | S, D           | M    | 69          | 27.7| N     | N           | N              | Cough, headache, shortness of breath, heaviness in chest | 104                            | N                   |
| BDH 164    | I, C, CL       | F    | 22          | 22.0| N     | N           | N              | Loss of taste and smell  | ~200-250                      | N                   |
| BDH 166    | I, C, CL       | M    | 50          | 33.8| N     | N           | N              | Cough, diarrhea, fatigue | 129                            | N                   |
| BDH 167    | I, C, CL       | F    | 51          | 36.1| N     | N           | N              | Asymptomatic, close contact | 79                             | N                   |

†=ileum, C=colon, CL=colon liquid, S=stomach, D=duodenum; ‡=male, F=female; ∗=D.M.=Diabetes mellitus, N= no, Y=yes; ♯=Immunosuppr.=Immunosuppressive therapy
Table 3. Detection of SARS-CoV-2 nucleic acids, protein and infectious virions in gastrointestinal samples collected from asymptomatic endoscopy patients.

| Patient ID | Cohort | Sample Type  | Sample Type (RT-PCR) | N1 (CT) | N2 (CT) | E (CT) | RNaseP (CT) | Virus isolation | Nucleocapsid pos. cells/ 10 colonic crypts \( ^a \) |
|------------|--------|--------------|----------------------|---------|---------|---------|-------------|-----------------|--------------------------|
| BDH 121    | A      | Colon tissue |                      | 38.0    | 38.2    | 36.7    | 33.1        | negative        | 0.00±0.00               |
| BDH 154    | B      | Colon liquid |                      | 30.0    | 31.8    | 31.4    | 33.0        | negative        | 1.96±0.22               |
| BDH 156    | B      | Colon liquid |                      | 33.1    | 35.4    | 35.7    | 33.2        | negative        | 2.89±0.71               |
| BDH 160    | B      | Colon liquid |                      | 38.0    | 36.9    | n.d.    | 31.8        | negative        | 2.59±0.25               |

n.d.; not detected; * Mean ± SD from two independent researchers. 75 or more crypts were evaluated in each biopsy sample.
Figure Legends

**Figure 1. SARS-CoV-2 detection in wastewater and GI samples.** (A) Community SARS-CoV-2 waste water surveillance data during the period of sample collection, with corresponding reported case numbers in Gallatin County, MT. Cohort A (n=100) represents randomly selected individuals who had no COVID-19 symptoms and cohort B (n=12) represents patients with a previous diagnosis of COVID-19 within 9 months prior to the endoscopy procedure. Arrows indicate dates when SARS-CoV-2 positive samples were collected. (B) Detection of SARS-CoV-2 RNA in colon liquid samples from three patients in cohort B. Comparison of inverse Ct values for quantitative PCR detection of SARS-CoV-2 genes N1 and N2 in colon liquids to number of days since COVID-19 diagnosis. Boxes around data points indicate data from one patient.

**Figure 2. Detection of SARS-CoV-2 protein in colonic tissue by immunohistochemistry.** Paraffin-embedded tissue sections were immunostained for SARS-CoV-2 nucleocapsid protein (NP, brown) and the counter stained with hematoxylin (purple). (A) Colon from a subject with no known previous SARS-CoV-2 infection and negative SARS-CoV-2 PCR results in intestinal samples does not contain any NP-positive cells. (B-D) Detection of SARS-CoV-2 nucleocapsid protein in the colonic crypts of cohort B patients BDH154 (B), BDH156 (C) and BDH160 (D). Arrowheads identify infected crypt epithelial cells. All scale bars equal 10 μm.
Figure 3. Inactivation of SARS-CoV-2 by *in vitro* incubation with colon liquids. (A) To investigate inactivation of SARS-CoV-2 in colon liquids, 10^6 PFU/mL SARS-CoV-2 was spiked into six cohort B colon liquids, then a plaque assay using VeroE6 cells was performed after incubation at 37°C, 5% CO₂ for 10 minutes, 1 hour, or 24 hours to determine the remaining viral concentration. Each data point represents an individual donor; bars and error bars indicate mean ± SD. Results are plotted on a log scale, so samples where virus was fully inactivated (PFU/mL = 0) are not shown. N.d.; not detected. *P ≤ 0.05, 2-way ANOVA with Friedman test for multiple comparisons. (B) Comparison of SARS-CoV-2 inactivation after 10 min incubation in the presence of colon liquids that tested negative (n=3) or positive (n=3) for SARS-CoV-2. SARS-CoV-2 was also incubated in PBS for comparison of inactivation over time.
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A. Public health data
- Case count
- Wastewater data
  - N1
  - N2

B. SARS-CoV-2 RNA (log10 copies/mL)

Cohort A

Cohort B

Patient
- N1
- N2

Days Since SARS-CoV-2 Positive
