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Highly socially vulnerable communities exhibit disproportionately increased viral loads as measured in community wastewater

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ABSTRACT

Wastewater surveillance has proven to be a useful tool for evidence-based epidemiology in the fight against the SARS-CoV-2 virus. It is particularly useful at the population level where acquisition of individual test samples may be time or cost-prohibitive. Wastewater surveillance for SARS-CoV-2 has typically been performed at wastewater treatment plants; however, this study was designed to sample on a local level to monitor the spread of the virus among three communities with distinct social vulnerability indices in Shreveport, Louisiana, located in a socially vulnerable region of the United States. Twice-monthly grab samples were collected from September 30, 2020, to March 23, 2021, during the Beta wave of the pandemic. The goals of the study were to examine whether: 1) concentrations of SARS-CoV-2 RNA in wastewater varied with social vulnerability indices and, 2) the time lag of spikes differed during wastewater monitoring in the distinct communities. The size of the population contributing to each sample was assessed via the quantification of the pepper mild mottle virus (PMMoV), which was significantly higher in the less socially vulnerable community. We found that the communities with higher social vulnerability exhibited greater viral loads as assessed by wastewater when normalized with PMMoV (Kruskal-Wallis, $p < 0.05$). The timing of the spread of the virus through the three communities appeared to be similar. These results suggest that interconnected communities within a municipality experienced the spread of the SARS-CoV-2 virus at similar times, but areas of high social vulnerability experienced more intense wastewater viral loads.

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1. Introduction

Since its designation as a global pandemic in early 2020 by the World Health Organization (WHO), the coronavirus disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has impacted the world by prompting wide scale closing of businesses, schools, and government offices; it has also overloaded the healthcare infrastructure. As of May 2022, a little over a year after the Food and Drug Administration approval of multiple vaccines for SARS-CoV-2, the latest Delta and Omicron variants of concern (COVID-19 variants B.1.617 and B.1.1.529, respectively) have swept through the United States (U.S.) at a pace more contagious than that of any previous variant (Twohig et al., 2022; Shiehzadegan et al., 2021; Liu and Rocklöv, 2021). The intersection of the infectiousness of COVID-19 and aspects of modern life, which gives people the ability to connect in larger numbers and faster than ever before, exposed many vulnerabilities in our current understanding of and monitoring methods for infectious diseases (Chinazzi et al., 2020).

The use of wastewater-based epidemiology (WBE) to estimate COVID-19 cases has greatly expanded since the beginning of the pandemic. WBE, a technique used to estimate substance consumption and disease prevalence in municipalities via composite or grab samples of wastewater, has been used to monitor other human viruses and drugs of abuse (e.g., opioids, amphetamines, etc.) (Sims and Kasprzyk-Hordern, 2020; Farkas et al., 2018). SARS-CoV-2 detection in wastewater measures the concentration of non-infectious RNA shed in municipal wastewater by those infected with the virus (Wang et al., 2020). WBE is generally conducted via the influent or primary solids at the wastewater treatment plant (WWTP), where viral genome copies, illicit substance, population marker, or other analytes can be quantified (Sims and Kasprzyk-Hordern, 2020). While this information has proven to be of great value, it provides a composite measure across all communities served by a WWTP (often on the order of 100,000 or more) and does not allow for the separation of patterns within distinct communities served by the WWTP. Because of the lack of samples collected within distinct populations served by a WWTP, there is a dearth of information on the patterns of the viral spread between different communities within a single municipality. Understanding how the spread of the virus developed in different communities throughout a city could help determine how these populations transmit the agent/substance/disease at different rates and times. Targeted wastewater surveillance (TWS) is a specific approach to the current WBE conducted at WWTPs. TWS is performed at the sub-sewershed level with the aim of observing input from specific neighborhoods, communities, hospitals, and/or businesses (Scott et al., 2021). While TWS may offer significant value, the paucity of research using this technology in outbreak settings and the potential challenges of testing for viral RNA requires cautious optimism. For example, according to the Centers for Disease Control and Prevention (CDC), there is greater variability in the load of SARS-CoV-2 in wastewater samples as smaller populations are assessed. This is because the SARS-CoV-2 RNA accumulated in wastewater has less opportunity to diffuse and is thus characterized by intermittent pulse inputs associated with the behavior of individuals, and the volume of human waste diminishes the further upstream it is from the WWTP (CDC, 2022).

One of our aims in this study was to ingrate demographics by using social vulnerability. The CDC uses U.S. Census Bureau data to determine the social vulnerability of every census tract by creating an index, known as the social vulnerability index (SVI). The CDC gathers information on the SVI by compiling an area susceptibility to factors such as disease outbreaks and natural disasters, and collate this information with socioeconomic factors such as income and poverty (CDC, 2020). The full range of these categories is explained further in Methods 2.1. To better understand the dynamics of viral spread within subcommunities served by a single WWTP, this study was also designed to determine timing and magnitudes of SARS-CoV-2 RNA viral loads in wastewater in three economically stratified communities. These neighborhoods were separated in population and substantially by the SVI so that we could find if more socially vulnerable communities were disproportionately affected. Finally, this study uses data collected from twice monthly grab samples, a low-resource model of WBE, in order to assess its utility for TWS in resource constrained communities.

2. Methods

2.1. Community selection and collection

The sites chosen for this study are described using anonymized identifiers to protect the privacy and confidentiality of those communities. Each collection site was approaching a lift station that collected from the entire community. The selected communities have 1) a similar size population, 2) greater than 5% of the population served by the same WWTP, 3) a major hospital located within that community, and 4) both residential and commercial areas. They varied in their social vulnerability index (SVI), a socio-demographic indicator that includes characteristics such as education, median household income, poverty level, median age, marital status, age, and employed adults based on their American Community Survey 5-Year Estimates 2015–2019 (ACS) (US Census Bureau, 2019) (Table 1). The patient population/community area population was large enough to maintain individual anonymity ($\geq$10,000). Three communities in Shreveport, LA were chosen based on their publicly available socioeconomic data and vulnerability, obtained from the U.S. Census Bureau and the CDC. Community A is classified as having a low SVI (Table 1), while Communities B and C are both classified as having a high SVI (Table 1). Collection of wastewater data for Communities A, B, and C occurred twice monthly from September 30, 2020, to March 23, 2021. The weekly total cases for Caddo county, the state of Louisiana, and the United States during the time of the study are presented in Supplemental Fig. 1.

Another variable used in community selection was zip code, which represents the wastewater service area collected at the manholes contained in 5 (Community A) or 6 (Community B and Community C) census tracts. Along with the zip code demographics from the U.S. Census Bureau, we included the SVI average for each community. The SVI is a metric developed by the CDC that produces a relative comparison of census tracts based on the proportion of the population who are/have living below the federal poverty level, unemployed, below-average income, lacking a high school diploma, aged 65 or older, aged 17 or

Table 1
Population Characteristics of Communities A, B, and C. Population dynamics are described by zip code data from the American Community Survey 5-year Estimates (2015–2019). The Social Vulnerability Index (SVI) was provided by the Centers for Disease Control by census tract and averaged for census tracts contained within a zip code. Communities B and C are considered low-SES and vulnerable to most vulnerable (quartile III and IV, respectively).

Site	Community A	Community B	Community C
Population[a]	~20,000	~20,000	~10,000
No HS Diploma[a] (25+ years old)	<5%	>20%	>20%
Median Household Income[a]	>$55,000	<$25,000	<$25,000
Below Poverty Level[a]	<15%	>40%	>35%
Median Age[a]	>40	>30	>30
Married[a]	>40%	<30%	<30%
>65 years of age[a]	15%	15%	25%
Employed Adult Males[a]	>80%	<60%	<70%
Percent African American[a]	<25%	>80%	>80%
Average SVI[b] (95% CI)	0.13 (0.03–0.22)	0.74 (0.57–0.91)	0.83 (0.72–0.94)

[a] Source: U.S. Census Bureau, American Community Survey 5-Year Estimates (2015–2019).

[b] Source: CDC SVI 2018, https://www.atsdr.cdc.gov/placeandhealth/svi/documentation/SVI_documentation_2018.html.

younger, civilians with a disability, living in single-parent households, from a minority group, speaking English "less than well," living in multi-unit structures, living in mobile homes, in crowded living conditions, lacking a vehicle, and living in group quarters. The SVI is scored between 0 and 1, with '0' representing socially privileged and '1' representing socially deprived/vulnerable (CDC, 2020; Flanagan et al., 2011). The CDC uses the SVI as an indicator of the impact of external factors, such as disease outbreaks, natural disasters, and exposure to chemicals, on human health. The reduction of the score (from 1 to 0) results in less economic loss and human suffering. The intended goal, which is to reduce the score, can be achieved by creating a plan for the distribution of essential supplies, better distribution of emergency personnel, and provision of emergency shelters and routes of evacuation, including routes designed specifically for those with special needs (e.g., no vehicle, elderly with no support, language barriers) (CDC, 2020). We downloaded the SVI for the census tracts within these communities from the CDC and averaged them within each community's zip code.

2.2. Polyethylene glycol (PEG) precipitation

Twice monthly, 500 mL of raw wastewater grab samples were taken from the gravity sewer mains located in the three communities between 7:20–10:15 a.m. and transferred to low-density polypropylene bottles (total collections n = 33) by the Shreveport Water and Sewage Department sewer team. The wastewater collection occurred concurrently from community to community (A, B, and C) into a collection set (collection sets n = 11), however the day of collection varied from collection-to-collection within the collection week (typically occurring on a Tuesday or a Wednesday). Some exceptions occurred of the collection weeks between November–January were due to Thanksgiving, Christmas, and New Year's U.S. holidays where the collection set was postponed a week. The delay between the February 2, 2020, collection set and the March 3, 2020, collection set was due to an extreme winter weather event that disabled much of the U.S. South infrastructure. The collection set was stored at 4 °C in an ice chest until transferred to the testing laboratory in the early afternoon.

Using a method adapted from Scott et al. (2021), 100 mL from each raw wastewater grab sample was transferred to another low-density polypropylene bottle. Polyethylene glycol 8000 (8%) (Fisher Bioreagents; Hampton, NH) and NaCl (0.2 M) (Fisher Bioreagents; Hampton, NH) were added to the bottle with the raw sewage. Farkas et al. (2018, 2017) demonstrated that the PEG/NaCl combination had the greatest recovery after 16 h. The solution was mixed for 2 h at 4 °C and allowed to settle overnight. The remaining volume of wastewater grab sample was archived at −20 °C. After the PEG precipitation, the entire precipitated sample volume was centrifuged at 6000×*g* for 1 h, forming a PEG-virus pellet. 100 mL of supernatant was then discarded from the sample and the pellet was resuspended in the remaining supernatant (~6 mL volume). The resuspended pellet was then transferred into low-adhesion microcentrifuge tubes (Eppendorf; Hamburg, Germany) in 6–1 mL aliquots and stored at −80 °C until RNA extraction (typically 14–22 days).

2.3. Positive clinical test data

The positive zip code clinical test data were provided without private health information by the Center of Excellence for Emerging Viral Threats (CEEVT) in tabulated data form (total number of clinical tests, positives, and zip codes). The CEEVT compiles SARS-CoV-2 screening information from testing centers associated with LSUHS, including grades K through 12, nursing homes, and hospital screenings. The positive SARS-CoV-2 screening tests used included PCR molecular and rapid-antigen positive tests. Positive test data from 7 days prior, 7 days post, 14–21 days, and 28–35 days from the date of wastewater collection were compared to the PMMoV-normalized wastewater concentration with a Spearman correlation to develop a possible, indirect relationship.

2.4. Whole process control

We chose bovine respiratory syncytial virus (BRSV), a single-stranded, enveloped RNA virus, as the whole process control to assess the matrix recovery of the extracted SARS-CoV-2 viral RNA from wastewater because the two viruses share similar properties and structures (Boxus et al., 2005), as also because there have been other studies that stated BRSV's capability for this purpose (Gonzalez et al., 2020; Kantor et al., 2021). A 125 μL aliquot of 125 INFORCE 3 cattle vaccine, a lyophilized vaccine product, (PBS Animal Health; Massillon, OH) resuspended in 10X PBS, was seeded into each 100 mL wastewater sub-sample (ratio: 50 μL cattle vaccine to 40 mL wastewater established from IDEXX Laboratories) and run through the SARS-CoV-2 PEG precipitation and RNA extraction procedures (described in 2.2 and 2.6.1, respectively). For BRSV seeded in two sample batches (n = 24), the average recovery rate for BRSV after PEG concentration, ultracentrifugation, and RNA extraction was 23.4% [CI: 19.6%, 27.2%].

2.5. PMMoV normalization

Pepper mild mottle virus (PMMoV) has been shown worldwide to be a consistent indicator of the concentration of human feces in wastewater and proposed for use in water quality assessment (Haramoto et al., 2013; Rosario et al., 2009). PMMoV is a plant virus endemic to humans and commonly ingested without incident; therefore, PMMoV is a reasonable candidate for normalization of SARS-CoV-2 RNA measurements in wastewater to account for the variation in fecal content of individual grab samples (Rosario et al., 2009; Bwire et al., 2021; D'Amico et al., 2020). Several studies have found the prevalence of COVID-19 in the communities is better understood when SARS-CoV-2 RNA concentrations are normalized with PMMoV, to eliminate the possibility of extreme amounts of waste (and therefore dilution), and not extreme COVID-19 transmission (D'Aoust et al., 2021). SARS-CoV-2 was normalized in two ways, 1) by dividing the detected SARS-CoV-2 RNA by the ratio of that sample's PMMoV GC/PCR reaction and the distinct median of the PMMoV concentration on a site-by-site basis (SARS/PMMoV site) (Equation (1)), and 2) performed as D'Aoust et al. (2021) (Equation (2)) where the quantified wastewater SARS-CoV-2 RNA was divided by the quantified wastewater PMMoV RNA. We performed the first normalization to account for potential differences the overall and ongoing population contributing to each site (Symonds et al., 2019) and the second to account for the daily population contribution.

$$SARS-CoV - 2\ GC/L_{day\ n\ for\ site\ X} \Big/ \left(\frac{PMMoV\ GC\ per\ PCR\ reaction_{day\ n\ for\ site\ X}}{PMMoV\ GC\ per\ PCR\ reaction_{median\ for\ site\ X}}\right) \quad (1)$$

Where n = collection date, X = specific site.

$$\frac{SARS-CoV-2\ GC/L}{PMMoV\ GC\ Per\ PCR\ reaction} \quad (2)$$

2.6. Quantification of SARS-CoV-2 viral RNA via RT-qPCR

2.6.1. RNA extraction

RNA was extracted from 200 μL of concentrated wastewater using the QIAamp Viral RNA minikit™ (Qiagen; Hilden, Germany) following the manufacturer's instructions, eluted in 80 μL of buffer AVE (included in the minikit), and archived at −80 °C. The kit contains all the required reagents (buffers were rehydrated with 100% ethanol). We included a negative PCR grade water extraction control with each extraction batch.

2.6.2. Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR)

The CDC N1 and N2 TaqMan primer and probe sets (Supplemental

Table 1) were selected for detection of SARS-CoV2 in RT-qPCR amplification based on the previous study by Peccia et al. (2020). A non-infectious SARS-CoV-2 standard (2.0×10^5 genome copies, GC, per mL, 2.0×10^2 GC per μL) from Exact Diagnostics was used to develop a standard curve. We also used TaqMan primers/probe to detect human RNAse P (RP) (Supplemental Table 1) as a process control, which was detected in all samples. The RT-qPCR amplification was conducted using a CFX96 Touch Real-Time PCR Detection System (CFX96) (Bio-Rad; Hercules, CA).

Reverse transcription (RT) was completed at 55 °C for 10 min. Amplification consisted of 45 cycles of 10 s denaturation at 95 °C followed by 30 s extension at 55 °C, with an initial denaturation stage of 1 min at 95 °C. Each quantitative polymerase chain reaction (qPCR) included: 5 μL aliquot of extracted wastewater purified RNA, 1.5 μL N1/N2 primer (CDC Emergency Use Authorization) and probe set (all emergency use authorization CDC primers and probes were procured from Integrated DNA Technologies; Coralville, IA), 10 μL 2X Universal Probe 1-Step Reaction Mix (New England Biolabs; Ipswich, MA), 1 μL 20X WarmStart RT Enzyme Mix (New England Biolabs; Ipswich, MA), and 2.5 μL of DNA/RNA nuclease-free water (total volume per reaction: 20 μL). The PMMoV assay with the TaqMan primers and probes (Supplemental Table 1) was not run until after the collection period in this study ended. The PMMoV assay was conducted after a single freeze/thaw cycle from the extracted wastewater RNA (volume of extracted wastewater RNA after SARS-CoV-2 quantification: 75 μL). The standard curve was generated from a gBlock Gene Fragment (Integrated DNA Technologies; Coralville, IA) PMMoV Standard (1.0×10^8 PMMoV GC per 5 μL) ten-fold serial dilutions (1.0×10^8–1.0×10^2 PMMoV GC per 5 μL).

2.7. Data analysis and assays

All figures were illustrated with the GraphPad Software™ in the GraphPad Prism 9.3.1 program (Dotmatics; Boston, MA). RT-qPCR results were obtained directly from the reaction vessel in which RT-qPCR amplification was performed, CFX96, and displayed via the CFX Maestro Software (Bio-Rad; Hercules, CA). The amplified product for the N1 and N2 assays were measured in the C_q (limit of detection was ~1 SARS-CoV-2 GC/reaction, described later in this section) of the sample wells and plotted along the standard curve against the log of the starting quantity (SQ). The SQ mean of the sample duplicate was converted to SARS-CoV-2 genome copies/L. For the specific sample RT-qPCR N1 and N2 assay, the limit of detection (LOD) was determined to be a sample above 40 C_q (this was typically plotted at ~1 SARS-CoV-2 GC per 5 μL, 4800 SARS-CoV-2 GC per liter). For RT-qPCR N1 and N2 assays, samples were rerun after the collection period of the study alongside a standard curve of the SARS-CoV-2 standard (Exact Diagnostic; Fort Worth, TX) with a dynamic linear range of quantification from 1×10^3 to 1 genome copy per reaction. The slope of the standard curve for N1 PCR ranged from −3.232 to −3.384 with amplification efficiencies ranging from 97.5% to 103.9%. The Y-intercept for each standard curve ranged from 37.969 to 39.001. R^2 values ranged from 0.978 to 0.993. For N2 PCR, the slope of the standard curve ranged from −3.262 to −3.424 with amplification efficiencies ranging from 95.9 to 102.5. The Y-intercept for each standard curve ranged from 39.534 to 40.696. R^2 values ranged from 0.985 to 0.994. The amplified product for the PMMoV assay was measured and plotted as the N1 and N2 assays were with its own standard curve, mentioned in 2.6.2. For the PMMoV assay, the standard curve ranged from 10^8 to 10^2 GC per PCR reaction. The slope was −3.341 with an amplification efficiency of 99.2%. The y-intercept was 42.908 and R^2 was 0.993.

3. Results

3.1. Measured wastewater virus magnitude variation

3.1.1. SARS-CoV-2 RNA magnitude varies between communities

The N1 and N2 primer SARS-CoV-2 RNA quantities were strongly associated within Community B ($r = 0.959$, $p < 0.001$) and Community C ($r = 0.986$, $p < 0.001$), so we only included the N1 magnitudes for these two communities in their SARS-CoV-2 RNA in the figures and analysis. The N1 assay was the only assay used in Community A. The N2 assay for Community A was not used in our analysis as there were only two samples above our threshold of detection of the 11 total samples analyzed from Community A. SARS-CoV-2 RNA was detected within the 33 collections 84.8% and 42.4% of the time using the N1 and N2 primers, respectively.

There was not a statistically measurable difference in the mean concentration of the unadjusted N1 SARS-CoV-2 RNA/L in wastewater grab samples between the three communities (Kruskal-Wallis, $p > 0.05$) (Table 2, Fig. 1, below). There was a measurable difference when the population was adjusted for with PMMoV (Kruskal-Wallis, $p = 0.0127$) (Fig. 1). The quantified SARS-CoV-2 genome copies were normalized to PMMoV for N1 and N2 with two methods previously described. To assess a pairwise difference between in each community we used a Wilcoxon ranked sum exact nonparametric paired *t*-test. A pairwise difference was observed between communities A and C with PMMoV site median normalization and SARS-CoV-2/PMMoV normalization ($p = 0.0391$ and $p = 0.0195$, respectively) (Fig. 1). There were not pairwise differences in relation to Community B and the other 2 communities (Wilcoxon, $p > 0.05$).

3.1.2. PMMoV as a population indicator

Following the detection of SARS-CoV-2 RNA in the wastewater samples ($n = 33$), PMMoV was quantified from the stored, extracted wastewater RNA, PMMoV RNA was quantified in all wastewater grab samples (5.0×10^7—8.4×10^8 PMMoV GC/L, lowest detection to maximum detection). There was a measured difference in the communities PMMoV RNA concentration (Kruskal-Wallis, $p < 0.05$). Community A's mean PMMoV RNA genome copies per PCR reaction is greater than Community C (Wilcoxon rank sum exact test, $p < 0.01$). There was not a pairwise difference between Community B to Community A or B to Community C (Wilcoxon, $p > 0.05$) (Fig. 1).

Table 2
Virus Summary Statistics. PMMoV, raw SARS-CoV-2 RNA, and PMMoV-normalized SARS-CoV-2 are compared via mean genome copy and standard deviation of each community. Quantification determined by plotting the inverse of the starting quantity of each virus' standard curve on measured C_q via RT-qPCR. PMMoV is a stable, low-variability virus that is commonly excreted by humans. Normalization with PMMoV accounts for possible dilution or excess population waste contribution at each site on a per sample basis. N1 quantification of Community C was larger than Community A (Wilcoxon, $p < 0.05$) after population adjustment using PMMoV using the two normalization strategies.

	Community A	Community B	Community C
Mean PMMoV genome copies per PCR reaction (Standard Deviation)	73,650 (41,352)	57,303 (45,010)	38,622 (21,566)
N1 SARS-CoV-2			
Mean Raw SARS-CoV-2 genome copies per L (Standard Deviation)	8150 (4010)	18,150 (23,779)	33,057 (32,138)
Mean PMMoV-Normalized SARS-CoV-2 per site PMMoV median (Standard Deviation)	7905 (3462)	17,674 (13,790)	36,562 (34,992)
Mean PMMoV-Normalized SARS-CoV-2 per PMMoV RNA (Standard Deviation)	0.1107 (0.0485)	0.3619 (0.2824)	1.016 (0.9723)

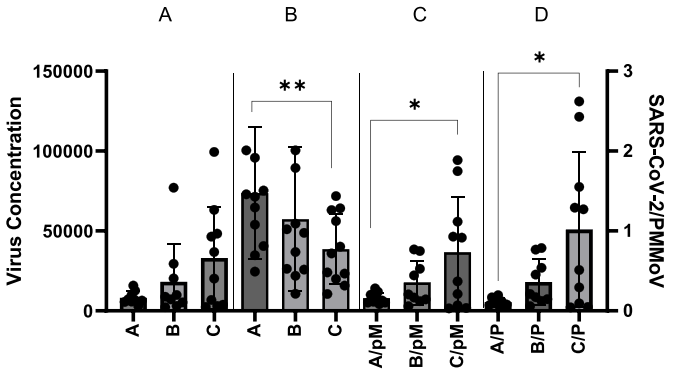

Fig. 1. SARS-CoV-2 and PMMOV wastewater concentration for communities. Left axis- RNA genome copies concentration. Right axis- SARS-CoV-2 genome copies per liter (L) normalization by dividing by quantified PMMoV RNA per 5 μL. The four sections are A) unadjusted SARS-CoV-2 per L, B) PMMoV per 5 μL, C) SARS-CoV-2 adjusted with PMMoV by accounting for the median PMMoV per 5 μL, and D) SARS-CoV-2 concentration when adjusting for the population by the daily PMMoV concentration measured from communities A, B, and C. Wastewater collection occurred at combined manhole sites that collected wastewater from each community before integration with the main city sewer line. All raw/PMMoV-normalized N1 samples from the collection period were included (represented by the dots) from Communities A, B and C. SARS-CoV-2 was normalized (first normalization) to the ratio of the site PMMoV concentration on collection to the median PMMoV concentration of each site (RNA per PCR reaction) as well as normalized to the daily concentration of PMMoV (second normalization). Significance of measurable difference determined from Kruskal-Wallis chi-square test of means. The mean concentration is represented by the bars. The error bars represent one standard deviation of the mean. The Pairwise comparison determined by Wilcoxon rank sum exact test. *$p < 0.05$, **$p < 0.01$, pM = PMMoV median, P=PMMoV RNA genome copies per 5 μL.

3.2. Beta wave wastewater SARS-CoV-2 duration by community

Using the twice monthly wastewater grabs, a picture formed from the wastewater RNA shedding of the 3 communities (Fig. 2). SARS-CoV-2 was above our threshold of detection (~4800 SARS-CoV-2 GC per L) for 154 days from September 30, 2020, to March 3, 2021, in Community C. Had a similar duration except there was no detection of SARS-CoV-2 RNA on December 4, 2020. Community A was above threshold from September 30, 2020, to February 2, 2021 (126 days). No community SARS-CoV-2 RNA was detected in wastewater on March 23, 2021.

3.3. Comparison of the communities with their zip code positive SARS-CoV-2 clinical tests

A Spearman correlation test was performed on the clinical test data and the PMMoV-normalized SARS-CoV-2 RNA wastewater concentration and showed that no significant correlation in the number of cumulative, positive individual tests in the associated zip code was found over the preceding week (-7D), next week (+7D), 14–21 days (+14D), or 28–35 (+28D) days ($p > 0.05$). The unadjusted SARS-CoV-2 RNA copies for each site were also not correlated with each other's individual positive tests in the zip code for the -7D, +7D, +14D, or +28D ($p > 0.05$). These time lagged correlations were also performed to establish a wave pattern that would show the route of COVID-19 through Shreveport, but we were unsuccessful.

This study also investigated the detection of RNA against the different time intervals—e.g., collection of Community A to next collection of Community B—of the other communities to unravel the waves of the individual variants beneath the overall wave of COVID-19 (30 September 2020–23 March 2021). This was done to specifically determine whether the communities were affected in a specific order. Using surveillance on these 3 communities could be too variable to establish this connection we were looking for with the parish cases/

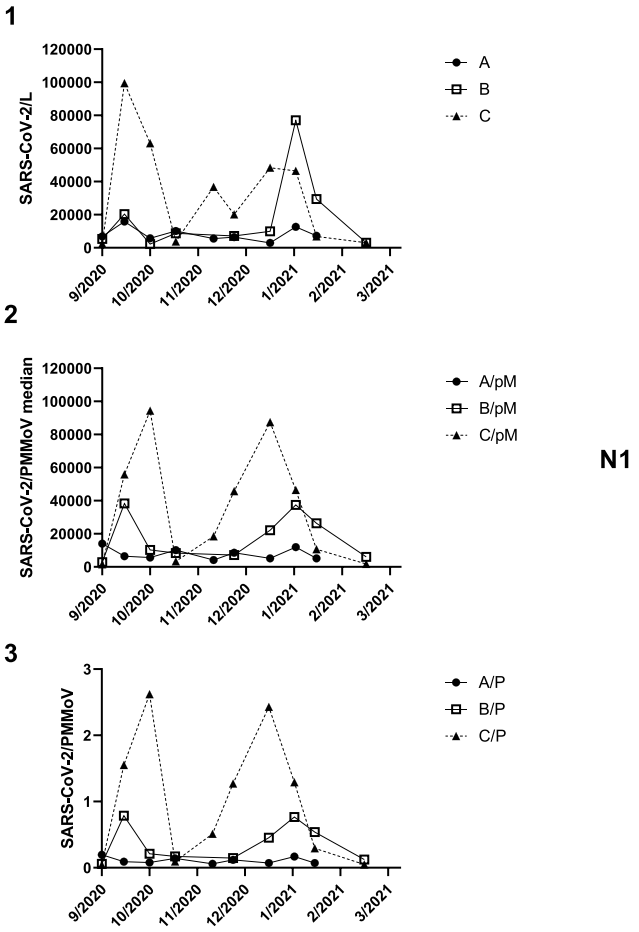

Fig. 2. Temporal SARS-CoV-2 in Shreveport wastewater. Collection period of study was September 30, 2020 to March 23, 2021. Major tick marks on x-axis signify end of the month (month/year). Community A quantification of SARS-CoV-2 above threshold of RT-qPCR for 126 days. Community B and C quantification above threshold for 154 days.

positives or the zip code positives as has been established with WWTP monitoring in the past. While the connection between the two previously mention disease prevalence indicators is not as strong, this study did show that COVID-19 affected each community differently.

The amount of SARS-CoV-2 RNA varied not only among the communities in their wastewater but also in the individual positive and total tests. Community C (n = 25,345) issued 23.7-fold and 27.4-fold more total tests than Communities A and B, respectively. This could lead to a potential confounding variable if positives were reported without the information from the total included.

4. Discussion

4.1. Wastewater sampling design

This study is among the first to integrate demographic information from the US Census Bureau by zip code, and compare wastewater retrieved SARS-CoV-2 viral load with the local SVI. Wastewater monitoring of SARS-CoV-2 has been increasing in the wake of the work beginning from early in the pandemic. However, the epidemiological value of wastewater monitoring that is focused on vulnerability and distinct prevalence patterns within a constrained community has been understudied. This project advances that knowledge by relating wastewater to US Census Bureau and SVI data. Other studies have confirmed

that, with COVID-19, the more at-risk more vulnerable populations have suffered disproportionately in terms of symptoms, hospitalizations, and deaths, but this relationship has not yet been established with wastewater, which is a valuable tool for community level monitoring, especially in resource constrained and vulnerable communities. Another feature of the design of this study was to focus on those lower-resource entities that can preferentially benefit from the twice-monthly grab samples. Frequency composite samples have been much more commonly used than grab samples, but are more personnel and resource intensive, and can require specialized equipment. The results of this study do show clear temporal trends as well as concentration differences between communities using this resource-efficient sampling approach. Finally, this study is also among the first to monitor concentration/temporal differences within a city at different community-level sites. The lower threshold of wastewater SARS-CoV-2 monitoring has not been established in times of high or low prevalence. We show in populations of 10–20 k that monitoring is possible and shows distinct trends, which allows for more focused monitoring and public health interventions than at a city-wide level. More work is needed at this level to establish the connection to other health metrics like hospitalizations and cases. The early-warning potential characterized by sampling from the influent of wastewater treatment plants of these metrics is not yet established on a community-level.

4.2. Public health policy implications

A significant problem for using testing of individuals to provide ongoing community level monitoring of public health is the prevalence of asymptomatic or pre-symptomatic individuals. These cases often do not fit the recommendations of symptomatic testing and can fall through the cracks of conventional testing (Zhao et al., 2020; Snider et al., 2021). Asymptomatic individuals are verified retroactively, and detection occurs only by testing symptomatic and asymptomatic individuals (Nikolai et al., 2020; Zhang et al., 2020). Since asymptomatic infections may be or likely are as infectious as symptomatic infections, transmission can occur without the knowledge of the affected individual (Alene et al., 2021; Ma et al., 2021). The global asymptomatic case rate is estimated to be 25% (Alene et al., 2021) to 40% (Ma et al., 2021); however, in select populations, this rate can account for a third (Ma et al., 2021) to 87% of cases (Subramanian et al., 2021). Monitoring these cases with individual nasopharyngeal swabs and rapid antibody-antigen tests is difficult and disproportionately affect lower SES/more vulnerable populations (Little et al., 2021; Ralli et al., 2021); wastewater surveillance can address the gap associated with under-reported cases (Bivins et al., 2020). The results of this study suggest that grab sampling can reasonably capture the general temporal trends of distinct communities in the same municipality. Community B experienced COVID-19 similarly and should be tested on par with Community C during an uptick, or the wastewater concentration deems they are both being affected. The wastewater detection of SARS-CoV-2 RNA is pointing to where the individual testing should go; it can help when there is an issue even if the cases are mainly asymptomatic.

University campuses have yielded indicators that show potential cases from dormitories before discovery by individual testing (Scott et al., 2021; Gibas et al., 2021). The key metric for these indicators is the trend line (general upticks) and not the absolute total of infected individuals. WBE is limited by factors such as varying viral shedding of individuals, SARS-CoV-2 stability in wastewater, and severity of illness. Assuming a low resource setting and these limitations, this model of WBE could still prove valuable during a community spread event. A SARS-CoV-2 upward trend in the wastewater could reinforce the power of individual testing like, as mentioned previously, Community C was tested 27.4-to-1 more times than Community B; yet Community B (Table 2 and Fig. 1) was much like Community C (Table 2 and Fig. 1) in terms of exposure and duration of SARS-CoV-2. This suggests that more wastewater sampling and/or clinical tests should have been performed in Community B. Moreover, the results of this study could yield an indicator of where to begin wastewater sampling for newly established practices, guided by the SVI or US Census Bureau to get a finger on the pulse of the sectors of a municipality. Information/warnings can then be disseminated to leaders of these communities to integrate a more efficient response and, most importantly to at-risk populations, healthcare intervention.

4.3. Vulnerability and RNA concentration

The SVI (5 or 6 census tracts averaged in each zip code) suggests that Community B (quartile III, vulnerable, SVI >0.500 but <0.750; Table 1) and Community C (quartile IV, most vulnerable, SVI >0.75; Table 1) are more vulnerable to any disaster or pandemic than Community A (quartile I, least vulnerable, SVI <0.25; Table 1). As indicated, the population adjusted wastewater concentrations of SARS-CoV-2 followed in the quartile of the SVI for Community A, to Community B, and Community C (Fig. 1). This met our expectation as previous work indicates lower SES are disproportionately affected with SARS-CoV-2 and clinical outcomes (Little et al., 2021).

The small population size relative to other PMMoV and SARS-CoV-2 studies could be more impacted by differences in diet or culture or daily population movements within the municipality. The individualized pockets of the three communities are so small and separated by SES that a separate population indicator from wastewater may need to be used. Communities A (20,000 residents) and B (20,000 residents) were approximately equally populated in 2019 and could not be statistically separated by PMMoV, but neither could Community C (10,000 residents) from Community B. It could be the case that the differences in concentration were driven by the differences in PMMoV. Community A's mean PMMoV (Fig. 1) was larger than Community C which would drive down the PMMoV-normalized SARS-CoV-2 in Community A relative to Community C.

4.4. Accounting for hospital association and other limitations

The populations in each community had no restrictions on movement within and between the other communities and their associated community hospitals; however, proximity to a hospital has been shown to be strongly correlated with the use of that hospital (Bergeron et al., 2015). There is no way to tell which member of which community is contributing to wastewater in other communities. The SARS-CoV-2 concentration could be affected by the 17 other hospitals in Shreveport, LA that are not covered in this study to which community members could also travel. Some of these other facilities are also integrated further upstream from the collection site. Compared to the other Shreveport hospitals not included in this study, the three hospitals located in the communities are the most capable during a spreading event that dramatically increases hospitalizations (e.g., size of hospital, emergency room/ICU bed capacity, and reputation).

This study design used grab samples as single snapshot of time to represent an entire 12–20 day period in our communities. This contrasts with the 12–24 h composite samples collected 2 to 5 times a week that are more common when using WBE for SARS-CoV-2 wastewater monitoring. The sample size of each community ($n = 11$) is also a concern. Nonetheless, the grab samples still yielded useful information indicating that sewage grab sampling is a viable approach in low-resource settings. It is also important to consider that a non-infected individual could experience an infection and recover in between these long-duration samples without their shedding contribution in waste; however, the temporal trends of spikes of COVID-19 and their resolution are still captured for the population (Fig. 2). There is uncertainty in how much or how long an individual may shed into the waste depending on symptoms, differences of variants, and viral load experienced.

SARS-CoV-2 and PMMoV were quantified after the study period was concluded from frozen ($-80\ ^{\circ}C$) extracted wastewater RNA. These RNA

could have degraded and limited accuracy. With that limitation in mind, PMMoV is quantified in all our samples, falls to within one order of magnitude, and is within range for the US South (Kitajima et al., 2018). This is the first wastewater study for northwest Louisiana, so comparative data was not available for SARS-CoV-2.

5. Conclusion

In this study, PMMoV-normalized SARS-CoV-2 wastewater RNA concentrations were assessed in three distinct communities in the same municipality that varied in their social vulnerability using a low-resource sampling strategy. Communities with higher social vulnerability exhibited greater viral loads but the timing of the spread of the virus through the three communities appeared to be similar. These results suggest that interconnected communities within a municipality experienced the spread of the SARS-CoV-2 virus at similar times, but areas of high social vulnerability experienced more intense wastewater viral loads. These data indicated that focused wastewater detection of SARS-CoV-2 RNA could potentially be used to direct tailored community-level medical interventions such as increased availability of vaccinations, increased availability of individual nasopharyngeal or rapid-antigen testing and attempts to increase individual awareness within a community. For example, wastewater data indicates Community B should have received individual testing at rates like Community C, yet it received 20-fold less individual SARS-CoV-2 tests. Finally, the use of PMMoV to correct for differences in the underlying population was effective in communities of between 10 and 20 thousand people, warranting continued use of this approach. These support the continued development, expansion, and refinement of focused wastewater surveillance of infectious disease burdens between communities and highlight the importance of attending to social determinants of health and vulnerable resource constrained communities.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

Alene, M., Yismaw, L., Assemie, M.A., Ketema, D.B., Mengist, B., Kassie, B., Birhan, T.Y., 2021. Magnitude of asymptomatic COVID-19 cases throughout the course of infection: a systematic review and meta-analysis. PLoS One 16 (3), e0249090. https://doi.org/10.1371/journal.pone.0249090.

Bergeron, P., Courteau, J., Vanasse, A., 2015. Proximity and emergency department use: multilevel analysis using administrative data from patients with cardiovascular risk factors. Can. Fam. Physician 61 (8), e391–e397.

Bivins, A., North, D., Ahmad, A., Ahmed, W., Alm, E., Been, F., Bhattacharya, P., Bijlsma, L., Boehm, A.B., Brown, J., Buttiglieri, G., Calabro, V., Carducci, A., Castiglioni, S., Cetecioglu Gurol, Z., Chakraborty, S., Costa, F., Curcio, S., de los Reyes, F.L., Bibby, K., 2020. Wastewater-based epidemiology: global collaborative to maximize contributions in the fight against COVID-19. Environ. Sci. Technol. 54 (13), 7754–7757. https://doi.org/10.1021/acs.est.0c02388.

Boxus, M., Letellier, C., Kerkhofs, P., 2005. Real Time RT-PCR for the detection and quantitation of bovine respiratory syncytial virus. J. Virol Methods 125 (2), 125–130. https://doi.org/10.1016/j.jviromet.2005.01.008.

Bwire, G.M., Majigo, M.V., Njiro, B.J., Mawazo, A., 2021. Detection profile of SARS-CoV-2 using RT-PCR in different types of clinical specimens: a systematic review and meta-analysis. J. Med. Virol. 93 (2), 719–725. https://doi.org/10.1002/jmv.26349.

CDC, 2020. CDC SVI 2018 Documentation. https://www.atsdr.cdc.gov/placeandhealth/svi/documentation/SVI_documentation_2018.html.

CDC. (2022, January 26, 2022). Sampling Strategy: where, How, and what to Sample. Centers for Disease Control. Retrieved February 7 from https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/developing-a-wastewater-surveillance-sampling-strategy.html.

Chinazzi, M., Davis, J.T., Ajelli, M., Gioannini, C., Litvinova, M., Merler, S., Piontti, A.P. y., Mu, K., Rossi, L., Sun, K., Viboud, C., Xiong, X., Yu, H., Halloran, M.E., Longini, I. M., Vespignani, A., 2020. The effect of travel restrictions on the spread of the 2019 novel coronavirus (COVID-19) outbreak. Science 368 (6489), 395–400. https://doi.org/10.1126/science.aba9757.

D'Aoust, P.M., Mercier, E., Montpetit, D., Jia, J.-J., Alexandrov, I., Neault, N., Baig, A.T., Mayne, J., Zhang, X., Alain, T., Langlois, M.-A., Servos, M.R., MacKenzie, M., Figeys, D., MacKenzie, A.E., Graber, T.E., Delatolla, R., 2021. Quantitative analysis of SARS-CoV-2 RNA from wastewater solids in communities with low COVID-19 incidence and prevalence. Water Res. 188, 116560 https://doi.org/10.1016/j.watres.2020.116560.

D'Amico, F., Baumgart, D.C., Danese, S., Peyrin-Biroulet, L., 2020. Diarrhea during COVID-19 infection: pathogenesis, epidemiology, prevention, and management. Clin. Gastroenterol. Hepatol. 18 (8), 1663–1672. https://doi.org/10.1016/j.cgh.2020.04.001.

Farkas, K., Cooper, D.M., McDonald, J.E., Malham, S.K., de Rougemont, A., Jones, D.L., 2018. Seasonal and spatial dynamics of enteric viruses in wastewater and in riverine

and estuarine receiving waters. Sci. Total Environ. 634, 1174–1183. https://doi.org/10.1016/j.scitotenv.2018.04.038.

Farkas, K., Hassard, F., McDonald, J.E., Malham, S.K., Jones, D.L., 2017. Evaluation of molecular methods for the detection and quantification of pathogen-derived nucleic acids in sediment [original research]. Front. Microbiol. 8 https://doi.org/10.3389/fmicb.2017.00053.

Flanagan, B.E., Gregory, E.W., Hallisey, E.J., Heitgerd, J.L., Lewis, B., 2011. A social vulnerability index for disaster management. J. Homel. Secur. Emerg. Manag. 8 (1).

Gibas, C., Lambirth, K., Mittal, N., Juel, M.A.I., Barua, V.B., Brazell, L.R., Hinton, K., Lontai, J., Stark, N., Young, I., Quach, C., Russ, M., Kauer, J., Nicolosi, B., Akella, S., Tang, W., Chen, D., Schlueter, J., Munir, M., 2021. Implementing Building-Level SARS-CoV-2 Wastewater Surveillance on a University Campus. medRxiv. https://doi.org/10.1101/2020.12.31.20248843.

Gonzalez, R., Curtis, K., Bivins, A., Bibby, K., Weir, M.H., Yetka, K., Thompson, H., Keeling, D., Mitchell, J., Gonzalez, D., 2020. COVID-19 surveillance in Southeastern Virginia using wastewater-based epidemiology. Water Res. 186, 116296 https://doi.org/10.1016/j.watres.2020.116296.

Haramoto, E., Kitajima, M., Kishida, N., Konno, Y., Katayama, H., Asami, M., Akiba, M., 2013. Occurrence of pepper mild mottle virus in drinking water sources in Japan. Appl. Environ. Microbiol. 79 (23), 7413–7418. https://doi.org/10.1128/AEM.02354-13.

Kantor, R.S., Nelson, K.L., Greenwald, H.D., Kennedy, L.C., 2021. Challenges in measuring the recovery of SARS-CoV-2 from wastewater. Environ. Sci. Technol. 55 (6), 3514–3519. https://doi.org/10.1021/acs.est.0c08210.

Kitajima, M., Sassi, H.P., Torrey, J.R., 2018. Pepper mild mottle virus as a water quality indicator. npj Clean Water 1 (19). https://doi.org/10.1038/s41545-018-0019-5.

Little, C., Alsen, M., Barlow, J., Naymagon, L., Tremblay, D., Genden, E., Trosman, S., Iavicoli, L., van Gerwen, M., 2021. The impact of socioeconomic status on the clinical outcomes of COVID-19; a retrospective cohort study. J. Community Health 46 (4), 794–802. https://doi.org/10.1007/s10900-020-00944-3.

Liu, Y., Rocklöv, J., 2021. The reproductive number of the Delta variant of SARS-CoV-2 is far higher compared to the ancestral SARS-CoV-2 virus. J. Trav. Med. 28 (7) https://doi.org/10.1093/jtm/taab124.

Ma, Q., Liu, J., Liu, Q., Kang, L., Liu, R., Jing, W., Wu, Y., Liu, M., 2021. Global percentage of asymptomatic SARS-CoV-2 infections among the tested population and individuals with confirmed COVID-19 diagnosis: a systematic review and meta-analysis. JAMA Netw. Open 4 (12), e2137257. https://doi.org/10.1001/jamanetworkopen.2021.37257 e2137257.

Nikolai, L.A., Meyer, C.G., Kremsner, P.G., Velavan, T.P., 2020. Asymptomatic SARS coronavirus 2 infection: invisible yet invincible. Int. J. Infect. Dis. 100, 112–116. https://doi.org/10.1016/j.ijid.2020.08.076.

Peccia, J., Zulli, A., Brackney, D.E., Grubaugh, N.D., Kaplan, E.H., Casanovas-Massana, A., Ko, A.I., Malik, A.A., Wang, D., Wang, M., Warren, J.L., Weinberger, D. M., Arnold, W., Omer, S.B., 2020. Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics. Nat. Biotechnol. 38 (10), 1164–1167. https://doi.org/10.1038/s41587-020-0684-z.

Ralli, M., Morrone, A., Arcangeli, A., Ercoli, L., 2021. Asymptomatic patients as a source of transmission of COVID-19 in homeless shelters. Int. J. Infect. Dis. 103, 243–245. https://doi.org/10.1016/j.ijid.2020.12.031.

Rosario, K., Symonds, E.M., Sinigalliano, C., Stewart, J., Breitbart, M., 2009. *Pepper mild mottle virus* as an indicator of fecal pollution. Appl. Environ. Microbiol. 75 (22), 7261–7267. https://doi.org/10.1128/AEM.00410-09.

Scott, L.C., Aubee, A., Babahaji, L., Vigil, K., Tims, S., Aw, T.G., 2021. Targeted wastewater surveillance of SARS-CoV-2 on a university campus for COVID-19 outbreak detection and mitigation. Environ. Res. 200, 111374 https://doi.org/10.1016/j.envres.2021.111374.

Shiehzadegan, S., Alaghemand, N., Fox, M., Venketaraman, V., 2021. Analysis of the delta variant B.1.617.2 COVID-19. Clin. Pract. 11 (4), 778–784. https://www.mdpi.com/2039-7283/11/4/93.

Sims, N., Kasprzyk-Hordern, B., 2020. Future perspectives of wastewater-based epidemiology: monitoring infectious disease spread and resistance to the community level. Environ. Int. 139, 105689 https://doi.org/10.1016/j.envint.2020.105689.

Snider, B., Patel, B., McBean, E., 2021. Asymptomatic cases, the hidden challenge in predicting COVID-19 caseload increases. Infect. Dis. Rep. 13 (2), 340–347. https://www.mdpi.com/2036-7449/13/2/33.

Subramanian, R., He, Q., Pascual, M., 2021. Quantifying asymptomatic infection and transmission of COVID-19 in New York City using observed cases, serology, and testing capacity. Proc Natl Acad Sci 118 (9). https://doi.org/10.1073/pnas.2019716118.

Symonds, E.M., Rosario, K., Breitbart, M., 2019. Pepper mild mottle virus: agricultural menace turned effective tool for microbial water quality monitoring and assessing (waste)water treatment technologies. PLoS Pathog. 15. https://doi.org/10.1371/journal.ppat.1007639.

Twohig, K.A., Nyberg, T., Zaidi, A., Thelwall, S., Sinnathamby, M.A., Aliabadi, S., Seaman, S.R., Harris, R.J., Hope, R., Lopez-Bernal, J., Gallagher, E., Charlett, A., De Angelis, D., Presanis, A.M., Dabrera, G., Koshy, C., Ash, A., Wise, E., Moore, N., Gunson, R., 2022. Hospital admission and emergency care attendance risk for SARS-CoV-2 delta (B.1.617.2) compared with alpha (B.1.1.7) variants of concern: a cohort study. Lancet Infect. Dis. 22 (1), 35–42. https://doi.org/10.1016/S1473-3099(21)00475-8.

US Census Bureau, 2019. American Community Survey 2015-2019 5-year Estimates. https://data.census.gov/cedsci/table?q=american%20community%20survey&tid=ACSST5Y2020.S0101.

Wang, W., Xu, Y., Gao, R., Lu, R., Han, K., Wu, G., Tan, W., 2020. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA 323 (18), 1843–1844. https://doi.org/10.1001/jama.2020.3786.

Zhang, J., Wu, S., Xu, L., 2020. Asymptomatic carriers of COVID-19 as a concern for disease prevention and control: more testing, more follow-up. BioScience Trends 14 (3), 206–208. https://doi.org/10.5582/bst.2020.03069.

Zhao, H., Lu, X., Deng, Y., Tang, Y., Lu, J., 2020. COVID-19: asymptomatic carrier transmission is an underestimated problem. Epidemiol. Infect. 148, e116. https://doi.org/10.1017/S0950268820001235. Article e116.