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Short Communication

SARS-CoV-2 concentrations in a wastewater collection system indicated potential COVID-19 hotspots at the zip code level

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HIGHLIGHTS
• Samples were collected in a wastewater collection system and treatment facilities.
• Different hotspots at the zip code level were identified for two COVID-19 surges.
• Sampling in collection systems improves the spatial granularity for surveillance.
• Strategic wastewater testing can inform local outbreak response efforts.

GRAPHICAL ABSTRACT

ABSTRACT

Wastewater based epidemiology (WBE) has been successfully applied for SARS-CoV-2 surveillance at the city and building levels. However, sampling at the city level does not provide sufficient spatial granularity to identify COVID-19 hotspots, while data from building-level sampling are too narrow in scope for broader public health application. The objective of this study was to examine the feasibility of using wastewater from wastewater collection systems (WCSs) to monitor COVID-19 hotspots at the zip code level. In this study, 24-h composite wastewater samples were collected from five manholes and two wastewater treatment plants (WWTPs) in the City of Lincoln, Nebraska. By comparing to the reported weekly COVID-19 case numbers, we identified different hotspots responsible for two COVID-19 surges during the study period. One zip code was the only sampling location that was consistently tested positive during the first COVID-19 surge. In comparison, nearly all the zip codes exhibited virus concentration increases that overlapped with the second COVID-19 surge, suggesting broader spread of the virus at that time. These findings demonstrate the feasibility of using WBE to monitor COVID-19 at the zip code level. Highly localized disease surveillance methods can improve public health prevention and mitigation measures at the community level.

1. Introduction

The severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) is the etiologic agent causing the coronavirus disease 2019 (COVID-19). SARS-CoV-2 is an enveloped, single-stranded RNA virus which can enter host cells by attaching its spikes (i.e., S protein) with angiotensin-converting enzyme 2 receptors (ACE2) present in hepatic, renal,
pulmonary, and gastrointestinal cells (Hoffmann et al., 2020; Tai et al., 2020; Zhou et al., 2020).

Because SARS-CoV-2 can attach to gastrointestinal cells, the virus has been detected in human feces from symptomatic patients (Gu et al., 2020; Song et al., 2020) as well as asymptomatic people (Tang et al., 2020). After the first symptom onset, the fecal shedding of SARS-CoV-2 has been reported to last up to seven weeks (Jiehao et al., 2020; Wu et al., 2020; Xiao et al., 2020) and its concentration may be up to $1 \times 10^8$ copies per gram of feces (Lescure et al., 2020; Pan et al., 2020; Wölfel et al., 2020).

There are clear public health advantages to wastewater surveillance for SARS-CoV-2. First, SARS-CoV-2 has been detected in samples collected in the influent of wastewater treatment plants (WWTPs) prior to clinical reporting of COVID-19 cases (Ahmed et al., 2020; Gonzalez et al., 2020; Haramoto et al., 2020; Medema et al., 2020). Second, wastewater surveillance for SARS-CoV-2 accounts for the presence of COVID-19 among asymptomatic carriers, providing a more accurate indicator of community spread (Yuan et al., 2021). While several studies have reported success in detecting SARS-CoV-2 in wastewater collected at wastewater treatment plants and correlating the viral concentration in wastewater with infected cases, this population-level approach does not provide the spatial granularity to monitor COVID infection at the community level (Thompson et al., 2020).

The objective of this study was to examine the feasibility of using wastewater from wastewater collection systems (WCS) to monitor potential COVID-19 hotspots at the zip code level. The working hypothesis is that wastewater collected from communities at the zip code level can provide adequate spatial resolution to guide potential short-term intervention practices. Wastewater samples were collected from the WCS of Lincoln, NE between July and September 2020. Autosamplers were utilized to collect 24-h composite wastewater samples at strategically selected manholes to represent five zip code areas of the city. Findings from this study can help optimize and standardize wastewater sampling strategies to empower wastewater based epidemiology (WBE) as a means for long term surveillance of SARS-CoV-2.

2. Materials and methods

2.1. Wastewater sample collection

Wastewater samples were collected at five manholes and two wastewater treatment plants in the City of Lincoln, NE, over a 12-week period from 7/7/20 to 9/22/20 (i.e., 7 locations × 12 sampling events = 84 samples total). Fig. 1 shows the locations of the manholes and treatment plants. Manholes were strategically selected based on the diameter of the pipe size (i.e., from approximately 53 cm to 119 cm, or greater than 119 cm). Four manholes collected wastewater from single zip codes: manhole # AA7-15 (zip code 68524), B1-292 (68516), B3-374 (68502), and B6-268 (68521) (Fig. 1). Manhole D2-42 collected wastewater from two zip codes (68506 and 68526). Within each zip code, there are multiple branches of WCS pipelines merging into the main pipelines (i.e., pipelines with diameters larger than 21 or 47 in., shown as green and blue lines in Fig. 1). Branches that collected wastewater from buildings exclusive to the target zip code(s) were chosen and manholes located close to the root of the branch (i.e., where the branches merge into the main pipelines) were selected for the study.

One of the WWTPs (Theresa Street Treatment Plant, or TSTP) received wastewater from areas represented by the five manholes as well as from other zip codes. The other WWTP (Northeast Treatment Plant, or NETP) is a newer and smaller facility. The City has the capability of diverting flows from different zip codes to NETP. NETP received wastewater from three zip codes (68504, 68505, and 68507) during the period of the study (Fig. 1). The average influent flow rate during the sampling period were $1.06 \times 10^5$ L d$^{-1}$ and $0.88 \times 10^5$ L d$^{-1}$, for TSTP and NETP, respectively.

24-h flow-weighted composite samples were collected from manholes and from influents to the two WWTPs every Monday to Tuesday during the 12-week period. Wastewater samples from manholes and WWTPs were transported to lab on ice and stored at -20 °C until RNA extraction.

2.2. RNA extraction and SARS-CoV-2 quantification

Frozen wastewater samples were thawed at 4 °C before being sequentially filtered through 8 μm and 0.45 μm membrane filters (Sartorius Biolab Products, Göttingen, Germany). RNA was extracted from both membrane filters with RNeasy Mini Kit (Qiagen, Hilden, Germany) and eluted with 50 μL of RNase free buffer.

Hepatitis G armored RNA (Asuragen, Austin, TX, USA) was used to determine the presence of inhibitors in RNA extracts for the reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay. Primers and probe were synthesized by Integrated DNA Technologies (Chicago, IL, USA) (Schlueter et al., 1996). For each Hepatitis G assay, a 25 μL final reaction volume comprised of 2.5 μL of Hepatitis G armored RNA, 2.25 μL of nuclease-free water, 1.25 μL of each primer, 0.25 μL of probe, 12 μL of iTaq universal probe reaction mix, 0.5 μL of iScript advanced reverse transcriptase (Bio-Rad, Hercules, CA), and 5 μL of RNA extracts. The Cq (quantification cycle) value of each sample was compared against that of non-template control, which contained nuclease-free water instead of RNA extracts. If the difference in Cq was greater than 1.0, the wastewater sample was considered to have PCR inhibitors. In a preliminary experiment using samples from different zip codes, RNA extracts with no dilution, 5-fold dilution, and 25-fold dilution were used to determine the impacts of inhibitors on PCR. Because 5-fold dilution consistently resulted in non-zero values, all samples in this study were diluted 5-fold.

SARS-CoV-2 in wastewater was quantified on a Mastercycler ep realplex (Eppendorf, Hamburg, Germany) following a previously published study (Nemudryi et al., 2020). RT-qPCR was performed using two sets of primer pairs (N1 and N2) and probes from 2019-nCoV CDC ELISA Kit (IDT # 10006606). For one-step RT-qPCR, a 20 μL final reaction volume comprised of 3 μL of nuclease-free water, 1.5 μL of primer and probe mix, 10 μL of iTaq universal probe reaction mix (Bio-Rad, Hercules, CA, USA), 0.5 μL of iScript advanced reverse transcriptase (Bio-Rad), and 5 μL of 5-fold diluted RNA extracts (Peccia et al., 2020). Nuclease-free water was used as non-template control (NTC) and as extraction blank.

A positive template control (PTC) plasmid (IDT # 10006625) was linearized using the Scal High Fidelity (HF) restriction enzyme (R3122S, 1000 units, New England BioLabs Inc., Ipswich, MA) and then cleaned using the Monarch PCR and DNA cleanup kit (New England Biolabs Inc., Ipswich, MA). Standard curves for N1 and N2 were generated using a dilution series of the linearized plasmid with concentrations ranging from 10 to 100,000 copies per reaction. Two technical replicates were performed at each dilution. The NTC showed no amplification over the 40 cycles of qPCR.

2.3. Total number of COVID-19 cases

The total number of COVID-19 cases in Lincoln (Nebraska, United States) during the sampling period was downloaded from Lincoln-Lancaster County Health Department Dashboard. The population for each zip code was obtained online from the website (zip-codes.com).

3. Results

3.1. COVID cases in Lincoln, NE

During the wastewater sampling campaign between July 7th and September 22nd, 2020, the number of new reported COVID cases in Lincoln had two waves (Fig. 2). The first wave of reported COVID cases started on July 7th and ended on August 18th with a peak weekly case of 360 reported on July 28th. The second wave started in late August and climbed to a maximum weekly case of 693 on September 15th.
3.2. SARS-CoV-2 concentrations in wastewater from manholes

Our sampling strategy allowed us to monitor the fluctuation of the SARS-CoV-2 concentrations in wastewater representative of various parts of the city. Standard curve parameters are reported in Tables S1 and S2 (Bivins et al., 2021). qPCR assay efficiencies ranged from 89 to 101% and standard curve R² values were above 0.99 (Tables S1 and S2). NTC and extraction blanks were tested negative for both N1 and N2. Thirty-five out of the total 84 samples (i.e., 12 time points × (5 manholes + 2 WWTPs)) were tested positive using both N1 and N2 primer sets. Most of the SARS-CoV-2 concentrations, measured with either N1 or N2, in these positive wastewater samples ranged from approximately 3.00 × 10³ gene copy L⁻¹ to approximately 1.80 × 10⁴ gene copy L⁻¹.

Fig. 1. A map showing the pipelines of the wastewater collection system in Lincoln, NE. The five manholes and two wastewater treatment plants are marked on the map.
For the samples collected from the five manholes, each location had 3 to 6 samples that tested positive for N1 and N2 during the 12-week sampling campaign between July 7th and September 22nd, 2020 (i.e., solid symbols in Figs. 3 and 4). Overall, the zip codes 68,521, and 68,506 + 68,526 showed fewer positive detections than the other zip codes (Fig. 4).

During the first wave of reported COVID cases between July 7th and August 18th, the wastewater collected from most of the five manholes tested negative. One exception was wastewater collected from zip code 68516. The SARS-CoV-2 concentration in wastewater at this zip code started to increase on July 21st and peaked on July 28th (Fig. 3C and D).

During the second wave of reported COVID cases starting August 25th, wastewater collected from all manholes appeared to have higher SARS-CoV-2 concentrations than during the first wave of reported COVID cases (Figs. 3 and 4). Most noticeably, wastewater samples from zip code 68502 had the highest SARS-CoV-2 concentrations.

Fig. 2. COVID-19 weekly cases in Lincoln, NE. The red diamonds represent the number of cases and the vertical red dash line separates the two waves in the number of cases during the sampling campaign.

Fig. 3. SARS-CoV-2 concentrations in wastewater for assay N1 (closed black squares) and N2 (closed red triangles) at zip codes 68,524 (A and B), 68,516 (C and D), and 68,502 (E and F). Open symbols indicate samples where no target was detected. Error bars represent standard deviations between the two technical replicates. The vertical red dash line separates the two waves in the number of cases during the sampling campaign.
among all the samples analyzed: $3.65 \times 10^5$ GC L$^{-1}$ N1 and $4.32 \times 10^5$ GC L$^{-1}$ N2 on September 22nd (Fig. 3E and F).

3.3. SARS-CoV-2 concentrations in wastewater from WWTPs

Wastewater samples from the two WWTPs had a higher frequency of positive SARS-CoV-2 detection than those from the manholes (Fig. 5). During the first wave of COVID-19 cases, the sample from NETP (68,504, 68,505, and 68,507) showed a positive detection on July 28th, while the sample from TSTP (the rest of the city zip codes) showed a positive detection on July 14th. During the second wave of COVID-19 cases, the samples from both WWTPs were consistently positive for SARS-CoV-2 and exhibited higher concentration than during the first wave (Fig. 5).

3.4. Correlations between wastewater data and COVID-19 cases

The surges of COVID-19 numbers in the two waves likely correspond to different hot spots in the city. The SARS-CoV-2 concentration at zip code 68516 peaked twice, on July 28th and on September 22nd, matching the two COVID surges. The SARS-CoV-2 concentration at the other zip codes match the second surge of the number of COVID-19 in Lincoln between September 8th and 22nd (Fig. 3-5).

4. Discussion

4.1. Spatial granularity at the zip code level

Current efforts in wastewater surveillance of SARS-CoV-2 are either conducted at downstream WWTPs (Ahmed et al., 2020; Gonzalez et al., 2020; Haramoto et al., 2020; Medema et al., 2020) or upstream locations, such as at specific facilities/buildings (Barich and Slonczewski, 2021; Betancourt et al., 2021; Gibas et al., 2021; Spurbeck et al., 2020). Sampling at WWTPs benefits from the convenience of existing infrastructures and the capability of obtaining composite samples from the entire area served. However, this sampling strategy cannot reveal the spatial distribution of the COVID-19 infections within the sewer network. Wastewater surveillance at locations within the WCS can provide the spatial granularity needed to plan early warning and targeted intervention for local communities.

Socioeconomic status (SES) is often clustered geographically (Link-Gelles et al., 2016). Neighborhood-level measures, such as prevalence of poverty by zip codes, allow researchers to assess SES ecologically (Agarwal et al., 2015). Hence, measuring SARS-CoV-2 concentrations in wastewater at the zip code level could allow for monitoring COVID-19 outbreaks among marginalized populations. Further, information at the zip code level could also guide rapid deployment of resources to neighborhoods in critical need. Publicly available COVID-19 epidemiology data are often aggregated at the zip code level (e.g., Lincoln, Chicago, etc.), making the comparison with wastewater data at the zip code level straightforward.

4.2. Comparison with previous studies

Measured SARS-CoV-2 concentrations in previous studies ranged from $10^2$ to $10^5$ GC L$^{-1}$ in wastewater influent (Gonzalez et al., 2020; Li et al., 2021; Medema et al., 2020; Miyani et al., 2020; Sherchan et al., 2020) and $10^6$ to $10^{10}$ GC L$^{-1}$ in sewage sludge (Peccia et al., 2020). In this study, SARS-CoV-2 concentrations in wastewater ranged from $3.3 \times 10^3$ to $4.3 \times 10^5$ genomic copies (GC) L$^{-1}$. Our numbers were close to the virus concentrations in wastewater influent to WWTPs.
Even though using WBE to monitor COVID-19 is a relatively new approach, WBE has been used to monitor other viral outbreaks via wastewater such as Saffold, Influenza A (H1N1), Hepatitis A and E, Norovirus, and Poliovirus (Bonanno Ferraro et al., 2020; Heijnen and Medema, 2011; Hellmér et al., 2014; Iaconelli et al., 2020; Lago et al., 2003; Lodder et al., 2012). For example, norovirus was detected at high concentrations in sewage 2 to 3 weeks before most patients were diagnosed with this infection in Gothenburg, Sweden (Hellmér et al., 2014). However, virus concentrations in wastewater do not always correlate with reported numbers of infections in a community. In one previous study, Saffold virus was detected in sewage samples in Italy, but there was no reporting of clinical infection in that community (Bonanno Ferraro et al., 2020). During the pandemic influenza A (H1N1) 2009, 2182 patients were hospitalized in the Netherlands, but the virus causing the pandemic was not detected in sewage (Heijnen and Medema, 2011).

4.3. Identification of hot spots

Compared to the second surge of COVID-19 cases, the first surge was likely concentrated within a smaller portion of the city. The total number of COVID-19 cases in the first surge ranged from 170 to 360 weekly cases. Except for its detection in zip code 68516, the virus was either absent or was detected sporadically at low concentrations in other zip codes studied during the first surge. This suggests that the zip code 68516 was a hot spot during the first surge. Zip code 68516 is the most populated area in Lincoln with 38,956 people according to the 2010 census. There are a total of 29 zip codes in the city of Lincoln, NE. The total population of Lincoln, NE is 266,032 according to the 2010 census. The population living in the six zip codes covered by manholes sampled accounted for approximately 51% of the total population in the city. Although we sampled six zip codes representing major residential areas, we did not cover all zip codes. Hence, it is possible that some of the COVID-19 hot spots during the first surge were not sampled in this study. In addition, it is possible that the flow of wastewater from the zip codes that sampled negative for the virus could dilute the wastewater flow from the hot spots and therefore result in low SARS-CoV-2 concentrations in the influent to TSTP.

The second surge was likely more widespread in the city. All zip codes in this study demonstrated more consistent detection with higher concentrations. The second surge in COVID-19 cases extended to October 16th, and therefore was not completely covered within the timeframe of this study. SARS-CoV-2 detection in multiple locations in the city hinted at a widespread surge of COVID-19 cases during this period.

4.4. Implications for public health

This study demonstrates that wastewater monitoring for SARS-CoV-2 can reveal COVID-19 hotspots at the local level. More localized WBE surveillance strategies may enable early detection of outbreak hotspots. Coordinated data sharing between municipal utilities and health services can allow for local monitoring of the correlation between virus concentration in wastewater and number of infections. Combined with geocoded sociodemographic information, data from WBE surveillance may be used by local health authorities to implement timely and precise intervention strategies tailored to hyper-local demographic characteristics. Swift and effective responses to detected outbreaks may prevent the disease from becoming more widespread throughout a municipality.
CRediT authorship contribution statement

Renys Barrios: Data curation, Formal analysis, Investigation, Methodology, Writing—original draft. Chin Lim: Investigation, Methodology, Writing—review and editing. Megan Kelley: Conceptualization, Funding acquisition, Methodology, Writing—review and editing. Xu Li: Conceptualization, Funding acquisition, Methodology, Supervision, Writing—review and editing.

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.

Acknowledgements

The authors would like to thank the support from Lincoln Transportation and Utilities (LTU). Particularly, we would like to thank Ben Kessler, Mark Barton, Nick Lind, Geoffrey Swanson, Travis Taylor, and Todd Bolding from LTU for helping with sample collection.

Funding sources

This project was financially sponsored by an internal grant from the University of Nebraska-Lincoln and in-kind support from LTU.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.149480.

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CRediT authorship contribution statement

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Science of the Total Environment 800 (2021) 149480

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