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Predicting COVID-19 cases in diverse population groups using SARS-CoV-2 wastewater monitoring across Oklahoma City

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HIGHLIGHTS

• SARS-CoV-2 concentrations in wastewater were used for predicting COVID-19 in diverse population groups in Oklahoma City.
• Viral concentrations in wastewater accurately predicted cases of COVID-19 with an average of 7 days lead-time.
• The lead-time varied significantly with population age, ethnicity and household income in any given location.
• The surveillance contributed to early warning of local outbreaks and helped guide COVID-19 public health action in the city.

GRAPHICAL ABSTRACT

Predicting COVID-19 Cases in Diverse Population Groups using SARS-CoV-2 Wastewater Monitoring across Oklahoma City

A B S T R A C T

SARS-CoV-2 was discovered among humans in late 2019 and rapidly spread across the world. Although the virus is transmitted by respiratory droplets, most infected persons also excrete viral particles in their feces. This fact prompted a range of studies assessing the usefulness of wastewater surveillance to determine levels of infection and transmission and produce early warnings of outbreaks in local communities, independently of human testing. In this study, we collected samples of wastewater from 13 locations across Oklahoma City, representing different population types, twice per week from November 2020 to end of March 2021. Wastewater samples were collected and analyzed for the presence and concentration of SARS-CoV-2 RNA using RT-qPCR. The concentration of SARS-CoV-2 in the wastewater showed notable peaks, preceding the number of reported COVID-19 cases by an average of one week (ranging between 4 and 10 days). The early warning lead-time for an outbreak or increase in cases was significantly higher in areas with larger Hispanic populations and lower in areas with a higher household income or higher proportion of persons aged 65 years or older. Using this relationship, we predicted the number of cases with an accuracy of 81–92% compared to reported cases. These results confirm the validity and timeliness of using wastewater surveillance for monitoring local disease transmission and highlight the importance of differences in population structures when interpreting surveillance outputs and planning preventive action.

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1. Introduction

While SARS-CoV-2 is primarily considered a respiratory virus, gastrointestinal symptoms such as nausea, vomiting, and diarrhea are reported in approximately 12% of cases (Parasa et al., 2020). Not surprisingly, SARS-CoV-2 RNA has been detected in municipal wastewater and in up to 59% of patient fecal samples (Gonzalez et al., 2020; Medema et al., 2020; Orive et al., 2020; Parasa et al., 2020; Wu et al., 2020). Studies have shown that the virus is even detectable in the feces of asymptomatic and presymptomatic cases (Jiang et al., 2020; Wu et al., 2020).

Wastewater-based epidemiology (WBE) is a population-based surveillance method that monitors for chemical or biological markers in wastewater to evaluate the health behaviors or infection status of communities (Gerrity et al., 2021). WBE combines analytical epidemiology with wastewater surveillance to provide objective community health information to public health officials and has previously been effective for monitoring outbreaks of norovirus, polio, and hepatitis A (Brouwer et al., 2018; Hellmér et al., 2014; Saguti et al., 2021) as well as predicting mortality from opioid overdoses (Gushgari et al., 2019). These findings indicate that WBE may be an ideal surveillance method to monitor the spread of SARS-CoV-2 in local communities.

At present, routine monitoring of wastewater to aid in SARS-CoV-2 surveillance and control is ongoing in many cities, regions, and municipalities across the world, as well as in hospitals, university dorms, and similar isolated populations (Ahmed et al., 2020b, 2020a; D’Aoust et al., 2021; Gonzalez et al., 2020; Hart and Halden, 2020; Medema et al., 2020; Peccia et al., 2020). Results from wastewater surveillance of SARS-CoV-2 have demonstrated that the virus can be detected in wastewater before human cases are reported in the area (Medema et al., 2020; Randazzo et al., 2020), offering several days and often up to weeks lead time in predicting outbreaks (Medema et al., 2020; Peccia et al., 2020; Randazzo et al., 2020). Specifically, results from the US have shown that SARS-CoV-2 concentrations were 6–8 days ahead of peaks in community cases (Nemudryi et al., 2020) and 2–4 days ahead of clinical PCR test results (Nemudryi et al., 2020) both of which correlate with observations from across the world ranging from 6 to 16 days lead time for community case reports (Medema et al., 2020; Randazzo et al., 2020) and up to 2–3 weeks for COVID-19 hospitalizations (Saguti et al., 2021).

Oklahoma City is the capital of the State of Oklahoma, located in the United States’ Great Plains region, with an estimated population of 655,000 persons in 2019. The city demographics are relatively diverse with 54% of the population reported as White Non-Hispanic origin, 20% Hispanic, 17% Black, 6% American Indian and Alaskan Native and 6% Asian in 2010 (US Census Bureau, n.d.). In the fall of 2020, a University of Oklahoma-based team established an interdisciplinary and cross-sectoral research group to monitor levels of SARS-CoV-2 in wastewater from selected areas of Oklahoma City with the aim of identifying local outbreaks and case clusters and providing a baseline of timely information for guiding disease prevention and control measures. In this paper we describe the methods employed and results achieved from this longitudinal surveillance of an important infectious disease across a larger metropolitan area.

2. Methods

2.1. Wastewater sampling

Sewage samples were collected twice per week from 13 locations across the sewershed that feeds into the largest wastewater treatment plant serving Oklahoma City. The sewer line samples were collected via manholes using Isco Avalanche refrigerated autosamplers (Teledyne Isco, Lincoln NE) as 900 mL grab samples collected at a single time between the period of 10:00 a.m. and 1:00 p.m. local time so as to sample during a likely peak time for human activity. All samples utilized proper chain-of-custody forms. Tubing, connectors, autosampler bottles, and strainers were cleaned after each sample collection using a multi-step protocol adapted from the U.S. Geological Survey (Chapter A7, Section 7.1, 2014) to ensure no contamination of samples. All samples were kept between 1 and 6 °C and processed within 24 h of collection.

2.2. Microbiological analysis

When samples were opened in the laboratory, they were handled in a class II, type A2 biosafety cabinet following COVID-19-specific biosafety procedures outlined by the Centers for Disease Control and Prevention (https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html). Triplicate subsamples (32 mL) were passed through a sterile, disposable 70 µm mesh cell strainer (e.g., Fisher Scientific 23-363-548) and into separate, clean, sterile Nalgene Oak Ridge High-Speed PFCO centrifuge tubes (Thermo Scientific 05-529-1D, max. vol. 42 mL) already containing 8 mL of a 5X PEG:NaCl solution (50% w/v PEG8000, 1 M NaCl). An aliquot of a reconstituted and diluted 1/1000 bovine vaccine containing Bovine Coronavirus (Calf Guard® Bovine Rotavirus Vaccine, Zoetis) was added to each sample, which was then vortexed thoroughly to mix. After thorough mixing by vortex, viral particles were precipitated in these tubes by incubating at 4 °C overnight (12–16 h) without shaking and mixing, and centrifugation at 14,600 x g for 45 min at 4 °C. The supernatant was decanted and the pelleted solids were used for total nucleic acid extractions following a protocol modified from the Bio On Magnetic Beads platform (Oberacker et al., 2019). The pelleted solids were lysed with a 6 M Guanidinium Thiocyanate solution (6 M Guanidinium Thiocyanate; 50 mM Tris HCl pH 8.0, 2% w/v Sarkosyl, and 20 mM EDTA pH 8.0), precipitated and bound to carboxylated magnetic beads (Sera-Mag SpeedBeads™Carboxyl Magnetic Beads; Cytiva 651512105050250) with an equal volume of isopropanol as described in detail in the Supplementary Materials. Following two washes with freshly prepared 70% ethanol, the nucleic acids were eluted with 100 µL of DEPC-treated water (CAS 7732-18-5; Sigma-Aldrich 693520), typically recovering between 85 and 90 µL. The eluates containing total extracted nucleic acids were stored on ice for up to 1 week for use for the analyzes (<1 h), and the remaining volumes were archived at −80 °C.

The SARS-CoV-2 viral RNA in each nucleic acid extraction was quantified using a 1-step Reverse Transcriptase Quantitative Polymerase Chain Reaction (RT-qPCR) and the N1 primer and probe reagents (IDT 10006606) used in the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use under CDC’s Emergency Use Authorization (EUA; https://www.cdc.gov/coronavirus/2019-ncov/lab/virus-requests.html). All primer and probe reagents are listed in Supplemental Table S1. Data for N1 represent the mean and standard deviation for triplicate reactions, where the slope for triplicate standards spanning 5 orders of magnitude across 72 different RT-qPCRs had a mean R² of 0.981 (±0.017). Additional parameters for the standard curves (slope, y-intercept, and efficiency) are given in Supplementary materials. Positive control sequences are also given in Table S2.

2.3. Epidemiological data and analyses

Numbers of notified COVID-19 cases per day were extracted from Oklahoma State Department of Health surveillance data. To obtain the daily count of notified COVID-19 cases per sewershed, geocoded cases were clipped to the sewershed polygon boundaries. The resulting clipped feature class was then overlaid over and summarized within the sewershed polygons and grouped by date. This allowed for extraction of daily total cases across sewersheds. The workflow was repeated weekly through a geoprocessing model created in Esri’s ModelBuilder to automate the process. Due to spatial heterogeneity of the sewer-line sewershed geography compared to census geographies, we used geoprocessing tools in Esri’s ArcMap and in Quantum GIS for quality control to clip the 2019 ACS 5-year census-tract demographic estimates with the sewershed polygon. Once clipped, we weighted each of the census-
Concentrations of SARS-CoV-2 (Copies per Liter) measured in wastewater, Oklahoma City, November 2020 through March 2021.

Table 1

| Location                        | Mean (SD) | Minimum (Month) | Maximum (Month) |
|---------------------------------|-----------|-----------------|-----------------|
| Lightning Creek 1               | 182,000 (356,000) | 310 (November)  | 2,042,000 (December) |
| Lightning Creek 2               | 705,000 (1,360,000) | 17,900 (January) | 7,291,000 (December) |
| Lightning Creek 3               | 473,000 (719,000)  | 10,500 (March)   | 3,912,000 (December) |
| Lower North Canadian 1          | 127,000 (178,000)  | 100 (November)   | 814,000 (January)  |
| Lower North Canadian 2          | 90,000 (140,000)   | 260 (March)      | 636,000 (December)  |
| Deep Fork 1                     | 423,000 (692,000)  | 310 (November)   | 3,051,000 (January) |
| Deep Fork 2                     | 143,000 (139,000)  | 5800 (March)     | 560,000 (December)  |
| Deep Fork 3                     | 290,000 (498,000)  | 1200 (March)     | 2,726,000 (December) |
| Deep Fork 5                     | 235,000 (517,000)  | 310 (March)      | 3,196,000 (December) |
| Deep Fork 6                     | 258,000 (536,000)  | 200 (December)   | 3,128,000 (December) |
| Deep Fork 7                     | 239,000 (391,000)  | 1300 (March)     | 2,565,000 (December) |
| Crutcho Creek                   | 172,000 (158,000)  | 6300 (February)  | 690,000 (December)  |
| Middle North Canadian           | 138,000 (289,000)  | 310 (February)   | 1,687,000 (November) |
| All Locations (Average)         | 268,000 (459,000)  | 3400 (March)     | 2,478,000 (December) |

3. Results

3.1. Wastewater concentrations

We monitored 13 sewersheds across Oklahoma City for 21 weeks for the concentration of SARS-CoV-2 RNA. During the study period, the average concentration of SARS-CoV-2 RNA fluctuated between 160 and 7.3 million copies per liter for all locations (Table 1). The highest average concentrations were observed in the Lightning Creek 1 sewershed and the lowest in the Lower North Canadian 2 sewershed (Table 1). Concentrations of SARS-CoV-2 RNA in wastewater peaked during November and December 2020 and showed a decline from February 2021 until the end of the study period (Fig. 1, Fig. 2). The viral concentrations in sewage were significantly higher in locations with a higher proportion of Hispanic and American Indian in the population and persons aged 65 or older (z = 940–2100, p < 0.001) and lower in locations with a higher proportion of Asians and a higher household income (z = −920–580, p < 0.001).

3.2. Epidemiological analyses

Between 1st November 2020 and 31st March 2021, a total of 15,129 cases of SARS-CoV-2 infection (6459 per 100,000 population) were reported from the Oklahoma City areas covered by the wastewater surveillance (Table 2). The accumulated incidence of reported COVID-19 cases per location during the study period ranged from 4763 in the Lower North Canadian area to 7603 cases per 100,000 population in the Middle North Canadian area (Table 2) and the number of new reported cases per location per day from zero to 71. During the study period, the reported number of cases in all locations combined decreased significantly (z = −18.2, p < 0.001) from an average high of 1140 cases per week in December to 114 in February (Fig. 2).

The incidence of COVID-19 was positively associated with a higher proportion of Hispanic (z = 25.5, p < 0.001) and American Indian (z = 18.9, p < 0.001) population and negatively associated with median income (z = −4.10, p < 0.01). Based on the correlation analysis, the number of reported cases in all areas combined was significantly associated with the concentration of SARS-CoV-2 RNA measured in wastewater 7 days previously (z = 4.42, R² = 0.87, p < 0.01, Table 2). The time lag for best predicting the number of COVID-19 cases from wastewater SARS-CoV-2 RNA in individual locations ranged from 4 to 10 days (mean 7.1 days). Different regression models based on wastewater viral concentrations and sociodemographic factors were fitted to reported COVID-19 cases as described above and the best results were obtained using a multivariate Poisson model. Fitting model predictions on 10% of the data points omitted from the analysis showed that the Poisson estimation predicted daily cases with an accuracy of 81% during the whole study period and 92% from November 2020 until end of January 2021 (Fig. 3). During the last two months of the study period (February and March 2021), the model predicted daily cases with an accuracy of 59% (Fig. 3), with predicted cases being on average 1.8 times higher than the number reported. The strength of the association between COVID-19 cases and viral concentrations in wastewater did not vary significantly with demographic factors (i.e. the model predicted equally efficient for all locations regardless of their ethnic and socioeconomic composition). However, the predictive time lag (early...
Fig. 1. Monthly average concentrations of SARS-CoV-2 (Copies/Liter) in wastewater from 13 locations across Oklahoma City in (a) November 2020, (b) December 2020, (c) January 2021, (d) February 2021 and (e) March 2021.

Fig. 2. Daily average concentrations of SARS-CoV-2 (1000 copies/Liter) in wastewater and daily SARS-CoV-2 cases from monitored locations in Oklahoma City, November 2020 through March 2021.
warning time) was significantly lower for areas with a higher household income \((z = -3.63, p < 0.001)\) and a higher proportion of the population aged 65 or older \((z = -2.18, p < 0.05)\) but higher for areas with a high proportion of Hispanic inhabitants \((z = 3.45, p < 0.01)\).

4. Discussion & conclusions

Our routine surveillance of SARS-CoV-2 RNA in wastewater from selected locations in Oklahoma City confirms the feasibility of using this measure as an early warning system for predicting increasing trends and local case clusters of COVID-19. Several other published studies have reported longitudinal monitoring of SARS-CoV-2 in wastewater (Gonzalez et al., 2020; Peccia et al., 2020; Saguti et al., 2021), however this is one of the first to account for different population structures and socio-demographic factors which are known to influence COVID-19 infection patterns and progression to hospitalization and severe disease. Our study highlights the fact that wastewater-based epidemiology is not a 'one size fits all' approach, particularly with respect to demographic characteristics, which is an important factor in understanding the risk and timing of outbreaks and case clusters among different population groups and for targeting testing and vaccine campaigns.

The results from the Oklahoma City monitoring indicate that increases in wastewater SARS-CoV-2 RNA concentrations preceed increases in reported COVID-19 cases by 4–10 days, depending on the population covered. This is similar to findings reported from Spain (Randazzo et al., 2020), The Netherlands (Medema et al., 2020), India (Kumar et al., 2020) and several US states and cities (Gonzalez et al., 2020; Nemudryi et al., 2020; Peccia et al., 2020). Having an advanced warning of increases in infection, independent of human testing, allows for prevention efforts to be set in motion at an early stage - including warning the population in the afflicted areas as well as preparing hospitals for a possible surge in infections. Because many cities and metropolises consist of small pockets of populations with different ethnic backgrounds, it is important to be able to account for these cross-city differences by not applying a 'one size fits all' approach to disease surveillance. This is especially relevant for COVID-19 which is known to be unevenly distributed across population types, particularly with regards to severity of disease outcomes. The results from our study show that it is possible to tailor wastewater-based disease predictions to the population covered by the sewersheds. We found that lead-times for an outbreak warning were significantly higher for areas with a large Hispanic population, possibly indicating that people within those sewersheds here wait longer before having their infection confirmed by a test. This can, in turn, reflect a population - and area - that is more vulnerable to larger outbreaks because a large number of unconfirmed infections increases the risk of community transmission. Additionally, these areas of Oklahoma City are also known for multi-generational, crowded housing which can further facilitate higher level of virus transmission. Conversely, the warning lead-times for areas with a high proportion of older and/or high-income populations...
were lower which signals a higher tendency to seek out a confirmatory test (either because of symptoms or known exposure) and possibly a lower risk for extensive community transmission. By combining infection signals from wastewater with knowledge about the population structure in a specific area or community, public health officials will be able to plan targeted and timely testing and vaccination efforts in order to reduce the likelihood of large transmission clusters.

Until late January 2021, our wastewater surveillance accurately predicted future peaks in infection (as indicated by notified cases) and public health action was taken on these predictions which ultimately may have resulted in preventing larger scale local outbreaks. Further, the predictions were also used for targeting vaccination campaigns in areas that showed not only a high level of community transmission but which were also known to be particularly vulnerable due to the population structure in that area. From February 2021 onwards, the reported number of SARS-CoV-2 infections in Oklahoma City (and the State of Oklahoma as a whole) declined rapidly. Although virus concentrations in the wastewater also decreased during this period, the rate of decline was less drastic than that of case counts. Through February and March, viral concentrations still indicated a significant level of community transmission of SARS-CoV-2 which was apparent in the predicted but not in the reported number of COVID-19 cases. This discrepancy could be due to several different factors. Firstly, the predictions were inaccurate and not reflecting real transmission in the community. Considering the accurate fit of the predictions until late January 2021, we believe that this is unlikely unless a sudden change in the wastewater-infection relationship occurred at this time. Secondly, the number of reported cases declined because of reduced testing activity rather than lower infection rates. Between January and March 2021, the daily number of tests performed in the State of Oklahoma decreased by more than 60% and the test positivity rate by almost 15% (Data, n.d.) - a trend mirrored across many other US states. While this could indicate lower infection rates, it may also be caused by ‘testing fatigue’ in the population, individual and public perception of important measures shifting from testing towards vaccine or even an early indication that COVID-19 vaccines were effective in reducing strong symptoms and therefore reducing testing levels. Overall, the discrepancy between predicted and observed COVID-19 cases in Oklahoma City in February and March was most likely caused by testing and reporting artefacts, and our surveillance results indicate significantly higher community transmission levels than reflected in the reported case numbers. This is a valuable lesson for future work and further cements the usefulness of wastewater surveillance in the absence of human testing or during periods when testing activity is influenced by non-disease factors.

The goal of the Oklahoma City wastewater surveillance was to set up a public health tool for monitoring SARS-CoV-2 RNA concentrations that was suitably specific at the temporal and geographical levels to allow for decision makers to prevent increases in community transmission. For the tool and outputs to be useful in a variety of settings and circumstances, it was also our aim to make the analytical and predictive process as simple as possible while still retaining high accuracy. With respect to analyzing and modeling the relationship between wastewater virus concentrations and COVID-19 cases, the linear approach of Poisson regression has the added advantage of simplicity which facilitates the use of the approach in other similar locations, particularly where time and resources for more sophisticated measures are scarce.

While our results point to a highly useful and accurate predictive role for wastewater-based epidemiology in COVID-19 surveillance, they need to be considered in light of several limitations. With regards to sampling, the viral concentrations measured at any location can be diluted by large sewage flows generated for instance by non-domestic industrial sources. This can introduce bias when comparing sites and calculating relationships between viral concentrations in wastewater and notified COVID-19 cases. In our study, the sampling sites were specifically selected to avoid major industries that would introduce variable proportions of wastewater from non-domestic sources, and therefore the comparison between different sites should not be drastically affected by flow. Generally, there is still little quantitative evidence for the relationship between an individual case of SARS-CoV-2 infection and the amount of virus excreted in the feces. Although there are examples of single infections being picked up from wastewater (Larsen and Wigginton, 2020), there is more work ahead to determine how much an infected individual will contribute to the community infection levels monitored in the wastewater. Timing of the sampling may also have caused bias in our analyses as the measured viral concentrations in wastewater reflect the actual day of sampling while notified human cases of COVID-19 reflect the day on which a person with a positive test result was reported to the authorities. While the latter is the best possible measure available, it does not accurately show neither infection time nor onset of possible symptoms. We acknowledge this as a general limitation which will be the same for most studies of this kind, based on disease surveillance data which are restricted in their timing accuracy. Another factor to consider in relation to this is how the strength of the infection (i.e. how symptomatic is the patient) relates to the number of viral particles excreted and, ultimately, how this impacts the overall concentrations in the wastewater. Further, the duration of excretion needs to be properly assessed and, if possible, accounted for when modeling the relationship between cases and wastewater concentrations, as a patient with long-lasting excretion may not drive community transmission levels as high as persons with new infections and excretions. This prolonged shedding of SARS-CoV-2 among convalescent patients could also partly explain the discrepancy between the observed and predicted cases from February onwards, assuming that a large proportion of the city population had already been infected at that point. Additionally, wastewater-based surveillance - unlike traditional clinical testing - will not distinguish between first infection and re-infections within a short period, thereby potentially giving an inflated estimate of the number of 'new cases' in a population. For COVID-19 in particular, this may however not be an important source of error as evidence suggests low re-infection rates among previously infected persons (Hall et al., 2021; Hansen et al., 2021). The notified number of COVID-19 cases relies heavily on access to testing and a population's willingness to become tested. Due to the structure of the reporting system, we were not able to collect data on location-specific testing activity and adjust for this in our model, however provided that such data are available, any wastewater-based epidemiology should make use of these. Finally, the analysis and predictions that we present in this study are based on notified cases of COVID-19 which is only reliable if testing is optimal and representative of the population being studied. As our results indicate, this was most likely the case for COVID-19 during the winter of 2020 and early 2021 and will probably be the same for any 'new' disease where testing-based surveillance is intense in the first period, or for a disease which is firmly established in an ongoing routine surveillance system. Provided that a strong baseline of reliable human testing data exists and covers at least several months, wastewater surveillance offers the ideal approach to take the surveillance to the next level by providing insights into under-reporting under ‘normal’ circumstances or by functioning as a stand-alone surveillance in exceptional situations.

In conclusion, our results confirm that surveillance of COVID-19 through wastewater offers a timely and useful addition to – or even standalone replacement of – traditional human testing, with the added advantage of significant lead warning time compared to ongoing case reporting. Given previous studies of this surveillance method for specific disease outbreaks, we believe that wastewater can also become an important future tool for monitoring other infections such as food- and waterborne diseases and emerging vector-borne diseases as well as general public health issues of concern, including antimicrobial resistance and substance abuse. By combining methods and knowledge from water engineering, microbiology and human public health, wastewater-based surveillance also represents important cross-disciplinary work which forms a crucial part of the One Health approach for combating infectious diseases. In future, veterinary and agricultural risk monitoring...
can easily be included in an established wastewater surveillance system to account for all One Health factors and optimize preparedness for newly emerging and emerging diseases.

CRediT authorship contribution statement

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are available upon request to the corresponding author.

ND, ER and ACM processed and interpreted data. All authors interpreted the analysis results and revised the manuscript.

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