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Assessment of a SARS-CoV-2 wastewater monitoring program in El Paso, Texas, from November 2020 to June 2022

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ABSTRACT

The border city of El Paso, Texas, and its water utility, El Paso Water, initiated a SARS-CoV-2 wastewater monitoring program to assess virus trends and the appropriateness of a wastewater monitoring program for the community. Nearly weekly sample collection at four wastewater treatment facilities (WWTFs), serving distinct regions of the city, was analyzed for SARS-CoV-2 genes using the CDC 2019-Novel coronavirus Real-Time RT-PCR diagnostic panel. Virus concentrations ranged from 86.7 to 268,000 gc/L, varying across time and at each WWTF. The lag time between virus concentrations in wastewater and reported COVID-19 case rates (per 100,00 population) ranged from 4–24 days for the four WWTFs, with the strongest trend occurring from November 2021 - June 2022. This study is an assessment of the utility of a geographically refined SARS-CoV-2 wastewater monitoring program to supplement public health efforts that will manage the virus as it becomes endemic in El Paso.

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Border; wastewater-based epidemiology; COVID-19; binational; minority population

Introduction

In the weeks following the international declaration that the novel coronavirus disease 2019 (COVID-19) was a public health emergency, wastewater monitoring was quickly identified as a non-invasive tool that could monitor the circulation of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) within communities. SARS-CoV-2 viral RNA has been found in biological fluids (fecal waste and urine) in both symptomatic and asymptomatic individuals, remaining detectable for several weeks in untreated wastewater (Ahmed et al. 2020). Incidentally, wastewater-based epidemiology (WBE) has emerged as a tool to characterize SARS-CoV-2 transmission in a defined sewage catchment area. As SARS-CoV-2 wastewater monitoring programs are implemented globally, program priorities have evolved from general virus detection (Ahmed et al. 2020) to evaluating whole genome sequences in wastewater in effort to identify emerging variants before clinical surveillance (Vo et al. 2023). However, the importance of establishing wastewater

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programs for individual communities, due to diverse demographics, political boundaries, and experiences, should not be overlooked.

WBE has historically and is currently being used to monitor pathogens (e.g. poliovirus) (Moore 1951), yet, its utility for early and ongoing detection of COVID-19 and future epidemics, is still evolving as data are interpreted for public health management (Faraway et al. 2022). The decline in diagnostic COVID-19 testing (due to nearly 45% of infected individuals experiencing no symptoms) (Oran and Topol 2020; Ma et al. 2021) hinders the accuracy of using active infections as a benchmark for public health decision-making. Wastewater monitoring for COVID-19 has been further adapted as an epidemiological tool to characterize infection trends in a community and provide timely forecasting to identify resurges of infections, therefore informing community public health decisions (Medema et al. 2020; Gerrity et al. 2021). Moreover, as the use of at-home testing increases and reporting of infections to public health authorities decreases, wastewater monitoring may serve as a critical ongoing surveillance tool for individual communities trying to manage the health, economic, and social impacts of the virus, as well as aid response measures for future pandemic and even extreme weather events.

Clinical testing, while cost-intensive and not optimally designed for continuous mass surveillance (Daughton 2020), has been found to be selectively biased for those seeking testing (symptom severity, occupational requirements, etc.), as well as a factor in the increased disparities faced by minorities during the pandemic (Garcini et al. 2022). Tracking and monitoring SARS-CoV-2, especially its variants, in wastewater has proven to be a successful complementary approach in the public health "tool box", as it has consistently identified mutations and spikes in clinical cases 1 to 3 weeks in advance, regardless of the location of sampling (Yu et al. 2022; Diamond et al. 2022).

El Paso, Texas, is a borderland community located in far west Texas adjacent to Ciudad Juárez, Mexico. Nearly 83% of the El Paso county population identifies as Hispanic (U.S. Census Bureau 2022a). As of 19 October 2022, the borderland city had experienced a cumulative total of 3,606 COVID-19 related deaths, of which 36% and 16% of those deaths were also associated with underlying conditions of renal disease and heart disease, respectively (City of El Paso 2022). The Hispanic community in El Paso, with 52% experiencing a low socioeconomic status and 48% lacking health insurance, have been disproportionately impacted medically, socially and economically throughout the COVID-19 pandemic (Cione et al. 2020; Cervantes et al. 2021). Compounding the disproportionate impact of the pandemic on underserved Hispanic populations, misinformation regarding testing and mistrust of government authorities may discourage the consistent and ongoing use of diagnostic tests (Garcini et al. 2022). The unique demographics of El Paso reinforce the importance of establishing and continuously supporting a wastewater monitoring program to track SARS-CoV-2 trends, hopefully to mitigate future surges in infections, hospitalizations, and deaths.

The City of El Paso is served by four wastewater treatment facilities (WWTFs) that process an average of 55 million gallons of wastewater per day, produced by an estimated population of 751,982 individuals. Each WWTF serves a distinct region of the city and therefore sewershed, which are influenced and physically divided by the Franklin Mountains. The four sewersheds are defined as: John T. Hickerson WWTF-west side, Haskell WWTF-central and downtown, Fred Hervey WWTF-northeast, and Roberto Bustamante WWTF-east side (Figure 1) (El Paso Water 2022). Since May 2020, a collaboration among the University of Texas Health Science Center at Houston School of Public Health, the Baylor College of Medicine, and El Paso Water (Link-Gelles 2022) has yielded nearly weekly wastewater sampling and analysis for SARS-CoV-2 at the four WWTFs.

The aim of this paper is to evaluate wastewater monitoring data collected in El Paso to assess SARS-CoV-2 trends in wastewater samples across four distinct sewersheds, and the correlation between measurements of the virus in wastewater samples and reported COVID-19 cases. As the use of at-home testing increases, and fewer individuals test for SARS-CoV-2 through medical providers that would previously report infections to public health authorities, validation of this

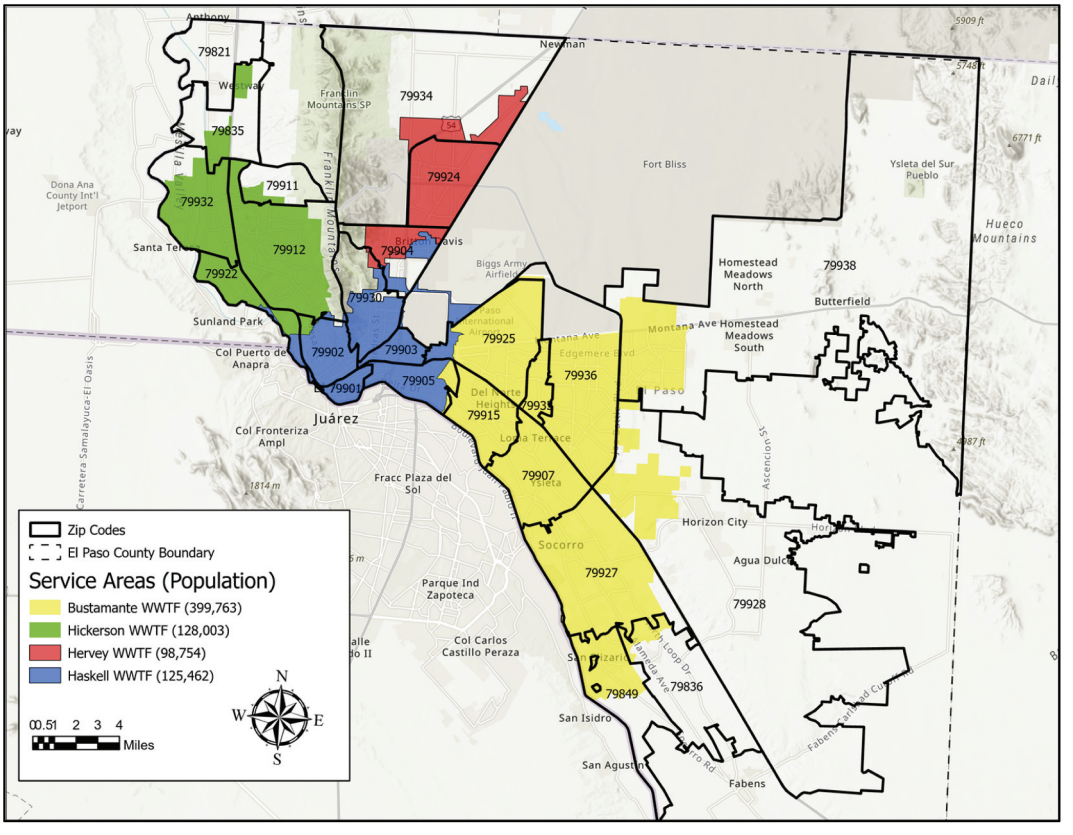

Figure 1. Wastewater service areas, ZIP codes, and populations in El Paso County, TX.

correlation to support ongoing wastewater monitoring is needed. This study is one of the first to monitor SARS-CoV-2 in untreated wastewater in a U.S.-Mexico border community. The assessment of community-specific wastewater monitoring programs is critical to identify the accuracy and utility of data collected for informing future public health management of the COVID-19 disease, as well as for routine monitoring of other infectious diseases.

Materials and methods

Wastewater sample collection

Wastewater samples were collected from the four El Paso WWTFs before treatment on a nearly weekly basis from 18 November 2020 to 1 June 2022. Composite (24 hour) samples (500 mL in volume) were collected and shipped overnight on ice to the [Baylor College of Medicine in Houston, TX]. Once received, samples were stored at 4°C and analyzed within one week. The total number of samples collected per month and at each WWTF (Figures S1 and S2) are described in the Supplementary Materials.

Molecular analysis

Samples were concentrated by electronegative filtration, then extracted according to the manufacturer's instruction associated with the chemagic Viral DNA/RNA 300 kit special H96 (CMG-1033-S, Perkin Elmer) along with the chemagic 360 (2024–0020, Perkin Elmer) automated platform. The

CDC 2019-Novel coronavirus (2019-ncoV) Real-Time reverse transcription-polymerase chain reaction (RT-PCR) Diagnostic panel was used to quantify the N1 and N2 genes, of which N1 was the primary focus for this study. Detailed methods and the quality control measures are described in the Supplementary Materials and adapted from Petrosino et al. (2020).

COVID-19 case data

Case data for reported infections (both diagnostic PCR and at-home tests) of COVID-19 in El Paso were retrieved from both the El Paso Fire Department and Public Health Department from November 2020 to June 2022. New daily COVID positive cases were aggregated by each of the 27 ZIP code tabulation areas (ZCTA) in El Paso County. ZCTAs encompassing Fort Bliss Army Base (located in northeast El Paso), and the University of Texas at El Paso campus were excluded due to case data not being available for these areas. Cases specific to each WWTF service area were identified using case data compiled in Microsoft Excel (Microsoft Corporation, Redmond, WA) and ArcGIS Pro software (Esri Corporation, Redlands, CA). The population of each WWTF service area was aggregated using the 2020 census block population estimates (U.S. Census Bureau 2022b). For each WWTF, we developed a ratio to estimate the proportion that each ZIP code was covered by a WWTF (Equation (1)).

$$WWTF\ Coverage\ (per\ ZIP\ code) = \frac{Area\ of\ WWTF\ service\ area\ within\ ZIP\ code}{Total\ Area\ of\ the\ WWTF\ service\ area} \quad (1)$$

This WWTF coverage ratio was then multiplied by the ZIP code-level COVID-19 case counts to estimate COVID-19 cases within each portion (ZIP code) of each WWTF service area. These values were then summed to find the total number of COVID-19 cases per day for each WWTF from November 2020 to June 2022.

Statistical analyses

A time series of daily wastewater measurements was created to correlate with the daily case data. Previous work has applied an interpolation approach to "fill in the time gaps" and impute the daily measures of SARS-CoV-2 in wastewater (Faraway et al. 2022). Here we used the approach of "last observation carried forward", so that the wastewater measurement remained the same until a new measurement was made. For the reported COVID-19 case data, we applied a 7-day rolling average to the daily COVID-19 cases to avoid any bias due to variation in reporting time and provide a more stable estimate of the case rate. We used time-lagged regression analyses to assess the temporal relationship between the SARS-CoV-2 RNA measures in the wastewater and the COVID-19 case data. R^2 and the standard error of the residuals (SE) from the regression were used to assess model fit, and the model with the largest R^2 and the smallest SE was identified as the best-fit model for the most probable time lag. This analysis was completed for each WWTF. All statistical analyses were performed in R using RStudio (R Core Team 2020; RStudio Team 2020).

Results

Wastewater concentrations of SARS-CoV-2 and reported COVID-19 case data for El Paso

Summary statistics of the SARS-CoV-2 concentrations (using the N1 gene assay) and COVID-19 case rates were calculated for each WWTF service area, stratified by three-time windows: 11/1/2020-3/31/2021 (first wave), 4/1/2021-10/31/2021 (stable time), and 11/1/2021-6/1/2022 (second wave) (Table 1). The Fred Hervey WWTF had the greatest mean and median of SARS-CoV-2 concentrations from 11/1/2020-10/31/2021, while Roberto Bustamante had the greatest values from 11/1/2021-6/1/2022. All WWTFs experienced the highest SARS-CoV-2 concentrations during the

Table 1. Wastewater service area case rates and SARS-CoV-2 concentrations in wastewater.

	Fred Hervey	Haskell	John T. Hickerson	Roberto Bustamante
Time window: 11/1/2020 – 3/31/2021				
COVID-19 Reported Case Rate (per 100k population)				
Mean (SD)	56.3 (45.4)	82.8 (79.8)	51.3 (42.4)	48.7 (27.1)
Median [Min, Max]	45.6 [9.11, 339]	61.4 [15.1, 616]	40.6 [3.91, 277]	44.4 [12.3, 164]
N1 (cp/L) in 10k				
Mean (SD)	6.43 (5.07)	2.91 (1.73)	5.10 (3.47)	2.91 (1.73)
Median [Min, Max]	7.30 [0.556, 16.3]	2.87 [0.661, 6.06]	4.33 [0.832, 11.6]	2.87 [0.661, 6.06]
Time window: 4/1/2021 – 10/31/2021				
COVID-19 Reported Case Rate (per 100k population)				
Mean (SD)	11.1 (8.29)	16.6 (16.3)	9.82 (7.41)	11.4 (7.62)
Median [Min, Max]	10.1 [0.00, 37.5]	14.4 [0.00, 159]	8.59 [0.00, 46.9]	10.8 [0.500, 32.3]
N1 (cp/L) in 10k				
Mean (SD)	1.07 (1.18)	0.943 (0.623)	0.454 (0.537)	1.01 (0.862)
Median [Min, Max]	0.739 [0.0113, 5.10]	0.945 [0.00867, 2.82]	0.325 [0.0587, 2.57]	0.865 [0.0387, 3.37]
Time window: 11/1/2021 – 6/1/2022				
COVID-19 Reported Case Rate (per 100k population)				
Mean (SD)	51.0 (62.3)	72.2 (91.3)	54.1 (68.4)	52.6 (65.9)
Median [Min, Max]	30.4 [0.00, 421]	36.7 [0.00, 487]	28.1 [0.00, 393]	28.0 [1.75, 399]
N1 (cp/L) in 10k				
Mean (SD)	4.25 (5.47)	4.03 (5.61)	3.06 (6.17)	4.63 (6.41)
Median [Min, Max]	1.30 [0.0273, 19.3]	1.13 [0.0367, 21.3]	0.554 [0.0507, 26.8]	2.01 [0.0220, 21.3]

first wave (11/1/2020-3/31/2021) and the lowest during the stable time window (4/1/2021-10/31/2021), consistent with the reported COVID-19 case rate. The lowest detected concentration of SARS-CoV-2, 86.7 copies/L (cp/L), occurred between 4/1/2021-10/31/2021 at the Haskell WWTF; the greatest detected SARS-CoV-2 concentration, 268,000 cp/L, was detected at the John T. Hickerson WWTF between 11/1/2021-6/1/2022. Contrary to the WWTFs that were identified to have the greatest viral concentrations in wastewater, the reported COVID-19 case rate (mean and median) for the Haskell WWTF service area, which serves the central and downtown communities of El Paso, was consistently the highest across all three-time windows. All but the Roberto Bustamante WWTF service area experienced a reported case rate of 0 at some point between November 2020 and June 2022.

Time series analysis of observed cases and wastewater concentrations

Observed SARS-CoV-2 measurements in wastewater and 7-day rolling average of reported COVID-19 case rates (per 100,000 population) visually indicate a general trend of virus concentrations increasing in wastewater first, followed by an increase in reported infections (Figure 2). This relationship is most evident following the second wave beginning in November 2021. All four WWTFs experienced a decrease in observed cases and SARS-CoV-2 concentrations in wastewater from April 2021 to September 2021. Some deviation by individual WWTFs was also observed. There was a slight spike in daily cases at the Haskell WWTF on 10 May 2021, but not at the other facilities. Similarly, there were spikes on 14 March 2022 at both the Fred Hervey and John T. Hickerson facilities. The cause of these individual spikes in cases remains unknown but could be potentially due to localized outbreaks. The time series of SARS-CoV-2 concentrations quantified by N1 and N2 assays and reported COVID-19 case rates for each WWTF (untransformed and log transformed) are described in Figures S3 and S4 in the Supplementary Materials.

A time-lagged regression model was developed to assess the temporal relationship between SARS-CoV-2 RNA concentrations in wastewater and the COVID-19 case rates. The most probable lagged time was identified by the model with the largest R^2 (red dots) and the smallest *SE*. The time

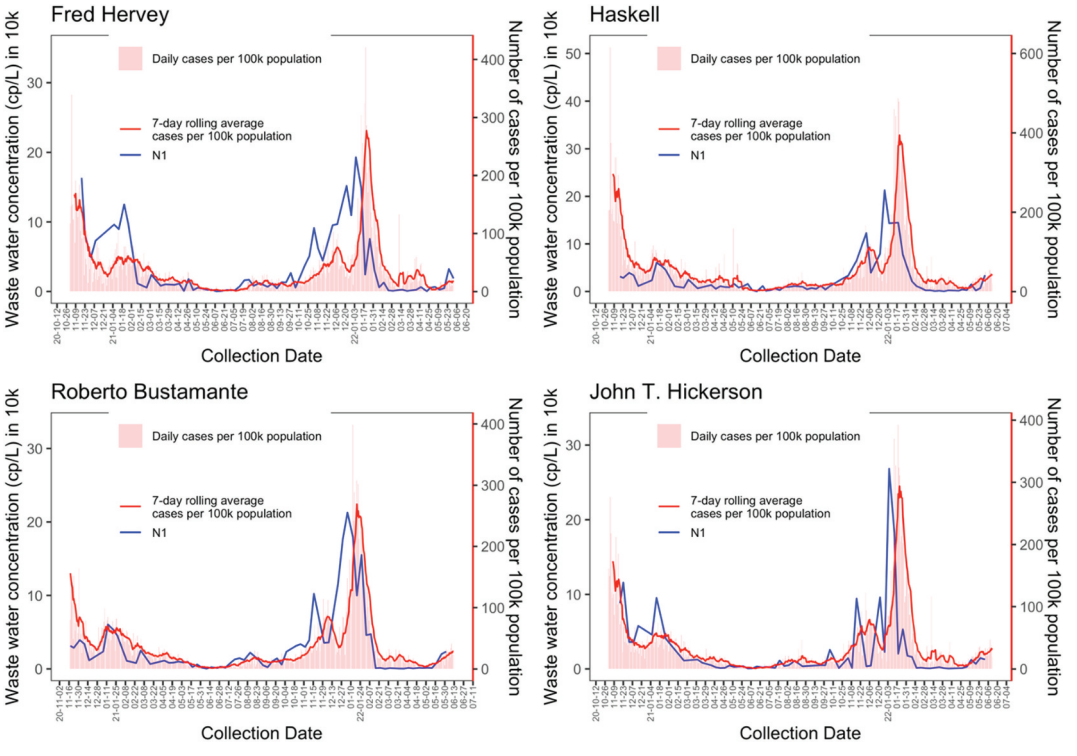

Figure 2. Time series of the SARS-CoV-2 wastewater concentrations quantified by the N1 assay and reported COVID-19 case data. Data are presented for each of the four WWTFs in El Paso, Texas.

lag across all four WWTFs ranged from 4 to 24 days, comparable to the estimated 1 to 3 weeks that has been previously reported (Yu et al. 2022) (Figure 3). The Haskell WWTF had the shortest (4 days), while Fred Hervey WWTF had the longest estimated lag time (24 days). However, it is important to note that the R^2 and *SE* are relatively similar from days 10 to 24 for the Fred Hervey WWTF, indicating that the time lag could potentially range for this WWTF. Roberto Bustamante and John T. Hickerson had similar estimated lag times of 10 to 11 days.

Discussion

This study evaluated trends of SARS-CoV-2 concentrations in wastewater and their relationship with positive COVID-19 cases in El Paso, Texas. Four WWTFs representing different sewersheds were included, further refining the scale of the temporal analyses.

Effective guidance from a SARS-CoV-2 wastewater monitoring program requires quality assurance and confidence in the interpretation of data collected (i.e. increasing SARS-CoV-2 signal reflecting increasing COVID-19 trends). Monitoring data collected from the four WWTFs generally indicate a lag time of 4 to 24 days between wastewater concentrations and reported cases; while this range in lag time is quite large (approximately 0.5 to 3.5 weeks), this monitoring program aids in providing an "on-time" snapshot of the virus circulating in the community. Relying solely on clinical surveillance methods results in a reactionary and delayed public health response. Recently, efforts to reevaluate biological or environmental samples for anti-SARS-CoV-2 antibodies or from cases identified as "COVID-19 like pneumonia" have been pursued to determine if SARS-CoV-2 was circulating in communities before the pandemic. These studies have resulted in mixed findings, with no early circulation of SARS-CoV-2 being identified in Rome, Italy (Capalbo et al. 2020), while anti-SARS-CoV-2 antibodies were detected in blood donors and patients in Saudi Arabia, who had

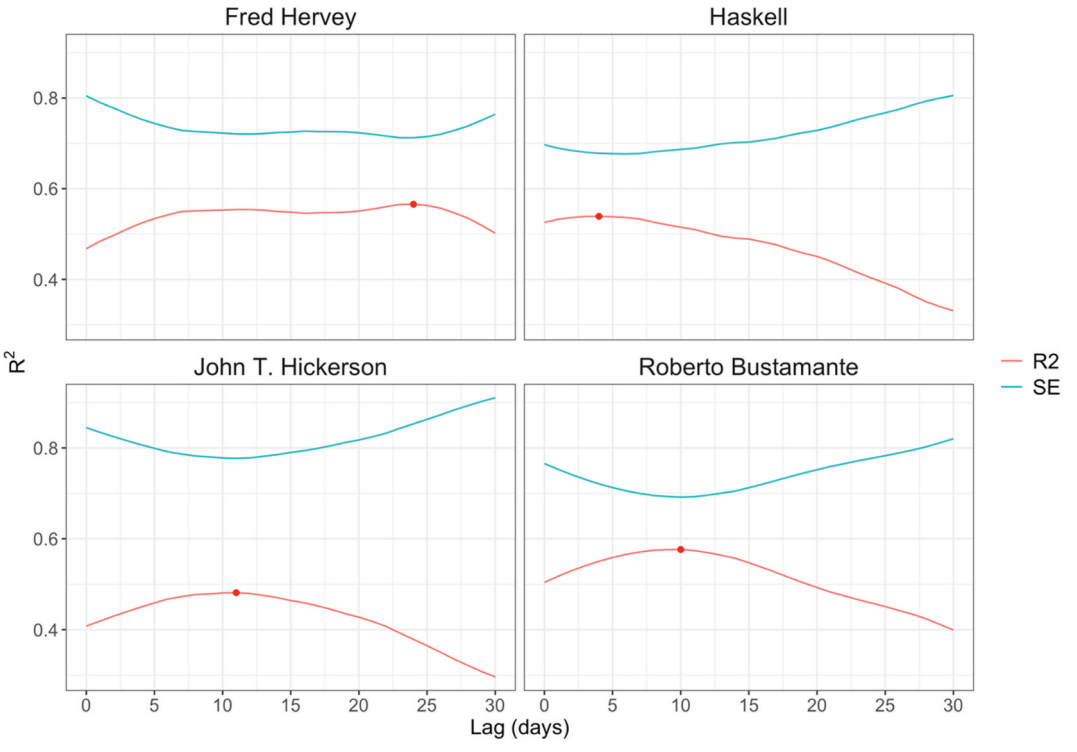

Figure 3. Panel plot displaying the $R^2$and *SE* by time lag (x-axis) in wastewater measurements and COVID-19 case data. Largest R^2 (red dots) and smallest *SE* indicate the most probable time (in days) that wastewater data can predict reported COVID-19 cases.

traveled to China before the pandemic (Mahallawi and Ibrahim 2022). Additionally, low- and middle- income countries, such as Indonesia, have found success with SARS-CoV-2 WBE programs, even given the limitations associated with mixed sewer and non-sewered sewage systems (Murni et al. 2022).

Site-specific wastewater monitoring data can be used to target and direct public health resources to mitigate impacts of COVID-19 in a community. The increased spatial granularity that is present in the City of El Paso sewer network, due to the four distinct wastewater service areas, is a significant advantage for this wastewater monitoring program. Localized or spatially refined wastewater monitoring can assist in identifying communities that are experiencing a likely increase in COVID-19 infections and direct needed public health interventions, since often marginalized populations are clustered geographically (Link-Gelles et al. 2016). Further, by quantifying SARS-CoV-2 in wastewater and determining the number of reported infections within a particular sewershed, mathematical models predicting community transmission can be developed; however, more information is needed regarding excretion patterns associated with SARS-CoV-2 infections, as well as virus detectability in wastewater, which can be impacted by inhibitors in the sewage and the efficacy of the laboratory detection method. In addition, although some reported COVID-19 cases include those who are asymptomatic, it is likely that many infected people did not test and are, therefore, not included in the data. The availability of at-home tests, coupled with the lack of consistent reporting mechanisms, suggests that even infected individuals exhibiting symptoms may not be included in the data. While COVID-19 cases were reported by ZIP code – given the population dynamics of this border community – it is likely that some cases were reported within a ZIP code not reflective of the case's dwelling. Although these limitations introduce uncertainty and variability when developing and interpreting predictive models for community transmission, current data can inform additional studies to better understand SARS-CoV-2 survival in

wastewater, improve laboratory detection, and inform dose-response. These types of studies will provide the information needed to better predict community transmission by identifying the factors that drive health risks and enable public health decision-makers to allocate resources and implement best practices within identified geographic areas to reduce virus transmission.

Neighborhoods served by the WWTFs all experienced a decrease in infection rates along with a decrease in SARS-CoV-2 sewage concentrations during the stable time window of 4/1/2021-10/31/2021. While this time window coincided with the emergence of the Delta variant, the cause of the decline in both the reported case rates and wastewater concentrations is challenging to determine. One possible explanation is the high vaccination rate in El Paso County, which was one of the highest vaccinated communities in Texas at that time (75.1% of residents were fully vaccinated by 13 September 2021) and public health leaders publicly recommended wearing masks (Harris 2022). An increase in the infection rate and SARS-CoV-2 in wastewater is evident in the following time window (11/1/2021-6/1/2022), coinciding with the emergence of the Omicron variant.

Homes, businesses, and other establishments not connected to a wastewater system, such as those on individual septic systems or decentralized wastewater treatment systems, are not captured by large-scale centralized wastewater monitoring. Nearly 20% of new homes built in Texas are connected to a septic system and an estimated 2.2 million permitted septic systems already exist throughout the state (Texas Water Resources Institute 2020; Texas Commission on Environmental Quality 2022). Colonias, unincorporated residential areas that lack essential services and basic infrastructure (such as water and wastewater systems), are prevalent along the U.S.-Mexico border, especially in El Paso County (Anders et al. 2010). Identifying measures to integrate these communities and areas into wastewater monitoring programs is critical not only for COVID-19, but to potentially monitor other infectious diseases of concern (e.g. enteric viruses, *Cryptosporidium*, etc.)

Wastewater treatment processes can generate and disperse aerosols, potentially exposing WWTF employees to SARS-CoV-2. Since the virus has been detected in wastewater aerosols (Pourakbar et al. 2022), quantitative microbial risk assessment has been applied to estimate potential health risks associated with worker exposure (Zaneti et al. 2021; Gholipour et al. 2021). However, no infectious SARS-CoV-2 have been measured wastewater, nor has there been any epidemiological evidence of individuals becoming infected with COVID-19 following exposure to wastewater aerosols (Sobsey 2021). Personal protective equipment currently used by wastewater treatment employees, and mitigation methods employed to limit exposure to other wastewater-derived pathogens are likely to suffice in reducing the risk of infection from SARS-CoV-2 wastewater aerosols.

Instances of divergence between increasing and decreasing trends of SARS-CoV-2 concentrations in wastewater and COVID-19 reported cases may be due to variations in virus shedding rates and amounts, virus stability in wastewater, emerging variants, disease stage of infected individuals, and other host-related factors. In addition, variability in the recovery efficiency of the laboratory methods used, as well as the occurrence of different constituents in the sewage samples collected from the different wastewater plants, may also contribute to inconsistent results. Further, the environmental conditions during sewage sampling at each of the sites – and over time – were not reported. Moreover, the testing of people – whether a suspected asymptomatic case or merely an exposed individual – is inconsistent. The timing of testing is also an issue since testing too early may result in a false negative (Liu and Rusling 2021). Finally, the mechanism for the reporting of positive (and negative) cases within the City of El Paso were not consistent among healthcare providers during the study period.

Identifying a baseline for the endemic phase of the SARS-CoV-2 virus circulating in the community would be useful to inform public health guidance. A baseline threshold would likely be unique to each individual community (due to population dynamics, economic activities, etc.), but reinforces the importance of establishing and evaluating community-specific wastewater monitoring programs. This study not only presents an evaluation of a wastewater monitoring program for uniquely diverse community located on the U.S.-Mexico border, but it also reinforces the importance of establishing these programs for individual communities to provide accurate

public health surveillance of the COVID-19 disease. El Paso is fortunate to have four distinct sewersheds within city limits, as this spatial granularity can be used to specifically target communities experiencing surges. It also exemplifies the different SARS-CoV-2 trends that may be occurring within the same community, but in separate locations, which would likely be overlooked by one centralized WWTF.

Future work should utilize wastewater monitoring data to inform human health risk assessments, which will be needed for improved strategic planning to successfully manage virus transmission with minimal disruption to daily life, the local economy, and hospital systems. Wastewater monitoring programs, especially in communities with a high density of septic systems or colonias, need to consider developing and implementing sampling protocols to incorporate decentralized wastewater infrastructure into their programs. Further, continued evaluation of wastewater monitoring programs for individual communities should be conducted due to the continuously evolving nature of the virus and the importance of the program for public health disease surveillance and guidance.

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author, AG. The data are not publicly available due to the sensitive nature of the collected data.

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