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Feasibility of Wastewater-based Public Health Monitoring Systems in Texas' Small Rural Communities

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Abstract: In recent years, there has been much focus on the use of wastewater-based epidemiology (WBE) in urban centers, particularly for SARS-CoV-2 monitoring. However, less is known about the application of WBE in rural settings or in areas of limited resources. Most WBE programs in low-resource communities have occurred outside the United States. To reap the benefits, WBE would need to be tailored to better reflect the socioeconomic challenges, technical barriers, communication limitations, and variable wastewater infrastructures associated with rural communities. The objective of this review is to evaluate the potential opportunities and challenges of deploying the current SARS-CoV-2 monitoring methodologies in small, rural communities, with a particular focus on rural Texas. For this, we conducted an inventory of rural communities in the state of Texas and their wastewater infrastructure. Based on specific rural examples, we evaluated the potential of current WBE methodologies used in urban settings to monitor for emerging biological agents of concern such as SARS-CoV-2. Our findings include an overview of rural wastewater capacity across rural Texas, a look at current WBE efforts to detect SARS-CoV-2, and recommendations for future implementation in two cities in rural counties, Kerrville and Valentine. WBE is a rapidly evolving public health tool with several notable advantages associated with cost, access, and adaptability. It is of particular use in resource-limited communities that often exhibit healthcare disparities. This study presents the first overview of the feasibility of implementing WBE in the rural settings of Texas. We provide several recommendations and suggest alternatives that may be of use when planning an expansion of WBE into these areas.

Keywords: *rural, Texas, WBE (wastewater-based epidemiology)*

Wastewater-based epidemiology (WBE) is a means of examining public health concerns (disease, drug use, toxins in the human body) using wastewater as the medium of investigation rather than direct testing on individuals. WBE has several notable advantages over individual testing (Xagoraraki and O'Brien 2019; Wu et al. 2022). WBE uses samples that are derived from populations rather than individuals, allowing for anonymized monitoring of human diseases or other excreted biological or chemical markers. Another advantage is that the method is passive, making use of either grab or automated sampling of the water at the source. Individuals need not be present or provide their samples to test for the agents directly (Polo et al. 2020; Safford, Shapiro, and Bischel 2022; Wu et al. 2022). The wastewater flowing through a sampling location serves as a record of human health because of modern sanitation engineering.

Determining the role of contaminated water in the spread of infectious agents within a community can be traced back to the 1850s when Dr. John Snow deduced that the Broad Street Pump was the

Research Implications

- Wastewater-based epidemiology (WBE) has enabled surveillance for community transmission during the COVID-19 pandemic and helped inform policy actions based on infection trends at the specific wastewater catchment level.
- WBE has great potential for the detection of public health concerns including emerging infectious diseases, antibiotic-resistant pathogens, pharmaceuticals, and emerging toxins, such as PFAS. However, the implementation of WBE in rural areas of the U.S. has been limited.
- A tailored approach to WBE in rural communities would account for limited resources and technical and socioeconomic barriers, and provide supporting data for public health providers and decision-makers at the community level.

source of a cholera outbreak in England (Buechner, Constantine, and Gjelsvik 2004). In the 1930s, U.S.-based researchers began using wastewater from the city treatment plants to monitor the spread of the poliovirus in large communities such as Charleston (South Carolina), Detroit (Michigan), Windsor (Massachusetts), and Buffalo (New York) (Trask and Paul 1942). Salmonella bacteria were isolated from sewage in Belfast, Ireland as early as 1928 (Wilson 1933). WBE would continue to be of value in the detection of water-borne pathogens throughout the 1950s, 1960s, and into the late 1980s across the world (Brouwer et al. 2018; Joseph-Duran et al. 2022). It is the preferred method for polio surveillance around the globe today (GPEI 2023). Contemporary applications of WBE include the pandemic outbreaks of the 2000s such as H1N1, Ebola, Zika, Middle East Respiratory Syndrome (MERS), and Severe Acute Respiratory Syndrome (Joseph-Duran et al. 2022).

While there has been much focus on the use of WBE in urban centers, particularly in recent years owing to the COVID-19 pandemic, less is known about the application of WBE in rural settings or in areas of limited resources. Most documented studies in such areas have occurred outside the United States in countries such as China, Bangladesh, Finland, Australia, and New Zealand (Lai et al. 2013; Kankaanpää et al. 2016; Hou et al. 2020; Price et al. 2021; Jakariya et al. 2022). Several of these studies combine rural communities with urban communities rather than treating them as distinct entities. One of the first documented WBE applications in rural communities in the U.S. was in the late 1930s, when researchers studied wastewater from a rural community in Michigan for periodic examination for polio (Trask and Paul 1942). Over the years, very few studies have employed WBE to detect pathogens within rural areas of the United States (Bishop et al. 2020; Margetts et al. 2020; Jarvie et al. 2023).

The reasons for the low utilization of WBE in these communities are varied and complex. Unlike their urban counterparts, rural communities are faced with specific challenges that distinguish them from urban counterparts. First, there are several socioeconomic challenges. Studies have shown that rural residents tend to be older, impoverished, and lacking in access to job opportunities and adequate healthcare resources (Mueller et al. 2021; Rural Health Information Hub 2023). Rural communities are less resilient to outbreaks and experience a disproportionate number of negative outcomes (Perry, Aronson, and Pescosolido 2021). Such negative outcomes are exacerbated by a lack of financial capital at the local level and lower funding from federal programs (Perry, Aronson, and Pescosolido 2021). Rural communities also face challenges with access to staff who are available and trained to support wastewater testing. Wastewater testing protocols need to be validated and verified, including the use of blind testing, controls, and matrix spike-ins, to name a few (APHL 2022). Management of a wastewater testing laboratory with a focus on microbiology would now require advanced molecular microbiology training. However, training programs in WBE are rare at the present time, particularly in the use of cutting-edge techniques such as next-generation sequencing for variant detection and digital polymerase chain reaction (PCR). The bulk of the current protocol development for SARS-CoV-2 detection and other emerging pathogens, such as the human *Monkeypox virus,* is spearheaded by partnerships with academic laboratories. The technology is costly and beyond the budget of most utilities, which likely outsource testing to local or statewide/federal environmental laboratories. Most environmental laboratories are at staffing capacity, busy fulfilling regulatory and compliance testing needs; time and resources are lacking for research and development to broaden a multi-targeted approach for WBE (US EPA 2015a; Switzer, Teodoro, and Karasik 2016). As of March 15, 2024, according to the Texas Commission on Environmental Quality, there are 167 labs (out of 245) certified by the National Environmental Laboratory Accreditation Program to test non-potable water.

Lastly, rural communities are different from their urban counterparts from a wastewater infrastructure perspective. There can be wide variability in treatment unit selection, quality and quantity of the wastewater profile, expense on a per-capita basis, choice of a centralized or decentralized treatment system, and numbers of employees dedicated to wastewater treatment in the community (Boller 1997; Tokich and Hophmayer-Tokich 2006). With these socioeconomic and wastewater infrastructure challenges, it is imperative for rural communities to have information that best reflects their own community.

In Spring 2021, the Texas Legislature established the Texas Epidemic Public Health Institute (TEPHI), located at The University of Texas Health Science Center at Houston (UTHealth Houston) (Clark et al. 2023). The institute has a mandate that includes working collaboratively with state, local, and federal agencies, academic institutions, professional associations, businesses, and community organizations to better prepare the state for public health threats. We hope that our efforts will synergize with that of TEPHI's mission, with our specific focus on rural communities and the role of academic institutions located in these rural regions to support state-wide efforts. The need to tailor WBE efforts to best reflect the socioeconomic issues, communication barriers, and infrastructural challenges associated with rural communities remains.

In this paper, we present a synthesis of the potential opportunities and challenges of deploying WBE methodologies in small rural communities. Specifically, we identify rural communities in terms of their demographic characteristics and their wastewater infrastructure. We also address how WBE would be useful in two rural communities in Texas to provide: (a) representative, unbiased information on community health, (b) information in a timely manner, and (c) specific information needed for public health and regional leaders to make informed decisions. Ultimately, our goal is to provide a framework whereby rural communities could identify indicators of public health for events such as outbreaks of infectious diseases. We expect that this framework will help rural communities establish an early warning strategy that is cost-effective, in house, informative, and responsive to the concerns and needs of the community.

Methods

Answering the question, "What is rural?" is not a simple task. There are several U.S. governmental agencies that provide varying guidance on how to define rural areas in the form of individual neighborhoods, city boundaries, and counties (Ratcliffe et al. 2016; Surbhi et al. 2021; Sanders and Cromartie 2024b). Rather than attempt to define "rural" to fit all situations that can be found in Texas, we take a more conceptual approach. The concept of rural generally connotes a human population area which exhibits some or all of the following characteristics (as compared to urban areas) – less dense in population, farther from city amenities (e.g., hospitals, professional sports venues, large office buildings), less diverse in demographic characteristics, larger travel distances for daily commutes, and smaller local government with lower service capacity. These markers are not meant to assess the quality of life in such places. They merely help researchers, planners, and demographers to better define rural beyond strict determinations. Therefore, in this work we have made specific assessments of rurality according to data availability involving delineation of rural communities, which is explained in the following subsections. Further details on the diversity of definitions for rurality are in the Supporting Information at the end of this paper, for those interested to see the underpinnings of our conceptual understanding.

Texas Rural Population and Demographics

To determine the potential application of WBE for Texas rural communities, we measured the extent of rural communities in Texas. We used the U.S. Census definition based on population number (population size of < 5,000) (CDC 2008). Using the U.S. Census definition, we looked at the population density of rural cities and sampled four rural cities around the state in the approximate north, south, east, and west regions to look at the racial demographics in the cities. To cover a more inclusive extent of rural communities, and in addition to the U.S. Census definition of rurality, we discussed implications of considering *metro/non-metro* and urban/not-urban characteristics. Our population and location data were obtained from the Texas Legislative Council Capital Data Portal, which is based on population and city geographic extent taken from the 2020 U.S. Census. We converted all city boundaries to centroid point locations.

Wastewater Flow Data - Wastewater Infrastructure

To evaluate the potential need, opportunity, and viability of WBE for rural Texans, we determined the scale of wastewater generation by defining cities with centralized wastewater treatment, their dispersion within the state, their population density, and their wastewater generation rate per capita. To define rural cities in this section, we used the U.S. Census population number of less than 5,000 persons. We collected all the wastewater treatment plant (WWTP) facility information from the Environmental Pollution Agency (EPA) Permit Compliance System (PCS), accessed through EPA Envirofacts as of April 20, 2023.

WBE in Texas

We reviewed the published literature for sampling, concentration, extraction, and detection methods currently being used in Texas in the context of SARS-CoV-2 as an example of current technical needs for detecting a biological agent of concern. Keywords used in the literature search included: 'SARS-CoV-2', 'wastewater', 'surveillance', 'wastewater-based epidemiology', 'rural', and 'detection'. We considered the methods used, the frequency and nature of sampling, and the equipment and procedural approaches currently implemented in Texas cities (Table 1).

Case Study–Application of WBE in Rural Communities

To understand how WBE can be implemented in rural communities, we conducted an analysis of the distribution of rural communities across the state and considered the nature of wastewater infrastructure in those communities. For a more detailed look, we examined two cities in two different rural counties in the State of Texas - Valentine (Jeff Davis County; population 133 in 2019) and Kerrville (Kerr County; population 24,477 in 2021). Kerrville has a population over 24,000, defined as rural in terms of residing in a *non-metro* county per the United States Department of Agriculture (USDA) Economic Research Services (ERS). According to the USDA-ERS, a county is considered *non-metro* if it meets at least one of these three criteria—"open countryside, rural towns less than 5,000 people and 2,000 housing units, and urban areas with populations ranging up to 50,000 people that are not a part of larger labor areas (metropolitan areas)" (Sanders and Cromartie 2024a; 2024b). Considering Kerr County as a rural county is also consistent with other definitions as described by the Texas Department of Agriculture and Texas Department of Housing and Community Affairs (Texas State Office of Rural Health 2012; Texas Department of Housing and Community Affairs 2022). It is interesting to note that Jeff Davis County, the county in which Valentine is located, can also be considered a *non-metro* county.

The factors analyzed in both cities include the rural demographics, sewer network characteristics, distance from a major center city (defined as having a population greater than 200,000 people with an entity that has current support for WBE activity), and type of WWTP. Kerrville is in a rural county, and is, along with Valentine, representative of one of the two types of centralized treatment plants in Texas (Figure 1). Valentine has a pond/lagoon system, characterized by a series of holding ponds, and Kerrville has an activated sludge system, characterized by an oxidation ditch system. The characteristics of both cities are detailed in Table 2.

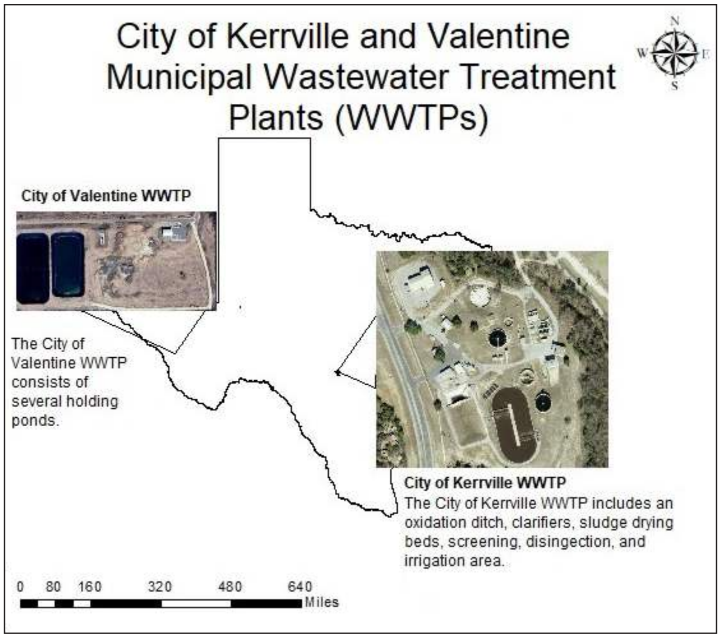


Figure 1. Characteristics of the centralized wastewater treatment plants in Kerrville and Valentine (Texas Department of Transportation 2015; 2024; ESRI 2021; Google Earth V 7.3 2024a; 2024b).

Health Outcomes and Literacy Data. The 2010 community health outcomes (HO) for Texas are publicly available from County Health Rankings produced by the University of Wisconsin, Population Health Institute. HO are a combination of length of life and quality of life, and can be influenced by a variety of factors, such as access to clean water, affordable housing, the quality of medical care, and the availability of well-paying jobs, all of which are influenced by policies and programs (UWPHI 2023a). The HO rankings are based on an ordering of composite z-scores weighted according to the model in the report, which assigned weights to specific measures of heath, standardized the measures within each state using a z-score, calculated weighted sums of the standardized measures within each state and sorted these composite scores to create an ordering of counties which determined the rank (UWPHI 2023a). Because the HO ranks are based on z-scores they do not have units, and they range from 1 (healthiest) to 221. The following counties had insufficient data to be ranked in 2010: Armstrong, Borden, Briscoe, Coke, Concho, Cottle, Dickens, Edwards, Foard, Glasscock, Hemphill, Irion, Jeff Davis, Kenedy, Kent, King, Lipscomb, Loving, Mason, McMullen, Menard, Motley, Oldham, Reagan, Roberts, Schleicher, Shackelford, Sherman, Sterling, Stonewall, Terrell, Throckmorton, and Upton. We used the HO rankings broken down by location to understand why HO might differ across a county. We focused on our two rural cities of Kerrville and Valentine, in Kerr and Jeff Davis counties, respectively, both of which are rural counties (UWPHI 2023b). We also examined the health literacy (HL) scores for our two communities (National Health Literacy 2010). HL is defined as the ability to find, understand, and easily use health information and services to make informed decisions and take informed actions (Health Literacy Texas 2023). The HL scores on the dashboard range from 177 to 280, with higher numbers indicating a higher level of HL. Rural and urban communities with low literacy may exhibit difficulties with reading and interpreting basic health information such as pamphlets about a condition.

Results and Discussion

Texas Rural Population and Demographics

Using the U.S. Census definition based on population number, there are 868 rural cities (population size of < 5,000) found in Texas, out of

Table 1. Published WBE schemes in Texas.

City	Study Purpose	Collection Location	Collection Frequency*	Wastewater Concentration Method(s)	RNA Extraction Method	RNA Detection Technique(s)	Reference
Houston (2021 Pop: 2,288,250[1])	Evaluation of varying methods for wastewater sampling, concentration, RNA extraction, and RNA detection	WWTP influent from six city treatment plants	One sample every hour for 24 hrs	Each sample is spiked with bovine coronavirus (BCoV) before concentration. HA extraction with bead beating. HA extraction with elution. PEG precipitation. Ultrafiltration	Chemagic Prime Viral DNA/RNA 300 Kit H96	One-Step RT-ddPCR Advanced Kit for Probes on QX200 AutoDG Droplet Digital PCR System with N1 and N2 SARS-CoV-2 primers; BCoV primers. qPCRBIO Probe 1-Step Go Separate-ROX on QuantStudio. Real Time PCR System with VGP pMMoV primers.	LaTurner et al. 2021
Austin (2021 Pop: 964,177[1])	Use wastewater and daily COVID-19 cases to identify sewersheds (via zip codes) in which COVID-19 was the most prevalent	WWTP primary clarifier effluent from two city treatment plants	Two to three times a week for 39 weeks	Pasteurization and centrifugation	MagMax Microbiome Ultra Nucleic Acid Isolation kit on the KingFisher Flex System	ViiA7 Real Time System with CDC nCOV_N2 primer	Nelson et al. 2022
Houston (2021 Pop: 2,288,250[1])	Assess how effective wastewater can be as a forecasting tool in comparison with other indicators of "disease surveillance" across a city	WWTP influent from all 39 city treatment plants	Weekly for 86 weeks	Electronegative filtration	Chemagic 360 automated platform, Viral DNA/RNA 300 Kit H96	One-Step RT-ddPCR Advanced Kit for Probes on QX200 AutoDG Droplet Digital PCR System and C100 Thermo Cycler. Water SARS-CoV-2 RT-PCR test kit	Hopkins et al. 2023
Houston (2021 Pop: 2,288,250[1])	Employment of modeling software to simulate SARS-CoV-2 presence in various sized sewersheds, and to identify preferable sampling locations in a watershed	WWTP influent from six city treatment plants	Not listed	HA filtration with bead beating	Qiagen Allprep Powerviral DNA/RNA kit	One-Step RT-ddPCR Advanced Kit for Probes on QX200 AutoDG Droplet Digital PCR System	McCall et al. 2022

Table 1, continued. Published WBE schemes in Texas.

City	Study Purpose	Collection Location	Collection Frequency*	Wastewater Concentration Method(s)	RNA Extraction Method	RNA Detection Technique(s)	Reference
Austin (2021 Pop: 964,177[1])	A comparison of methods, specifically wastewater concentration, sample collection, and RNA extraction/detection	WWTP primary clarifier effluent from two city treatment plants	Six times throughout an eight-week period; 14 times throughout a 39-week period	Samples are first either pasteurized and/or centrifuged. Samples undergo vacuum filtration and nanofiltration regardless of being pasteurized or centrifuged	MagMax Microbiome Ultra Nucleic Acid Isolation kit on the KingFisher Flex System	ViiA7 Real-Time System with CDC nCOV_N1 primer	Palmer et al. 2021
San Antonio (2021 Pop: 1,451,853[1])	A temporal study of the change in viral concentration entering a city WWTP	WWTP influent	Weekly for 12 weeks every Tuesday	Adsorption-extraction by means of electronegative membranes. Bovine coronavirus as surrogate for recovery validation.	RNeasy Power Microbiome Kit with QIACube Connect	BioRad QX200 Droplet Digital PCR System with one-step RT-ddPCR	Al-Duroobi et al. 2021
El Paso (2021 Pop: 678,415[1])	An assessment of a two-year city monitoring program	WWTP influent from four city treatment plants	Weekly for 98 weeks	Electronegative filtration	Chemagic Prime Viral DNA/RNA 300 Kit H96	7500 Fast Dx Real-Time PCR Instrument	Gitter et al. 2023

*24 hr. composite samples were taken in each case.

Table 2. Characteristics of two cities in rural Texas, Valentine, and Kerrville.

City	Population	Sewer Network Characteristics	Distance from Major City Center	WWTP Description	Data Source
Valentine	133 (2019)	● Three different pipe sizes— 19,000 linear feet 6-10", 38 manholes ● Two locations with 200 linear feet, 16" steel casing ● Lift station, 15,000 linear feet, 3-inch force main; unknown staff number	159 miles southeast of El Paso	Pond system (bar screen, facultative lagoon, storage pond)	City of Valentine 2003; City-Data 2023
Kerrville	24,477 (2021)	200 mi of collection lines, 3,163 sewer manholes, 27 lift stations; 2.2 MGD daily average flow; a staff of 13	65 miles northwest of San Antonio	Preliminary (screening, grit, equalization), aerobic/anoxic, oxidation ditch, clarifiers, rapid sand filters, chlorine disinfection, dechlorination	City of Kerrville, Texas 2023; U.S. Census Bureau 2023a

a total of 1,223 municipalities. These rural cities are distributed all over the state (Figure 2), with 75% having populations of less than 2,000.

The size distribution and population density of all rural cities, as well as demographics of four representative cities taken from east, west, north, and south Texas are provided in Figure 3. The histogram (Figure 3a) highlights the right-skewed distribution of small city populations, indicating that many of the towns we examined are particularly small with about half (438 cities) having population sizes of less than 1,000 persons. We sampled four cities around the state in the approximate north, south, east, and west regions to look at the racial demographics in the cities (Figure 3b). The sample also contains cities that fit into one of the four quartiles for rural population size (Q1: 22-422, Q2: 423-989, Q3: 990-2,062, Q4: 2,063-4,974). In the far west and south of Texas a greater Hispanic population is evident while in the north and east, there is a much greater Anglo population. Varying cultural and social differences among these communities is likely to influence the level of acceptance and trust in emerging methods such as WBE. These trends are important to consider as inclusive, ethical, and effective strategies for implementation are developed (Medina et al. 2022; National Academies of Sciences, Engineering, and Medicine 2023). Using the population size of < 5,000, a statistical summary of rural Texas city population density is shown in Figure 3c. Population density helps validate the rural designation of these cities and informs some aspects of community cooperation that can be influenced by population density (Smailes 1996). Higher population density can increase human interaction, which has impacts on disease transmission and, potentially, residential interest in public health. It has been reported that rural public health workers engage with communities that tend to be skeptical of the role of government (Leider et al. 2020). Our results show that the mean population density of rural Texas cities in 2020 was 680 ± 470 people per square mile (mean ± sd); 867 cities were included with viable population density values, when notable outliers were excluded (5% of cities are outliers). This results in a coefficient of variation (CV) of 69%, indicating a fair amount of variation in rural community population density. Overall, 75% of Texas rural cities in this selection

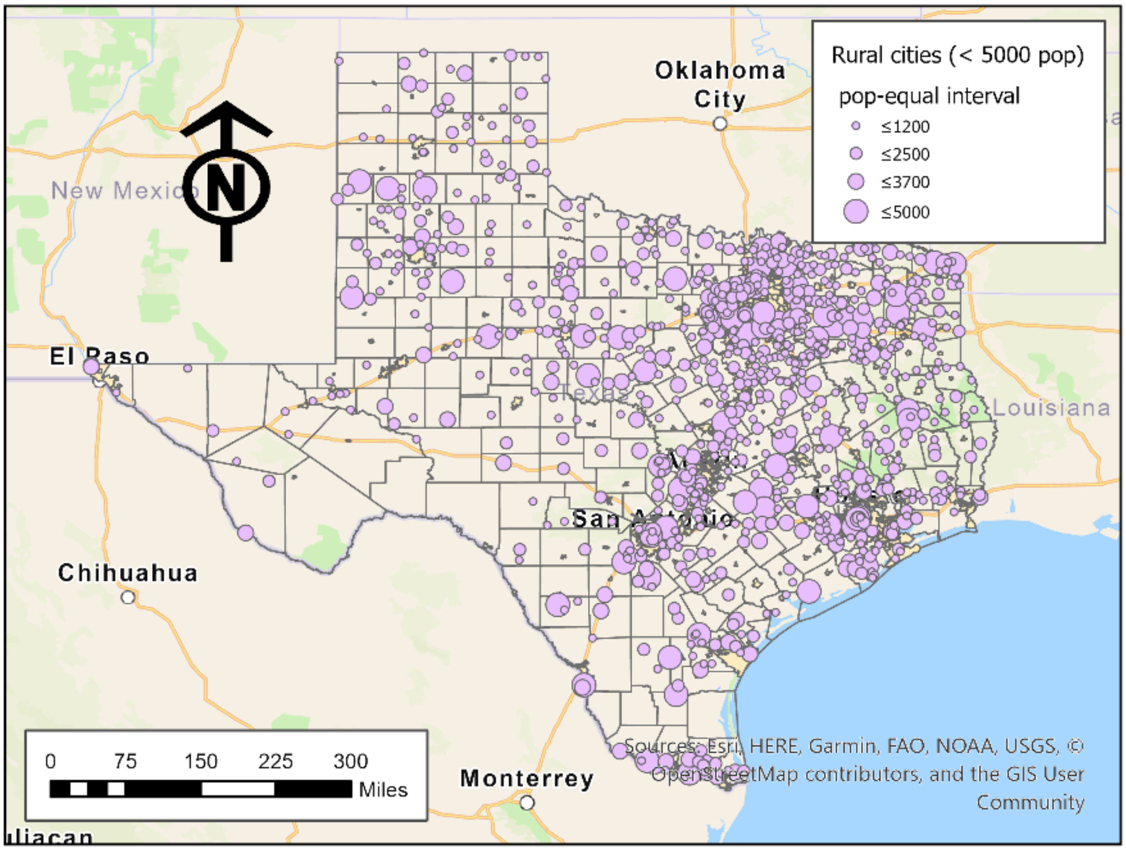


Figure 2. Distribution of rural communities (n=868) within the state of Texas. All data taken from the 2020 U.S. Census, with inclusion criteria of less than 5,000 in total population. Size classes are determined as convenient intervals from minimum to maximum size.

were at a density of < 1,000 people per square mile (pop/mi^2).

Using the < 5,000 population criterion, 71% of all cities in Texas are rural. However, the proportion of the population living in areas of the state considered rural is small. Out of a total Texas population of 29.1 million in 2020, rural cities account for 1.24 million people (4.3%) (U.S. Census Bureau 2023b). There is a sizable amount of the Texas population that lives outside of incorporated cities (8 million), while all incorporated cities have a total population of 21.1 million. This population may not necessarily be classified as "rural" if the population measurement of < 5,000 is used, i.e., they may live near a city limit boundary but just outside of it. Consequently, we consider it likely that communities outside of city limits could also be classified as rural. A more inclusive delineation of rural communities is found when considering *metro/non-metro* and urban/not-urban characteristics. A *metro* area is defined as a core area containing a large population nucleus with adjacent communities that are integrated to that core (U.S. Census Bureau 2023b). *Non-metro* counties are outside the boundaries of *metro* areas and could have or not have a population larger than 5,000. The Rural Health Information Hub (RHIhub) (2023) shows that if counties are determined to be either *metro or non-metro* based on various parameters, including eligibility criteria for federal programs, 10.8% of all Texans in 2020 live in *non-metro* areas. Using *non-metro* could be a more inclusive way to classify communities as being rural. The U.S. Census Bureau's (2023b) official fraction of rural for 2020 in Texas was reported as 16.3%. The Bureau defines any population which is not found in an urban designated area as "rural." Using a population residual calculation, the Bureau determines that "not urban" is equivalent

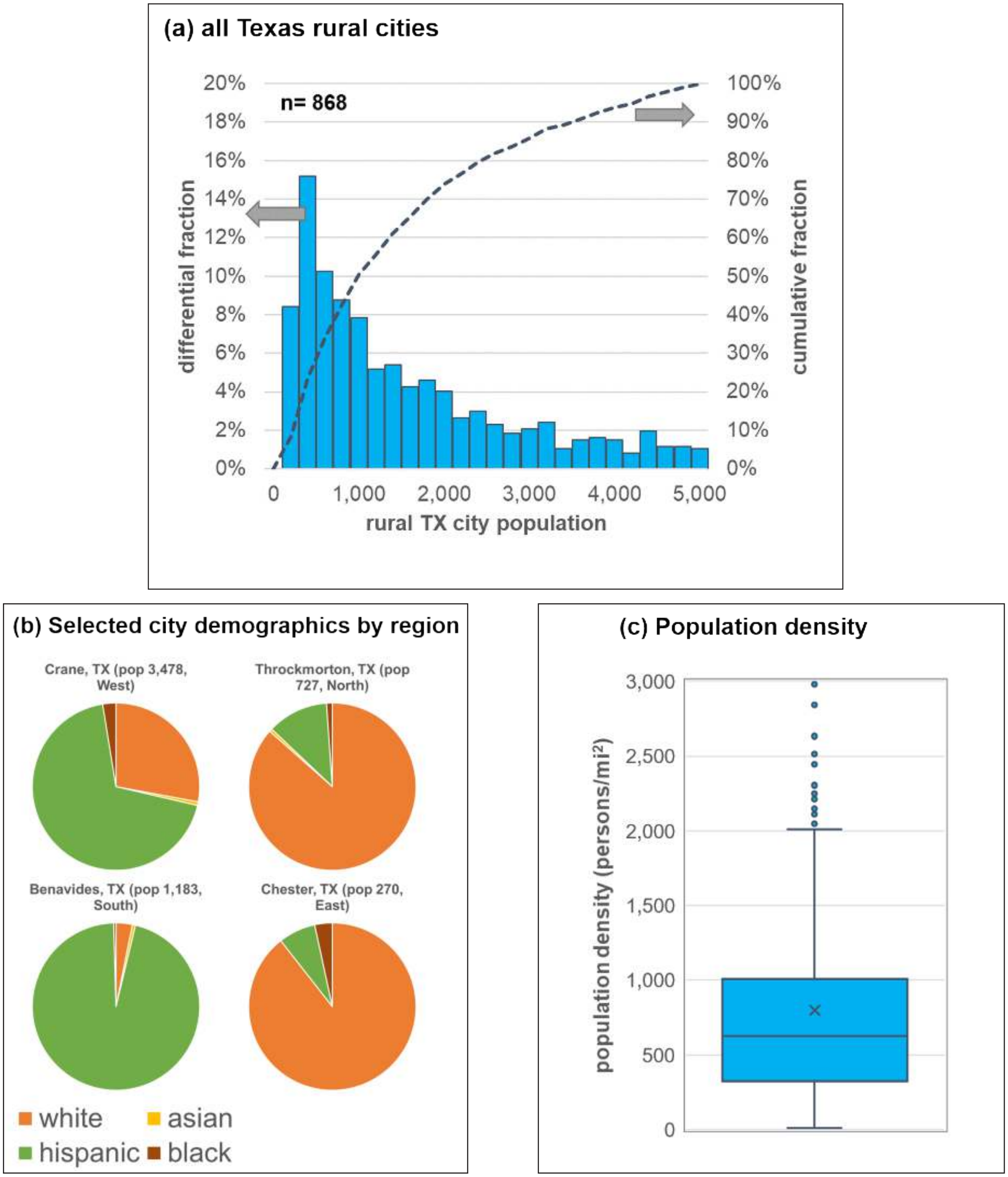


Figure 3. Texas rural city population data. (a) The statistical distribution of population size ranges from 22 to 4,974 as differential histogram and cumulative population (dashed line). (b) The demographics by race in four cities that span the four areas of Texas (North, East, South, West). Each city was selected so that one city each was in a different quartile of rural populations (Q1: 22-422, Q2: 423-989, Q3: 990-2,062, Q4: 2,063-4,974). (c) Rural Texas town population density; the sign "x" represents the mean. A total of 867 cities were included with viable population density values. The use of an outlier determination criteria of 1.5IQR reveals an upper outlier threshold of 2000 person/mi^2 which then identifies that 5% of cities are outliers in the rural Texas town class.

to rural. Based upon the data we have presented here at the city boundary level, Texas is in practice approximately 10% rural, with the fraction of rural population variable according to the region of the state, *non-metro* and "not-urban" characteristics.

Wastewater Flow Data - Wastewater Infrastructure

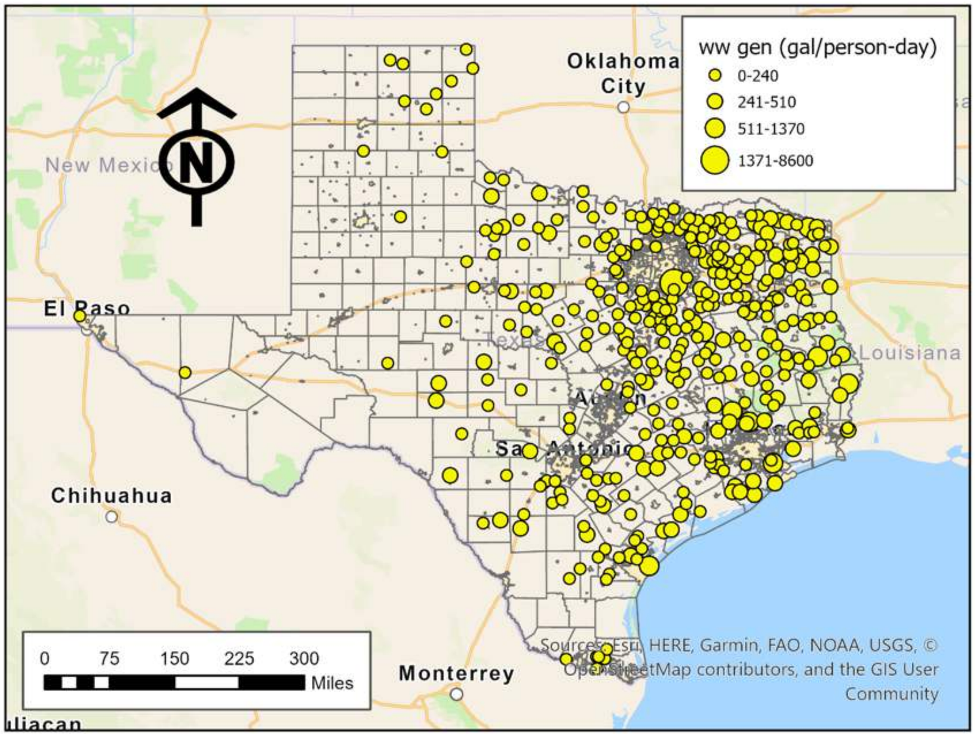
The use of population data could provide greater accuracy on daily wastewater flow and variation, but for the purpose of this study, we used the design flow of the WWTP to determine the scale of wastewater generation. We focused our study on which cities have centralized wastewater treatment, their dispersion within the state, their population density, and their wastewater generation rate per capita, in order to evaluate the potential need, opportunity, and viability of WBE for public health benefits for rural Texans.

The examination of wastewater quantity and infrastructure in particular communities we identified as rural requires that there be data in each community on both population and wastewater. We therefore had to find a match between the Census 2020 city name and the city name in EPA permit records. Out of 868 rural communities we identified (using the population < 5,000 threshold), we were able to identify 371 cities for which we could obtain wastewater flow data, which constitutes a match rate of 43%. We considered this subset of cities likely to represent what typically occurs in rural communities across the state, as the mean population for all the rural cities we identified (n=868 cities) was 1,430 (range of 22-4,974), whereas the mean value of cities that had matching wastewater data (n=371) was 1,724 (range of 116-4,969), a slightly higher population mean. We hypothesize that this may be because larger rural cities are more likely to have centralized wastewater treatment, and thus more likely to have current permit records available in the EPA permit records database. However, cities as small as Cuney, TX (pop. 115), reported a wastewater treatment permit for even a very small design flow of 0.05 millions of gallons per day (MGD), suggesting that size alone does not necessarily predict if a city has centralized wastewater treatment or if their treatment structure and/or strategy is available in the EPA permit system.

Figure 4 provides a spatial outlay of the rural cities where wastewater treatment matching was possible in terms of wastewater generated per capita. In Figure 4a, we see the variation in wastewater generation rate per capita. The wastewater generation rate per capita is 234±39 gal/person-day (mean±95% conf), and the 90th percentile of wastewater generation is 353 gal/person-day. Therefore, there are some unique instances where wastewater generation per capita is relatively high, but for most rural areas the 95% confidence span of 195-273 gal/person-day is representative. When comparing wastewater generation rate per capita in rural cities (< 5,000 pop.) and urban (> 5,000 pop.) cities we found a nominal decrease in the urban mean (199 gal/person-day, n=228) compared to the rural mean (234 gal/person-day, n=371). However, there was no statistical difference via an independent sample t-test ($p > 0.05$) between these means. We can conclude that the wastewater generation rate of urban cities versus rural cities is approximately the same, at least based on the treatment plant design flow rate (Figure 4b). The wastewater generation rates or design flow do not correlate strongly with geography. However, there is a spatial pattern of more rural centralized treatment systems with permits in the eastern third of the state. We think that this is due to a greater proportion of the Texas population overall residing in the eastern third as compared with the central and western thirds. The census data would support this hypothesis, despite the growth in population in certain counties along the border.

Figure 5 shows the overall change in the design flow rate of treatment plants (i.e., the general treatment capacity) across the state for rural cities. There are some notable examples of higher flow rates in the 2.4-3.2 MGD but only a few. Out of all wastewater flows, 95% of flows in these rural cities were 0.96 MGD or less. To put this scale of flow into perspective, we first compared it to larger cities of population > 5,000. In this dataset, 95% of all design flows were 24.7 MGD or less. At the median level, urban cities had flows that were about 10x larger (0.25 MGD rural vs. 2.6 MGD urban). We also considered the size of the inflow pipe to the single WWTP that a rural location would receive (typically, all rural cities

(a) Wastewater generation per capita



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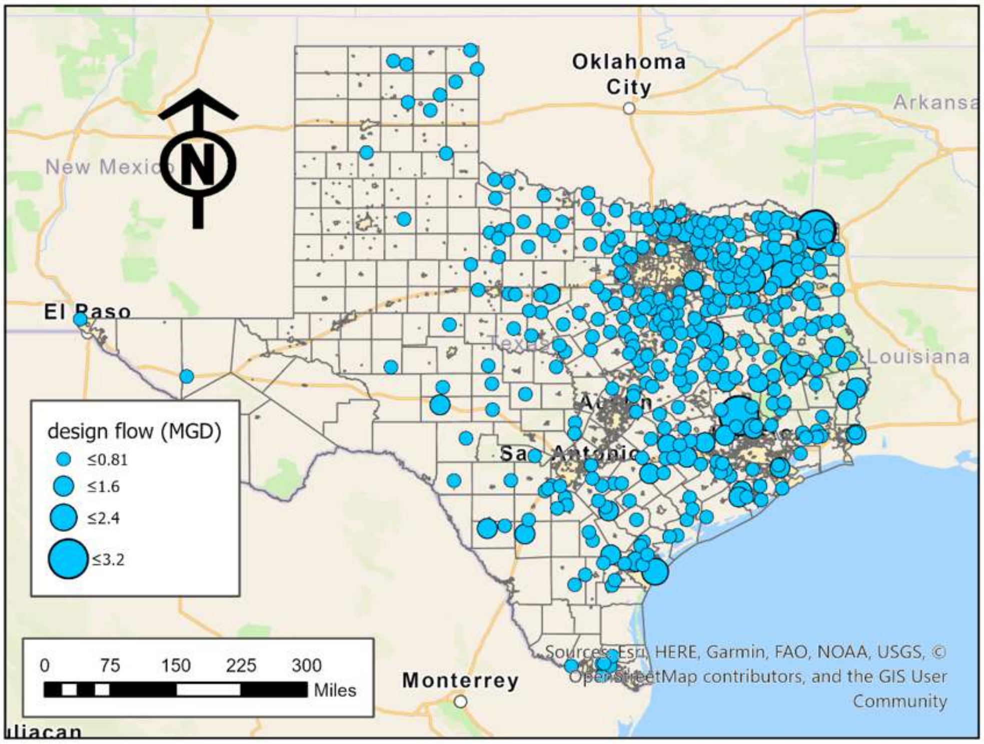
(b) Wastewater treatment design flows



Sources: Esri, HERE, Garmin, FAO, NOAA, USGS, © OpenStreetMap contributors, and the GIS User Community

Figure 4. Wastewater treatment and rural population data linkages in rural Texas cities. (a) The wastewater generation rate per capita as design flow of wastewater per unit of the 2020 Census population is provided as gallons per person per day. (b) The total design flow is for the entire rural community.

have only one centralized treatment facility). The median flow rate to wastewater facilities in a rural city is 0.25 MGD. Figure 5 illustrates the way such a flow would fill a sanitary sewer main feeding the plant influent, demonstrating how different pipe sizes would be filled at the rural wastewater flow rate for the entire town.

Given that a practical design of such an influent pipe at the design flow should be at 20-50% full (to balance cost and capacity to deal with flow variations), the influent pipe size for this typical small-town wastewater treatment facility would be 7-14 inches in diameter. A similar analysis for the urban median flow (2.6 MGD), if it were concentrated into a single facility influent, would be an 18–36-inch diameter pipe.

On average, rural cities in Texas have total wastewater flows which are 10x smaller than the typical urban setting. Such differences in pipe size and flow depth would impact the results of any WBE strategy. The water depths may be shallower, and the pipe sizes smaller. An operator's ability to easily obtain a wastewater sample would be affected by this flow depth; at times of lower flow it could become more difficult to obtain. Despite these challenges, most rural Texas cities are likely to have only one WWTP and outfall, which allows for the entire community to be evaluated for public health concerns at a single location. The fact that rural towns have slightly larger wastewater per capita generation rates may indicate that there is a greater dilution of fecal matter-influenced wastewater (the portion most used for WBE) with other wastewater sources (showers, sinks, local industry, car washes, etc.). This dilution could obscure the signal of pathogens or other WBE constituents of interest, another area of difficulty that rural WBE schemes in Texas would need to surmount.

WBE in Texas

As detailed studies of methods for SARS-CoV-2 are available, we focused on detecting a pathogen such as SARS-CoV-2 when determining an idealized protocol for a rural setting. We based our suggestions on comparing techniques used in Texas cities, when possible, as research in rural communities is lacking. These suggestions would also be applicable to sites and locations beyond Texas with similar processes and population sizes.

Sampling Frequency and Location. Developing a sampling protocol in a rural community would require balancing detection errors and resources. A sampling protocol would ideally include a daily sampling schedule and composite samples to avoid errors in non-detectable targets (Table 3) (Ahmed et al. 2022). However, daily sampling would not be feasible for most communities, nor would it be cost-effective. Sampling twice per week for SARS-CoV-2 was determined to be sufficient to avoid detection inaccuracies and was

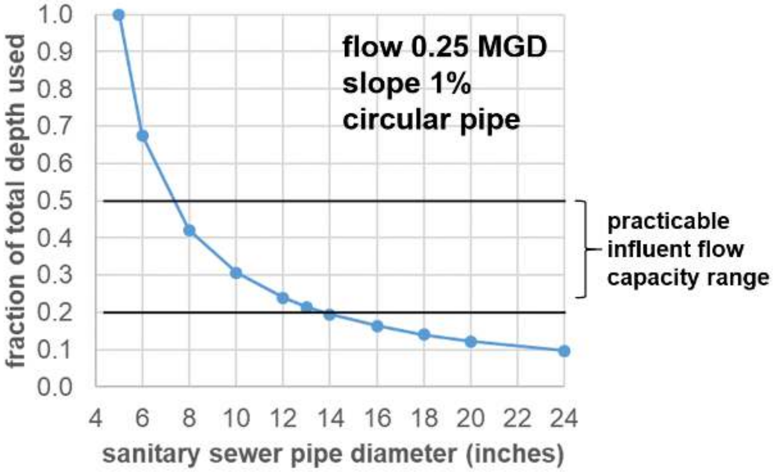


Figure 5. Representative influent pipe sizes for a single rural wastewater centralized treatment facility providing treatment for the entire city. Calculation conducted at uniform open channel flow at a flow of 0.25 MGD on a 1% in a concrete sanitary sewer circular pipe.

Table 3. Recommended practices for the detection of SARS-CoV-2 in wastewater samples.

Method Component	Description	Recommendation	Reference
Sampling		QA/QC protocols to avoid contamination during sampling based on drinking water sampling protocols	US EPA 2015b
		Composite sampling is recommended to minimize false negatives due to temporal fluctuations or collection of multiple grab samples within a 3-4 hr period using passive samplers. Single-grab samples are adequate if viral load is expected to be high.	Ahmed et al. 2022
		Large volume for collections (1-2 L) especially when low incidence of COVID cases is expected	Ahmed et al. 2022
	Composite influent samples over a 24-hr period sampled one to two days per week at 12 WWTPs and daily for five days per week for two WWTPs	A three-day per week sampling frequency evenly spaced apart will not compromise surveillance and a non-consecutive two-day per week frequency has minimal impact on surveillance	Feng et al. 2021
Concentration	Seeded wastewater with gamma-irradiated SARS-CoV-2	Aluminum-based precipitation with manual extraction (< 40%) was considered the best strategy due to time (less than 2h) versus an overnight incubation using PEG	Pérez-Cataluña et al. 2022
		If COVID cases are low, both liquid and solid fractions should be concentrated	Ahmed et al. 2022
	Grab samples of wastewater from a manhole and influent from two WWTPs	Solid precipitation that concentrated 400 mL sample by 100-fold provided detectable SARS-CoV-2 RNAs compared to methods using supernatant from initial centrifugation	Kitamura et al. 2021
	24-hr composite influent samples spiked with bovine coronavirus (BCoV) and quantified by ddPCR	HA filtration with bead beating provides SARS-CoV-2 RNA concentrations consistently above the LOQ compared to centrifugation followed by direct extraction	LaTurner et al. 2021
Extraction	Seeded wastewater with gamma-irradiated SARS-CoV-2	Automated extraction provides higher sensitivity	Pérez-Cataluña et al. 2022
	Grab samples of manholes or lift stations associated with individual buildings or clusters on university academic, dormitory, research, and hospital facilities	Smaller RNA extraction eluents improve sensitivity likely due to PCR inhibitory substances: 10 µL for elution (Ct 33) compared to 40 µL (Ct 35)	Sharkey et al. 2021

Table 3, continued. Recommended practices for the detection of SARS-CoV-2 in wastewater samples.

Method Component	Description	Recommendation	Reference
Assay	Seeded wastewater with gamma-irradiated SARS-CoV-2	Genomic RNA of SARS-CoV-2 as positive control when evaluating assay efficiency	Pérez-Cataluña et al. 2022
		A surrogate virus should be used as a positive sample process control for at least 10-20% of samples or an endogenous control	Ahmed et al. 2022
	24-hr composite influent samples spiked with bovine coronavirus (BCoV) and quantified by ddPCR	BCoV as a process control and normalization factor for recovery has low variability	LaTurner et al. 2021
	Influent wastewater samples with aluminum-based adsorption-precipitation method and RT-qPCR	N1 and N2 primers provide higher positivity rates compared to primer sets E, IP2, IP4, and RdRp	Pérez-Cataluña et al. 2022
	Composite influent samples over a 24-hr period sampled one to two days per week at 12 WWTPs and daily for five days per week for two WWTPs	If selecting one set of primers, N1 primers are more sensitive and have higher correlation to SARS-CoV-2 case numbers	Feng et al. 2021
	Grab samples of manholes or lift stations associated with individual buildings or clusters on university academic, dormitory, research, and hospital facilities	V2G-qPCR and N1 or N2 RT-qPCR similar in terms of limit of detection (LOD) or quantitation (LOQ) with N1 RT-PCR providing results higher than V2G-qPCR (approximately 10%)	Sharkey et al. 2021
	Detection of SARS-CoV-2 RNA isolate strain in diluted RNA from isolate extraction. Grab samples of wastewater from a manhole and influent from two WWTPs	Duplex RT-qPCR using N1 and N2 primers, compared to NIID_2019-nCoV_N primer sets, was more sensitive in detection (< 10 gc/reaction of diluted RNA samples) and detected higher numbers in wastewater samples.	Kitamura et al. 2021

sufficient to correlate with a 7- to 8-day lag time in case detection at two Austin WWTPs (Feng et al. 2021; Nelson et al. 2022). In El Paso, a nearly weekly sampling approach had a 4- to 24-day lag time (Gitter et al. 2023); therefore, a biweekly sampling strategy would be more practical when limited resources and personnel are considered, especially in a rural community (Feng et al. 2021). Extending the time between sampling events would be expected to increase detection errors for environmental monitoring; however, an increase in the concentration of biological or chemical contaminants observed at multiple sampling points within a community would then warrant a strategic increase in sample frequency and location of sampling points (Levine et al. 2014). Such a temporal and spatial approach would facilitate source tracking (chemical or biological) and localization of the problem while reducing the costs and barriers for utilities and laboratory personnel. The Balanced Approach Survey (BAS) considers spatial variation in sampling locations and targeted sampling sites based on a determination of more susceptible populations within an ecological context (Brown, Robertson, and McDonald 2015).

Our recommendation for the rural cities selected is that WBE should consider a multi-dimensional environmental sampling approach to reduce sampling size while capturing critical data. Regarding application of the BAS (Brown, Robertson, and McDonald 2015), sampling sites should be selected based on representation of wastewater in the geographic area (two-dimensional points) and include additional dimensions that determine the sensitivity of communities or severity of the environmental impact on subsections of the community. These could include limited public health resources, social and economic factors, and the health behaviors of a population such as those tracked to determine county health rankings (UWPHI 2023b). Considering the higher impact that SARS-CoV-2 had on minority populations, including more sampling points within these communities would be crucial (CDC 2020). From our review of the published literature, WBE sampling points in what we consider to be rural communities are currently rare in Texas, with the focus being on major metropolitan areas such as Austin, Houston, San Antonio, and El Paso (Table 1). Addressing this gap would preemptively address hospital stress resulting in higher deaths (Soria et al. 2021).

Techniques and Approaches to Sampling. Several cost-effective alternatives for sampling have been proposed, such as the "Moore swab," a gauze pad suspended by a string in water to provide a composite sample of human fecal matter by continuously filtering flowing water over a 24-hr period (Sikorski and Levine 2020). Testing of this sampling matrix could be incorporated into a citizen or community science program for water surveillance, such as one being conducted by Texas Stream Teams (a collaborative effort across the State looking at environmental water quality), in collaboration with academic or non-profit entities. Such programs would empower rural residents to participate and engage in local public health efforts. Research has demonstrated the feasibility of passive sampling as a viable method for the collection of wastewaters to monitor the changes in viral presence throughout the COVID-19 pandemic, many of which occurred either during times of low prevalence or in smaller wastewater communities such as universities (Hayes et al. 2021; Hayes, Stoddart, and Gagnon 2022; Li et al. 2022). In the only North American rural community wastewater surveillance study, sampling locations were compared between a pumping station upstream from a wastewater lagoon and a lagoon pool (D'Aoust et al. 2021). At both sampling sites, a 24-hour composite sample, taken every three to seven days for approximately five months, was collected using an autosampler. Pumping station samples had higher levels of SARS-CoV-2, likely due to the higher fecal-associated material present at the pumping station site which had degraded within the lagoon given the high residence time (80 h to 10 days), as well as to low water flow velocity and particle settling from the use of polyaluminum sulfate. Total RNA concentrations were up to five-fold higher at the pumping station, confirming the degradation of biological material for detection. Travel time from the wastewater source and sampling location require consideration of viral decay. In a Houston study, SARS-CoV-2 viral decay was ≥ 50% at wastewater sampling points with a higher number of remote regions (McCall et al. 2022). Depending on the location of the lagoon and the geographical area being served, upstream sampling may be needed.

For rural communities with lagoon wastewater treatment, such as Valentine, samples collected from the last pumping station within a series could represent the influent collection point of an urban wastewater treatment. Pumping station viral load data in the rural community study (D'Aoust et al. 2021) showed similar trends to the clinical positivity rate, indicating that the lagoon sampling location would not be representative of community trends. In rural communities where cost and energy supply must be considered, grab samples taken at a biweekly frequency would need to be sufficient. The analyses performed by the National Wastewater Surveillance System (NWSS) used a 15-day surveillance window for trend reporting (CDC 2023). There is emerging evidence that grab samples, depending on the context and sampling targets, are comparable to composite samples collected over 24 hrs using an autosampler (George et al. 2022). Unlike grab sampling methods, autosamplers, while ubiquitous at urban plants, are costly and it would be difficult to scale up sampling if many autosamplers were required to maintain a surveillance program in a rural setting.

Extraction Methods. The choice of concentration and extraction method also warrants consideration in the context of rural settings, as many of these methods are pathogen-specific and require equipment and technical prowess which may not be present in a rural, environmental laboratory. Detection of SARS-CoV-2 in suspended solids is likely to be more consistent than detection in the liquid phase (Palmer et al. 2021). In comparisons of extraction methods from raw wastewater, electronegative filtration (HA filtration) with bead beating was determined to be the best approach based on consistent results above the limit of quantitation (LoQ), and was the most sensitive in terms of C_t (cycle threshold) value with a strong correlation to clinical data (Ahmed et al. 2020; LaTurner et al. 2021; Sharkey et al. 2021). Direct extraction (centrifugation of a sample followed by RNA extraction from supernatant) was the cheapest method in terms of startup costs and consumables, and even provided the highest concentrations of SARS-CoV-2 based on genome copies per L of wastewater. However, direct extraction was less likely to have a positive relationship with N1 and N2 gene copy numbers (LaTurner et al. 2021). The structural form of SARS-CoV-2 and a surrogate control requires investigation to understand how the concentration method affects recovery (LaTurner et al. 2021; Palmer et al. 2021). The methods for SARS-CoV-2 recovery may not work for all pathogens and different kits would be required for other viruses and pathogens such as bacteria and parasites. There is no method currently that works for all agents and some commercial kits are very costly and require additional equipment. The WBE studies in Texas have also relied on automated methods which would not be present in a typical environmental testing lab. In summary, we believe that extraction methods are critical when designing monitoring methods and require extensive resources. This step might need high expertise involvement to define a clear prioritization for targeting agents of public health concern.

Detection and Quantification. To be able to rely on the results obtained one would need to include controls. Recovery controls can be used in two ways: to evaluate the entire processing of a sample (process control) and to confirm the presence of fecal matter (fecal indicator). Both have the potential to be used as a recovery factor for normalization of quantifiable data, critical given the range in population densities among rural communities. Pepper mild mottle virus (PMMoV) has been used as a human fecal indicator and as an internal control for normalizing SARS-CoV-2 detection between sampling events (Rosario et al. 2009; Kitamura et al. 2021). When compared to bovine coronavirus (BCoV) as a process control, PMMoV detection was more variable, yet had higher recoveries (LaTurner et al. 2021). In the rural community study by D'Aoust et al. (2021), PMMoV was used for the recovery of SARS-CoV-2 in wastewater and showed trends like that of clinical data. As a possible limitation of the study, however, the clinical data obtained was for a larger geographic region that potentially did not represent the regions being served by the rural wastewater lagoon treatment location (D'Aoust et al. 2021). Other markers such as CrAssphage and HF183 can be used as indicators for the presence of human fecal matter (Ahmed, Masters, and Toze 2012; Wilder et al. 2021; Sabar, Honda, and

Haramoto 2022). While BCoV would be suitable for normalization, it would need to be reexamined for rural communities where bovine fecal matter could be a potential contaminant (LaTurner et al. 2021). Human coronavirus 229E (HCoV 229E) spiked into samples was used as another effective surrogate for monitoring infections within college campus residences at the University of Arizona (Betancourt et al. 2021). An alternative to genetic data for normalization was used in South India; quantification of caffeine levels in influent samples had greater than a 75% concurrence with N1 and N2 gene copies (Chakraborty et al. 2021).

Another consideration is the use of quantitative or droplet digital PCR (ddPCR), with ddPCR rapidly becoming the gold standard owing to its advantages in dealing with PCR inhibitors and direct quantification capacity (Al-Duroobi et al. 2021; Ciesielski et al. 2021; LaTurner et al. 2021; McCall et al. 2022; Hopkins et al. 2023; Jarvie et al. 2023). Droplet digital PCR allows for absolute nucleic acid quantification, with higher sensitivity and specificity than other PCR methods (Hindson et al. 2013; Kojabad et al. 2021). The target analyte molecule, DNA/RNA, is encapsulated into nanoliter-sized droplets that serve as a reaction chamber for amplification. Ciesielski et al. (2021) compared SARS-CoV-2 levels in influent wastewater detected by RT-ddPCR and RT-qPCR and found that RT-ddPCR signals were detected earlier during the study, likely when viral loads were lower. The assay limit of quantification (ALOD) for RT-qPCR was greater (60 copies/reaction) than RT-ddPCR (0.25 copies/reaction) using the N2 gene of the SARS-CoV-2 virus. Although RT-ddPCR is more sensitive, it may be difficult to differentiate background levels at low concentrations of viral RNA (Park et al. 2021). Ahmed et al. (2022) suggested that RT-qPCR should be used for wastewater samples because of the subjectivity in differentiating between a positive and negative signal with RT-ddPCR.

In the D'Aoust et al. (2021) study, samples were concentrated using settling at 4°C for one hour followed by centrifugation for RNA extraction from pelleted solids (Qiagen RNeasy PowerMicrobiome kit). SARS-CoV-2 viral signals were quantified using the primers for the N1 and N2 regions of the gene and singleplex one-step RT-qPCR, followed by normalization using PMMoV detection. Based on internal control (vesicular stomatitis virus, VSV), extraction recovery was between 3 and 4.5%. As mentioned above, the quantification of SARS-CoV-2 showed similar trends to the community data; however, the epidemiological data was only available for the larger geographic region (population of approximately 200,000) and not the regional community (population of approximately 4,000) that represented only 2% of the obtained clinical data. The availability of localized clinical data may also present a challenge to establishing a rural WBE scheme.

Case Study–Application of WBE in Rural Communities

We have recommendations that are based on the characteristics of our two cities (Table 4), derived from our consideration of rural communities and the unique challenges associated with rural WBE strategies because of diversity in size, wastewater characteristics, and treatment method selection. The major limiting factor in the deployment of any WBE strategy is the cost.

Case Study Valentine. While establishing WBE can be challenging, the case study of Valentine provides support for the need to adopt WBE within rural communities. There appears to be no SARS-CoV-2 case data readily available for Valentine, and so county-level data must be used. Reviewing the epidemiological data (as of April 17, 2023) for Jeff Davis County, there was a reported total of 278 cases with 10 deaths (Huang et al. 2021). There are a few challenges to consider with data collection and reporting for a city the size of Valentine. First, this data represents the entire county, and not necessarily the city of Valentine. Valentine is not the county seat of Jeff Davis County–it is Fort Davis, a city slightly larger than Valentine and so one could infer that more of the cases reported for the county could be from citizens in Fort Davis. Further support for this inference can be seen in the second challenge– the lack of testing centers relatively close to Valentine. Currently, the closest testing center to Valentine is in Alpine, TX, a 60-minute drive away. This testing center could also be used by residents from Fort Davis, which is only 30 minutes away from Fort Davis residents. In addition, Fort Davis had at least one instance of testing being done in the community in June of 2020.

Table 4. Recommendations for considering a WBE approach in a rural community such as Valentine or Kerrville.

Factor	Valentine (pop size=133 in 2019)	Kerrville (pop size=24,477 in 2021)
Sample Collection	Grab sampling might be recommended for this city because the number of staff might not be adequate to complete more than just one task at a time.	Any sampling method might be applicable to this city if strategically planned appropriately.
Sample Location	Collection within the sewer network or after the bar screen.	Collection within the sewer network, at the headworks, or before the aerobic/anoxic tank.
Sample Frequency	Recommend using a similar schedule as employed for BOD5 collection.	Twice per week.
Sampling Processing and Assessment	Consider partnering with local university partners in El Paso or an environmental lab.	Consider an in-house method such as purchasing a turnkey device that detects the pollutant.

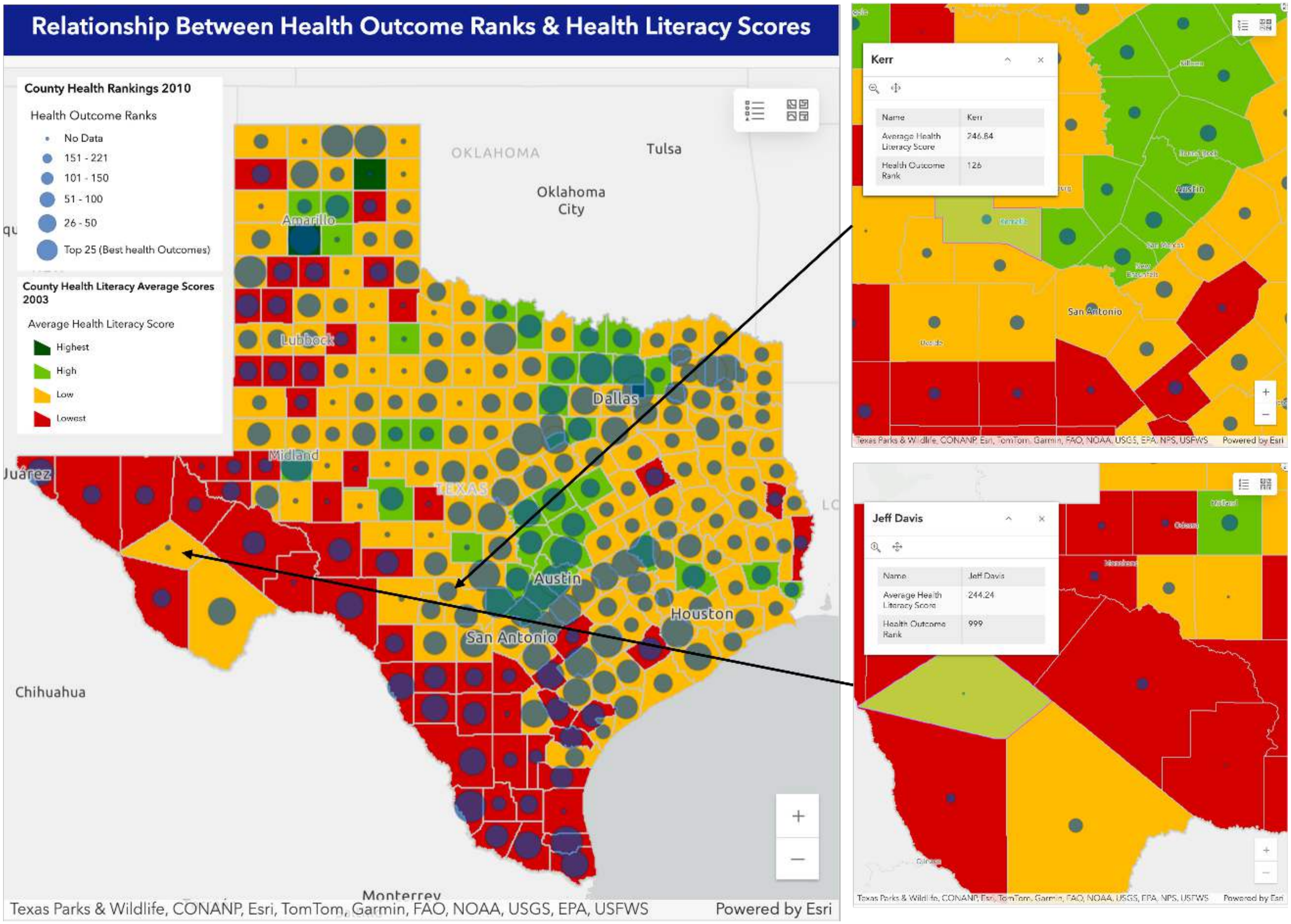
Note: Our recommendations consider technical features, however, social factors should also be considered, such as building capacity, communication with, and characteristics of the community.

Case Study Kerrville. If we consider the sampling processing and assessment for the city of Kerrville, using in-house methods would require the city to procure the necessary equipment to detect the agent of concern. Equipment costs can vary based on the precision and complexity of the instrument, along with the need to continue to invest capital for consumables, maintenance and training, and management of personnel. If enough personnel were trained and capable of using the equipment, the cost per sample would be less than outsourcing the work to a commercial lab, which can be beyond the budget of a rural city. The cost per sample analyzed, shipping costs, and sample and shipping preparation costs to an outsourced lab could be prohibitive. The locations of our rural cities also suggest the need for collaboration with a local academic unit or college to support WBE development. This would require funding support and the development of training programs to be implemented at universities and community colleges in or near rural regions of Texas.

HO and HL Data. The HO and HL for Kerr (home of Kerrville) and Jeff Davis (home of Valentine) counties are shown in Figure 6.

Both rural cities demonstrated low HL scores compared with their urban counterparts. Kerr had a HL score of 246.84 compared with that of Travis, home of Austin (248.41) and Bexar, home to San Antonio (237.72), while Jeff Davis had a score of 244.24, compared with that of Midland (247.23). The range of reported values is from 177 to 280, with higher numbers indicating a higher level of HL. When HO was included (ranging from 1 to 221, with a value of 999 indicating the county was unranked), the low HO for Jeff Davis (HO:999) and Kerr (HO:126) compared with Travis (HO:7), Bexar (HO:78), and Midland (HO:18) gave further support for the establishment of WBE in these rural communities. Certainly, with these challenges for Valentine, WBE monitoring would be an appropriate tool to complement the health center testing data and give the city officials of Valentine a more localized profile to track the spread of the virus within their community.

Regardless of the method selected, it is our opinion that the value of information derived from the analysis should drive decision-making. In communities with poor HO and low HL, this type of population-level screening could make a difference



Kerr

Name	Kerr
Average Health Literacy Score	246.84
Health Outcome Rank	126

Jeff Davis

Name	Jeff Davis
Average Health Literacy Score	244.24
Health Outcome Rank	999

Figure 6. Health literacy and health outcome scores for Texas with panels showing the scores for Kerr and Jeff Davis. The colors indicate health literacy, with dark green having the highest score and red having the lowest score based on quartile scores for health literacy. The size of the circles within each county indicates the health outcome score; the larger the circle the higher the score. Stars show the location of Kerr and Jeff Davis on the map.

in morbidity and mortality in the event of a public health emergency. Several reports and studies have recommended WBE for rural communities and made suggestions as to implementation (Shrestha et al. 2021; National Academies of Sciences, Engineering, and Medicine 2023). Importantly, the results must be useful enough to make informed decisions by providing valuable and timely information where clinical sampling is lacking or access to sampling is unavailable to the appropriate decision-makers at the utility, local government, and state and federal levels. A reliance on data from urban centers would likely miss emerging agents of concern in regions that lack clinical surveillance.

Challenges for Implementing WBE in Other Rural Systems and Implications for Stakeholders

Our goal was to understand the potential for rural communities to employ WBE for measuring agents of concern such as outbreaks of infectious diseases (Gruchlik, Linge, and Joll 2018). There are many practical considerations when developing a contextualized WBE strategy beyond socioeconomic and wastewater infrastructure concerns, including the sampling collection method, sample processing and assessment, sampling location, and sampling frequency (Figure 7).

Wastewater Surveillance in Septic Systems. An important consideration for wastewater surveillance within rural communities is implementation within septic systems, also known as on-site sewage facilities (OSSFs) or decentralized systems. According to estimates from the American Society of Civil Engineers (ASCE), approximately 20% of U.S. citizens, including 60% of rural residents (Maxcy-Brown et al. 2021), have their wastewater treated by means of OSSF (Texas Water Resources Institute 2024). In many places worldwide, decentralized treatment is still a primary method of processing municipal wastewater (Shrestha et al. 2021; Gonçalves et al. 2022). With Texans comprising 5.8 million of those residents on OSSF (Texas Water Resources Institute 2024), it is important to consider strategies that will enable surveillance to be easily implemented within communities that employ OSSFs.

There are several important questions that must be answered when thinking about the implementation of wastewater surveillance within these communities. This work will address two fundamental questions. First, can the samples collected from OSSFs best represent the population within a community, given that sampling will most likely occur in individual households? Septic systems have hydraulic and pollutant characteristics that are different from a municipal WWTP (Iwamoto et al. 2022). For example, it is suggested that ideal retention time for solids within a septic tank can range between 12 and 24 hours (Nnaji and Agunwamba 2012), while at municipal plants that time is a few days (Li et al. 2023). This can result in two different outcomes. On one hand, the concentration of viruses within an OSSF waste stream could be higher than at a municipal plant. Since the operation of an OSSF is different from a municipal treatment plant, viruses are not always removed as efficiently as in centralized facilities. This results in viruses being concentrated within the waste stream not only because the treatment methods employed are not designed to remove these agents of concern, but also because there are no dilution effects or pathways for viral reduction, as are present in municipal waste streams. Wastewater in an OSSF is not being transferred long distances from the wastewater source (i.e., homes, apartments, schools) through a distribution network, as it would be in a municipal system. This pathway presents the opportunity for the concentration of these agents to be reduced by the time the wastewater reaches the plant, and temperatures during hauling might increase viral decay (Gwenzi 2022; Li et al. 2023).

Another outcome is that the concentration of viruses within solids from OSSFs might be different from the concentration of viruses in municipal systems. This happens because the solids in a septic tank stratify from shallow to deep. Amongst this stratification, the virus attached to the solids typically will concentrate within the deepest layers of the tank, which Li et al. (2023) surmise might result in a possible increase in viral decay, depending on holding time of the sludge. Factors such as viral decay, accessibility to household tanks, temperature, and sampling depth within a tank could also result in a misrepresentation of viral load in samples collected (Aslan et al. 2020; Li et al. 2023). Also, unlike a municipal treatment

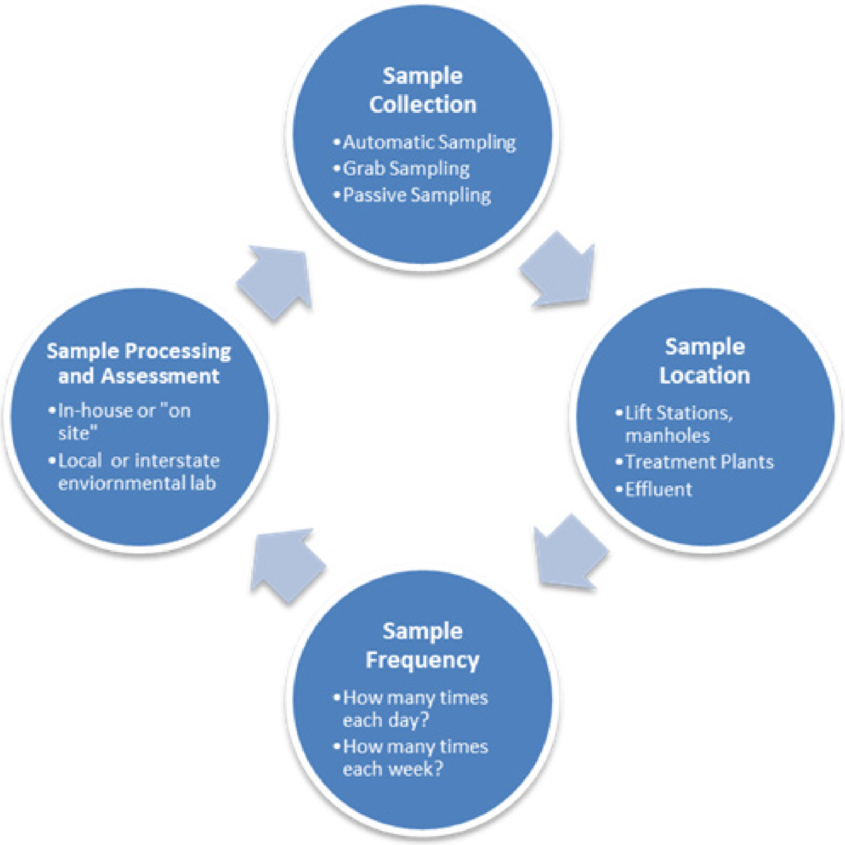


Figure 7. A diagram of the main factors that must be considered when instituting a WBE program. Each factor includes bullet points that outline key points and/or questions that provide context on how each factor relates to the establishment of WBE within a city.

facility where the wastewater amalgamates together giving some representation of the individuals in the community (Minnesota Pollution Control Agency 2024), OSSF wastewater is not always collated. In those scenarios, samples will need to be done at individual homes in order to obtain a community profile. This poses not only a time and financial constraint, but also raises potential ethical challenges as well (Shrestha et al. 2021). In cases such as these, it would be more feasible to sample pumped, hauled sewage rather than individual septic tanks (Li et al. 2023).

A second important question to address is where and with what methods do we sample? Currently, there have only been a few studies that have considered surveillance within OSSFs systems, and so guidance on this question is limited. Examples of studies published include the assessment of communities in Bangladesh (Amin et al. 2020; 2023), Japan (Iwamoto et al. 2022), Saudi Arabia (Hong et al. 2021), and China (Zhang et al. 2020; Dong et al. 2022). However, five of those six studies were assessing wastewater from hospitals (Zhang et al. 2020; Hong et al. 2021; Dong et al. 2022; Iwamoto et al. 2022; Amin et al. 2023), with three of those five facilities being temporary quarantine facilities for COVID-19 patients (Zhang et al. 2020; Hong et al. 2021; Iwamoto et al. 2022). A recently published study on wastewater surveillance in OSSF facilities evaluating wastewater from public beach restrooms in Malibu, CA (Li et al. 2023) was the only study found highlighting a United States study location. Please note that this study was not evaluating OSSFs in U.S. rural communities. However, this work does enable us to see that common public spaces (i.e., schools, community centers, churches) in communities employing OSSFs might provide a better way to sample wastewater within a community using septic systems, while at the same time resolve some of the logistical and ethical concerns of sampling at individual homes.

In closing, the lack of studies within U.S. rural communities at OSSFs presents an opportunity for future researchers to address the unknowns currently missing in literature. While there are other prevailing questions that must be addressed, the questions addressed in this study highlight and provide initial discussion topics on what should be considered.

Conclusions

It is a challenge to define what constitutes rural wastewater infrastructure in the United States and this has major implications when considering the feasibility (in terms of funding and available resources) of a public health strategy such as WBE. WBE has been shown to have utility for the detection of a wide variety of agents of public health concern, but an understanding of the challenges faced by rural communities is essential when attempting to design a feasible strategy for implementation.

Data Availability

All data analyzed in this paper are publicly available with data sources provided in the reference list.

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Ethical Approval

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Competing Interests

The authors declare no competing interests.

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Supporting Information

Rural Definition

The question, "What is rural?" for the purposes of demography is not a simple one. There are several ways to reach the definition, and they may all yield different results. Briefly, the most common ways are (1) communities with less than a threshold maximum population, (2) residing in a county which has a population density less than a maximum threshold, (3) residing in any place which is outside of a U.S. Census Metropolitan Statistical Area (MSA), (4) examining levels of isolation or distance from more heavily populated areas, or (5) the presence or absence of significant agricultural activities. As stressed by one USDA-ERS article, there is not a consistent definition of rural, and this may be appropriate. It is not that "rural" is meant to be a subjective concept. Rather, it is that there is generally a greater purpose (certainly from a planning or governmental perspective) in calling an area rural, urban, or peri-urban. The definition of rural should fit this purpose (Sanders and Cromartie 2024).

A short examination of other studies having many varied purposes illustrates the point of having a workable definition for rural, as opposed to a universal definition. We outline five studies that have strong emphasis on how to define rurality over the last 25 years in Table 1. A few common themes emerge. First, there is the need to relate rurality to the lived experiences of those who are being studied. Despite many demographic metrics that could be used, it is important to examine the finding from these metrics according to both the lived experience of residents and inquiring how they themselves define rurality (Berry et al. 2000; Krutsinger et al. 2024). Second, geospatial metrics of rurality would do well to consider at least two major types of data. The two most common are population density and distances to services, but most researchers acknowledge that more metrics could be added to these to improve the rural definition (Nelson and Nguyen 2023; Krutsinger et al. 2024). Third, it may sometimes be inaccurate to speak of rural and urban as a dualism or dichotomy. In these studies, there are instances where those in areas that were significantly labeled as urban core self-identified as rural. In other cases, the definition of rural versus urban is fuzzy (Bennett et al. 2019; Johnson and Scala 2022). It may not be as simple as either rural or urban since there is a continuum between some extremes. Leaning into the work of Bennett et al. (2019), we are being careful to define precisely what our definition of rural is, depending on the analysis we conduct and with some justification why that analysis is appropriate in each case of its use (Johnson and Scala 2022).

There are two definitions of rural that we use in our study.

Method 1 - Community Population Size

Given that it is our aim to examine the landscape of rural wastewater-based epidemiology potential, it seemed appropriate to think of rurality in terms of places that were smaller in size and fully incorporated with centralized water utilities. This is predominantly a size threshold approach. While there are certainly places outside of incorporated cities that are rural, these places are not very likely to have centralized sewerage. We selected a minimum population threshold of < 5,000 residents for this definition, based primarily on practical concerns. While there are communities larger than this which might be considered rural by some definitions, these locations are frequently more suburban and have a greater tax base and workforce to use WBE. This is the definition that we used to determine inclusion for rural communities on all statewide GIS analyses.

Method 2 – County Population and Presence of Metropolitan Area

Another method for rurality is to look at the population density of a county. A definition given by USDA-ERS is that a county should have rural towns < 5,000 people with urban areas with populations as high as 50,000 people, and not otherwise holding any metropolitan areas (Sanders and Cromartie 2024). This definition is admittedly fuzzier since it has room for towns which are

Table 1. Studies involving critical examination on the definition and conceptualization of rurality.

Study	Purpose	Method(s) for Identifying Rurality
Berry et al. 2000	Classifications of counties in the Western U.S.	Attempted to find U.S. Census metrics to describe rural according to interviews with county commissioners. Found three criteria that most fit with qualitative data-(1) population density, (2) population, and (3) agricultural land base.
Bennett et al. 2019	Rural health and creating more certainty in the definition of rural in general	Definitions of rurality are highly variable, and many reasonable definitions are possible. Thus, researchers should "include the specific definition and clearly define how rurality is operationalized in their work." Also, they encourage reporting rurality down to the smallest possible unit and to note any limitations in whatever definition is chosen in a given study.
Johnson and Scala 2022	Evaluation of U.S. political landscape by culture and geography	Examining political ideology, they find that rural and urban are two poles of extremes. Much of the U.S. is in a continuum between the dense urban core and the isolated community. They emphasize finding degrees of rural along the continuum.
Nelson and Nguyen 2023	Concerns about the inequities and disadvantages of those who are rural	Created a single metric, Community Assets and Relative Rurality (CARR), which evaluates rurality according to traditional population measures (remoteness, population density) and ease of access to services and amenities (geographic metrics of access and availability).
Krutsinger et al. 2024	Rural health and access to healthcare	Examined the viability of the Rural-Urban Commuting Area (RUCA) according to alignment with self-identification of people saying they reside in a rural or urban area. The lack of alignment between RUCA and self-perceptions points to a need to use more "patient-centered" definitions of rural in healthcare.

called "rural" and areas that are urban but non-metro. It is based on this definition that we selected case study communities for detailed examination into wastewater treatment process units and conveyances. More detail on more specific rural criteria for these communities is found in the presentation of the results.

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