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Research article

Fate-and-transport modeling of SARS-CoV-2 for rural wastewater-based epidemiology application benefit

Gabrielle Bognich [a], Nathan Howell [b], Erick Butler [b,*]

[a] Holland School of Sciences and Mathematics, Hardin-Simmons University, Abilene, TX, USA
[b] College of Engineering, West Texas A&M University, Canyon, TX, USA

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ABSTRACT

Wastewater-based epidemiology (WBE) for the detection of agents of concern such as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has been prevalent in literature since 2020. The majority of reported research focuses on large urban centers with few references to rural communities. In this research the EPA-Storm Water Management Model (EPA-SWMM) software was used to describe a small sewershed and identify the effects of temperature, temperature-affected decay rate, flow rate, flush time, fecal shedding rate, and historical infection rates during the spread of the Omicron variant of the SARS-CoV-2 virus within the sewershed. Due to the sewershed's relative isolation from the rest of the city, its wastewater quality behavior is similar to a rural sewershed. The model was used to assess city wastewater sampling campaigns to best appropriate field and or lab equipment when sampling wastewater. An important aspect of the assessment was the comparison of SARS-CoV-2 quantification methods with specifically between a traditional microbiological lab (practical quantitation limit, PQL, 1 GC/mL) versus what can be known from a field method (PQL 10 GC/mL). Understanding these monitoring choices will help rural communities make decisions on how to best implement the collection and testing for WBE agents of concern. An important outcome of this work is the knowledge that it is possible to simulate a WBE agent of concern with reasonable precision, if uncertainties are incorporated into model sensitivity. These ideas could form the basis for future mixed monitoring-modeling studies that will enhance its application and therefore adoption of WBE techniques in communities of many sizes and financial means.

1. Introduction

Wastewater-based epidemiology (WBE) is the strategy of monitoring wastewater for the presence of viruses such as the recent severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the virus that causes the COVID-19 disease. The purpose behind this strategy is to provide a way to track or document community spread [1]. Because the SARS-CoV-2 virus is detectable within the feces and urine of individuals, it has the potential of augmenting or supplementing clinical data, even if the nasopharyngeal swab test is negative for the virus [1–4]. Supplementation of clinical data is advantageous because it includes asymptomatic and presymptomatic individuals which can aid in preventing underrepresentation of all individuals infected with the virus in a community [5,6].

Urban rural communities, as defined by the United States Department of Agricultural Economic Research Service (USDA ERA) of

* Corresponding author.
E-mail address: ebutler@wtamu.edu (E. Butler).

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having a population range between 2500 and 49,999 [7,8], had several challenges during the peak of the COVID-19 pandemic. For instance, through the surveying of adults in the rural American West, Mueller et al. (2021) found that COVID-19 pandemic had negative impacts on areas of rural life such as an increase in unemployment and use of unemployment insurance, impacts that have a higher probability of being more damaging to these communities than their urban counterparts [9]. The current structure in place for rural communities such as the lack of physicians in rural communities [10] underscores the need for a comprehensive way to monitor the spread of a virus such as COVID-19 within a community. WBE can provide the mechanism necessary to support this endeavor.

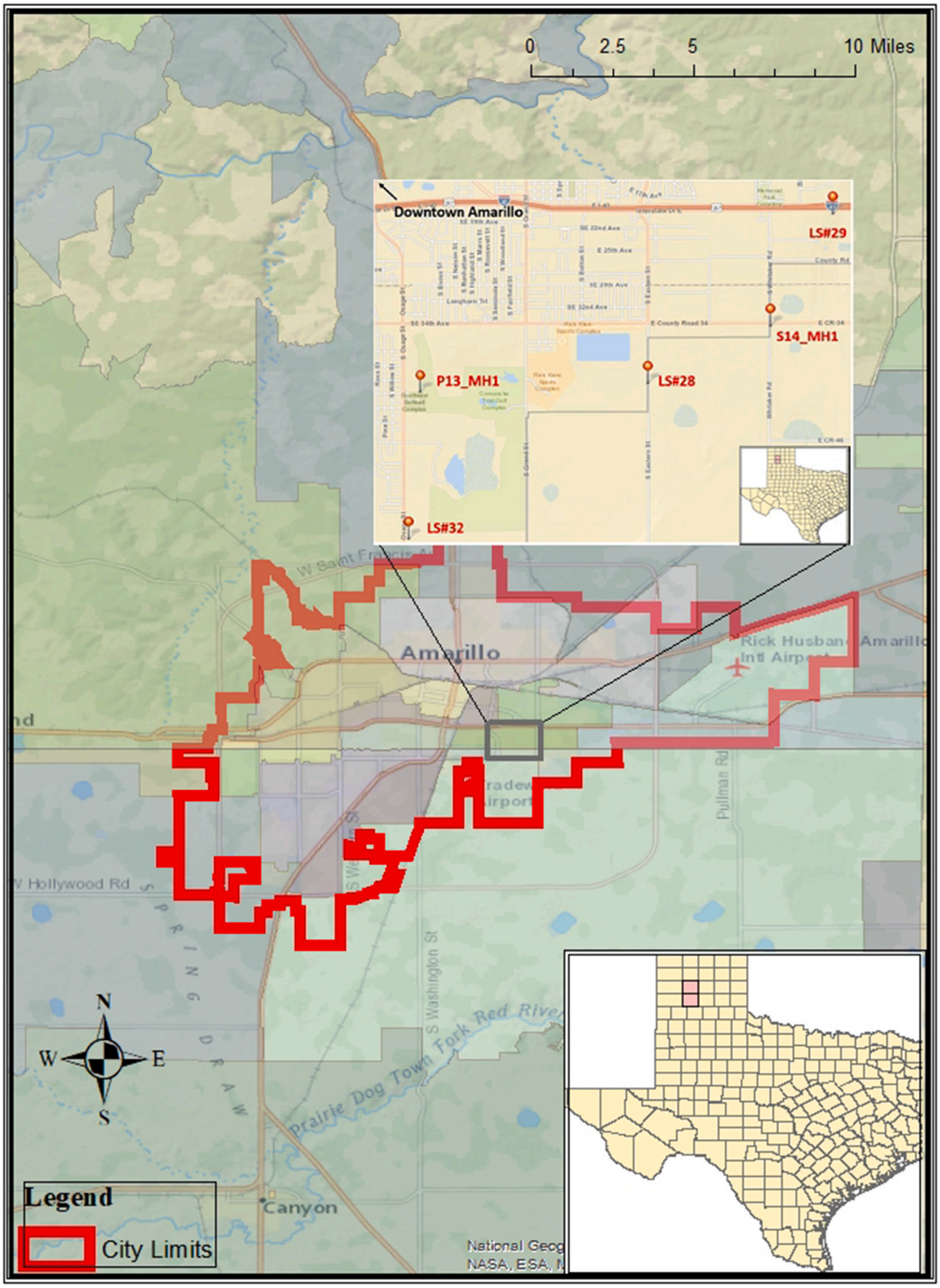

Fig. 1. A map of the City of Amarillo with an insert of the sewershed study area.

In conjunction with the challenges the pandemic posed to rural communities, there is an underrepresentation of rural communities employing WBE as a mitigation strategy. For example, Medina et al. (2022) concluded that 85% of all wastewater treatment plants in California employing WBE were in urban communities, while only 15% were in rural, with none in rural towns [11]. Aslan et al. (2020) observed that the COVID-19 infection rate in rural communities was higher than urban communities by 56% during the heightened part of the pandemic, so it can be beneficial to incorporate WBE as a strategy to benefit these communities [12].

There are several challenges that could create a barrier to entry for WBE use by rural communities [11,13]. In general, rural communities are isolated from environmental laboratory services which can impact their ability to obtain timely results on samples collected [14,15]. There also has been a limited amount of studies that instituted WBE in rural communities [13,15–19]. A summary table has been included in Table S1.

Modeling the COVID-19 virus in rural communities has been completed using various applications and methodologies [20–25]. Studies for other entities such as drugs have included rural communities as a case [26–28]. The objective of this work is to contribute to the modeling efforts for a rural community context. Wastewater operators in rural communities not only need to understand the lowest concentration of constituents that can be detected within a wastewater sample, and how to sample, detect, quantify, and interpret the presence of these constituents in wastewater.

2. Methods

The modeling method undertaken for the study concerns what might be called a case study in a rural context without any direct comparison with measured SARS-CoV-2 concentrations. Many water quality modeling studies involving the use of statistics use those statistics to categorize input data and present simulation outputs in a compact form as well to evaluate model accuracy and trustworthiness using fit statistics. Such statistics commonly used to illustrate the point include root mean square error (RMSE), Nash-Sutcliffe Efficiency (NSE), percent bias, relative error (RE), and mean absolute error (MAE). Data for generation of these types of comparisons is not possible in our context, indeed in many rural and small sewershed contexts, because SARS-CoV-2 data are not available in these places. This is much of the motivation for this study (to encourage more WBE sampling in rural spaces), but it is also a limitation of the modeling approach we present here. This approach could be classified as "make the best modeling assumptions we can and evaluate the findings with care." As the purpose is to aid rural wastewater operators in how WBE would work for them and to inform wise sampling practices (not to validate model accuracy), this approach can be considered justified. Additional details on the modeling approach can be found in the Supplemental Information which includes Table S2.

To develop the model, a small sewershed with a population between 18,000 and 24,000 [29] was used to help represent possible outcomes within more rural communities in America. This sewershed is located in the City of Amarillo, TX, USA (Fig. 1). Amarillo is the largest city in the more rural Texas Panhandle with a population slightly above 200,000 people [30]. Amarillo was chosen as the study location because it would be similar to an urban rural location as defined by the United States Department of Agriculture (USDA). This figure also includes the context of the sanitary sewer transport network (in the SE portion of the city boundary [29]). Other salient details of the study location and associated sewer main are provided in Table S3. Information on the definition of rural communities can be found in the Supplemental Information.

The following base case description provides the initial model conditions and any additional changes that altered the model in subsequent runs. The model conditions selected were those that would result in concentration values of 10^{5-6} copies/mL or 10^{2-3} copies/L, a point in which an individual would be considered infected with the virus. According to Phan et al. (2023), these conditions reflect the situation in which the virus would incubate within an individual for 3 days and infect an individual for 8 days [31]. The following is a summary of the computations employed within the base case. There are three wastewater-related values that will be put into SWMM–viral load, decay rate, and flow rate.

The first value is the computation of an estimated amount of virus or the viral load (in gram copies per second, or GC/s) that is

Table 1
A table describing the morning and evening fraction load variable calculations of Eqs. (1) and (2).

Variable	Description	Unit	Value	Source
n_{pop}	Number of individuals in sewershed population or to the upstream pipe section	people	25,000	Representative population estimates for this portion of the city.
r_p [a]	Daily infection fraction within the sewershed for Randall County, TX	fraction	0.01 to 0.045 (general range)	Randall County was chosen to represent the data across the region. Data from the map was extracted on November 21, 2022 [33].
g_{cap}	Individual fecal generation rate	g feces wet/person	200	Moderate value from Ref. [34].
C_f	The fecal shredding rate (FS) as provided in literature	GC/g feces wet	676,000	Value was selected from Ref. [35].
Δt_{flush}	Time range of the flush event in the equation. Could have multiple in the day.	minutes	120	Presume a 2.0 h typical morning flush time say 8:00 a.m. to 10:00 a.m.; and 4:00 p.m. to 6:00 p.m. This will allow for flush to move through the system. The definition of morning flush comes from Ref. [36].
60	Conversion from minutes to seconds	seconds	–	–
f_{am}, f_{pm}	morning load fraction evening load fraction	fraction	0.75 0.25	In this condition, there is an assumption that 75% of the total daily viral load appears in the morning and 25% of the total viral in the evening.

[a] Data for Harris County was accessed here: [22,37].

present in the sewershed each day. The viral load computation was determined from a 2-h event by which the virus is added to the system by human defecation. This is referred to as a flush event. Viral loads were computed for two separate flush events– one morning flush (8:00 a.m. to 10:00 a.m.) and one evening flush (4:00 p.m. to 6:00 p.m.). Each flush event was assumed to be independent. Two flush events accounts for wastewater sampling at two different time periods were used to capture a profile of the virus within the wastewater throughout the day. The daily morning and evening viral loads were monitored within a time period of twelve to sixteen weeks. A point in the pandemic in which there was an increase of daily infection rates was also chosen, as indicated by the reported SARS-CoV-2 positivity rate. The result is a peak at the highest daily infection rate at any point of the pandemic recorded for the area. This event occurred between December 19, 2021 to March 23, 2022, during the spread of the Omicron variant in Amarillo [32].

To simulate the viral load within the time of a flush event, two determinations were made—(1) a calculation of the total viral load based on fecal generation rates, population, and viral shedding; (2) the apportion of load between morning and evening flush events. In the base case, 75% of the load came in the AM flush event and 25% in the evening event. Equations (1) and (2) were used to compute the morning and evening loads. Complete details of variables in those equations employed can be found in Table 1.

$$morning\ load\left(\frac{GC}{s}\right) = \frac{f_{am} C_f g_{cap} n_{pop} r_p}{(\Delta t_{flush})(60)} \tag{1}$$

$$evening\ \ load\left(\frac{GC}{s}\right) = \frac{f_{pm} C_f g_{cap} n_{pop} r_p}{(\Delta t_{flush})(60)} \tag{2}$$

where f_{am} and f_{pm} = morning and evening load fractions, C_f = fecal shedding rate, g_{cap} = individual fecal generation rate, n_{pop} = population served by the sewershed, r_p = daily infection rate, Δt_{flush} = flush event time.

To take into account the reduction in virus across the sewershed during each flush event, a decay rate was included in the computation. There is not a standard decay rate, k, applied to SARS-CoV-2 across literature. Therefore, different scenarios were simulated with decay rates taken from literature to determine if the unknown decay rate standard significantly impacted the informative nature of sampling within the model. One was selected for the base case and others were selected for different scenarios. For the base case, a temperature-corrected decay rate was calculated from the linear relationship between temperature (°C) and a log-transformed kinetic rate using given recorded temperature and kinetic rates as reported in Ref. [38]. This is represented by Eq. (3) and is shown below:

$$\log_{10} k = 0.0159T - 2.5327 \tag{3}$$

where T = temperature in °C; k = kinetic rate (day^{-1}).

A temperature of 18 °C ± 1 °C was used because this is the general temperature of the wastewater in the sewershed during the time period and was the temperature employed for all decay rates in subsequent scenarios. The decay rate computed for the base case using Eq. (1) was about 0.134 day^{-1}. The decay rates for the subsequent scenarios were determined using linear interpolation from given kinetic rates in literature.

The flow data was received from the most downstream wastewater treatment plant in the southern network of the City of Amarillo. That data was recorded every 2 h on the odd hours of the 24-h day (01:00, 03:00, …) and collected from May 2021 to May 2022. The data was reconditioned to make it more amenable to numerical simulation and eliminate obvious errors in the flow measurements, such as times of lift station down-time and low flow errors. These instances were rare over the annual flow data and therefore were adjusted based on interpolated values for standard flow conditions. Complete details on the process are provided in the Supplemental Information.

From the base case, a series of fifteen model scenarios were completed in which either vary the viral load or the decay rate. These changes were divided s into four categories– manual adjustment to the infection rate (± 5% and ± 10%), the use of infection rates from either a different time period and/or a different location (Randall County, June 29-October 8, 2021; Harris County (Houston), December 6, 2021 to March 18, 2022), three different fecal shedding rates (66,000 GC/g feces, 2,300,000 GC/g feces, and 4.7 × 10^9 GC/g feces) computed by adjusting the virus concentration (GC/L), and a use of four different temperature-corrected decay rates based on experimentation from literature (0.648 day^{-1} [39], 0.044 day^{-1} [40], 0.204 day^{-1} [41] and 3.12 day^{-1} [17]). During the fifteen model scenarios, the base case was maintained for all variables with the exception of the one being changed.

To evaluate the base case and scenarios, thresholds for quantification that someone conducting an actual WBE campaign might experience was examined. The distinction is important because there are instances where a lower SARS-CoV-2 wastewater concentration would be seen by some laboratory methods and not by others. There are two methods of quantification—a traditional laboratory and a field method. Traditional laboratory methods involve wastewater sample collection and viral quantification by a laboratory grade qPCR with reagents. Examples of studies employing this method can be found here [6,13,17,40]. On the other hand, a field method enables a researcher to collect and complete viral quantification in the field through the use of a portable qPCR such as LuminUltra's GeneCount® Q-16 [42]. Studies that provide details on the methods for this device have been summarized here [43–45]. An important distinction between the two methods is the practical quantification limit (PQL). Example PQLs for SARS-CoV-2 for laboratory and field methods are 1 and 10 GC/mL (1,000, 10,000 GC/L), respectively [46].

These two thresholds were used to examine aspects of quantification, interpretation, and community spread awareness when using the two methods in a rural context. The field method can be conducted in house and at relatively low cost by non-experts with the disadvantage of lower sensitivity. The lab method is more expensive, requires the use of outside services, and/or has a larger time lag between sample collection and quantification. However, it has the advantage of being more sensitive by a factor of 10. These simulations will investigate how often the differences in PQL make a practical difference in interpretation and usefulness for a rural community. Please note that quantification of SARS-CoV-2 in the community was not completed.

To better understand the error around the model outputs, additional sensitivity analysis around the wastewater flowing through the sewershed by manually adjusting the flow rates by ± 5% and ± 10%, the fecal shedding rate by ± 5% and ± 10%, and the morning and evening load fractions ($f_{am} = 0.25$, $f_{pm} = 0.75$; $f_{am} = f_{pm} = 0.50$). A total of ten different runs were run in SWMM and each of these variables was individually varied. Table 2 is a summary table of the sensitivity runs completed.

The sewer network model in The EPA SWMM software (SWMM v 5.2; [47]) was developed using the following process outlined in Fig. S1. First, the pipeline network using pipeline (conduit), lift stations (storage units with pumps), and nodes/manholes (junctions) were created. Once the pipeline was positioned, the specifications such as pipe diameter, and length to reflect the true sewer network in the city of Amarillo were adjusted. Then, an Excel document was created to document dependent data such as flow rates, SARS-CoV-2 input loads, and pump data. After creating the Excel file, it was converted to a *.dat file (format preferred by SWIMM), and then uploaded it directly into the SWMM software. Based on the model specifications, a project file is created for each scenario and the constraints such as the decay rate are adjusted. This project was run in a SWMM file. The raw data was then collected, sorted, and presented. Additional details on data cleanup can be found in the Supplemental Information (Fig. S2).

Using the established SWMM model developed, an analysis on residence time within the sewershed was also conducted. The following section describes the process by which this was undertaken.

The purpose of the examination of a conservative tracer in the sanitary sewer model is to evaluate important aspects of the sewer system in this case when considered like a reactor. The residence time (RT) within the sewershed was examined by using a conservative tracer. Model simulations are provided in Table S4. Three days were chosen during the simulation period, one for each complete or near complete month during the simulation period. On each of those days, a 26-min triangular pulse of conservative tracer (total mass known exactly) was to be released according to the days and times shown in Table S5.

3. Results

Results from four selected sensitivity runs that adjust flow to analyze the impact of flow variation on the levels of SARS-CoV-2 that can be detected in the sewer network will be discussed below. Further details can be summarized in Figs. S3–S6 and Table S5. Fig. 2 shows the depth of the water in the pipeline from the model. The depth is generally proportional to the percentage of flow change, meaning, a 5% increase in flow will result in a 5% increase from the baseline condition. The remaining sensitivity runs did not have flow varied from the baseline condition and therefore the flow data is the same. When the flow is increased, the overall SARS-CoV-2 (GC/L) concentration is decreased. This is a result of dilution within the system because upstream viral inputs themselves are not changing in examination of flow rate sensitivity.

Based on the overall assessments of the sensitivity runs, the three main factors that impact the quantity of SARS-CoV-2 (GC/L) in the sewer network are increased flow, increased positivity rate or SARS input, and the time of day when the flush times (meaning SARS-CoV-2 fecal discharge events) are increased or decreased in the morning and the evening. Fig. 3 shows the four sensitivity runs that result in larger delineation from the baseline condition. A decrease in the morning and evening fraction loads by 25% and 50% results in a decrease in SARS-CoV-2 concentration by 50% and 25%, respectively (green lines). The dip in concentration concludes that the quantity and time of when the flush times are represented in the model has a significant impact on the viral load output from the model. This is an important factor when considering what time of day sampling should occur. It is possible that flush times will vary from community to community. And so if the region's maximum flush time is at a different time of day than what was shown here, the quantity of SARS-CoV-2 (GC/L) is impacted and the probability of sampling wastewater with a quantifiable viral concentration changes. These flush times were determined by a standard day of when people are leaving for work and when they return. A region, such as a rural farming population, may have different peak flush times and the model should be adjusted accordingly.

In order to establish the capabilities of multiple different scenarios that model real life in the SWMM, fifteen unique runs based on the standard baseline condition were performed. The purpose of these scenarios was to determine how changes in the quantity of SARS-CoV-2 in the sewer network impacts sampling schedules, strategies, and optimal times. The three components that were altered were the fecal shedding rate, the first order decay rate (k, day^{-1}), and the increased SARS-CoV-2 input load based on the infection rates. Additionally, two runs were created to observe the wastewater conditions of a separate location (Harris County, TX) and an

Table 2
A table listing all of the sensitivity runs completed to validate the SWMM model.

Run Number	Description
1	increase the base condition flow rate by 5%
2	increase the base condition flow rate by 10%
3	decrease the base condition flow rate by 5%
4	decrease the base condition flow rate by 10%
5	increase the base condition fecal shedding rate (C_f) by 5%
6	increase the base condition fecal shedding rate (C_f) by 10%
7	decrease the base condition fecal shedding rate (C_f) by 5%
8	decrease the base condition fecal shedding rate (C_f) by 10%
9	daily load fraction distribution changes to 0.25 for the morning load fraction (f_{am}) and 0.75 for the evening load fraction (f_{pm})
10	daily load fraction distribution changes to 0.50 for the morning load fraction (f_{am}) and 0.50 for the evening load fraction (f_{pm})

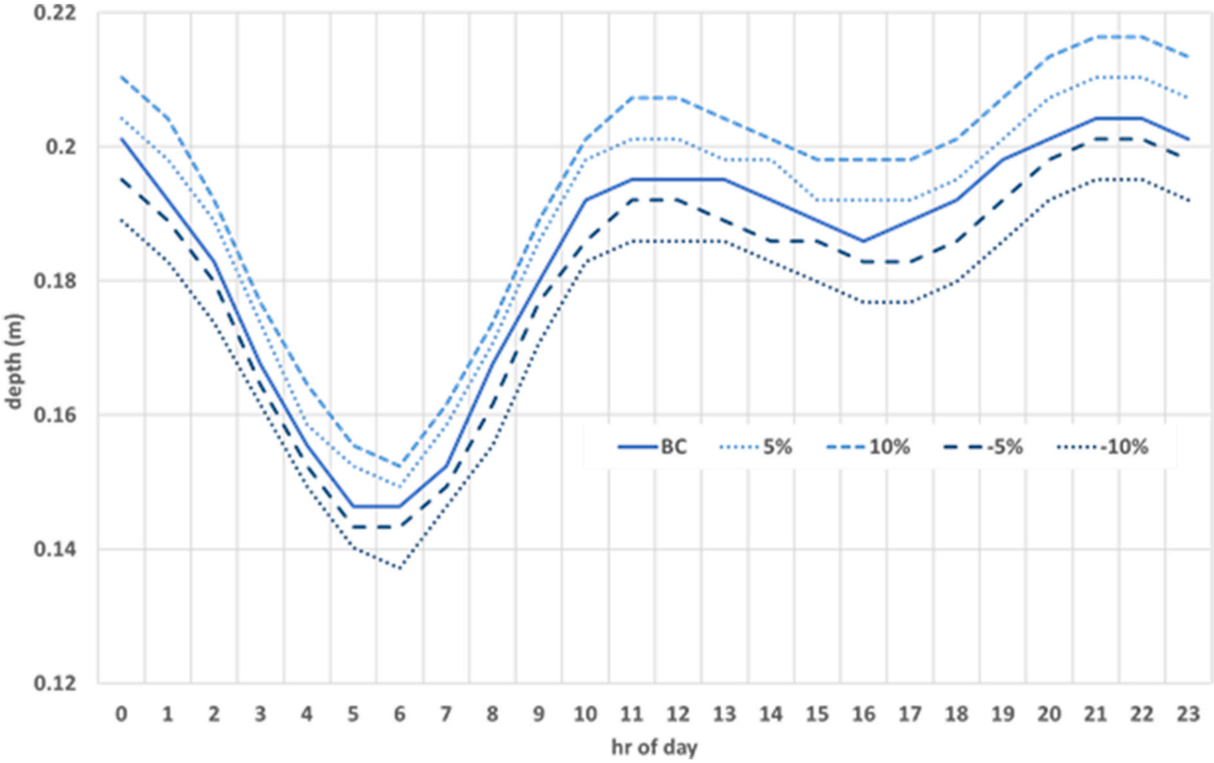

Fig. 2. A graph depicting the temporal change in sewershed water depth under selected model conditions.

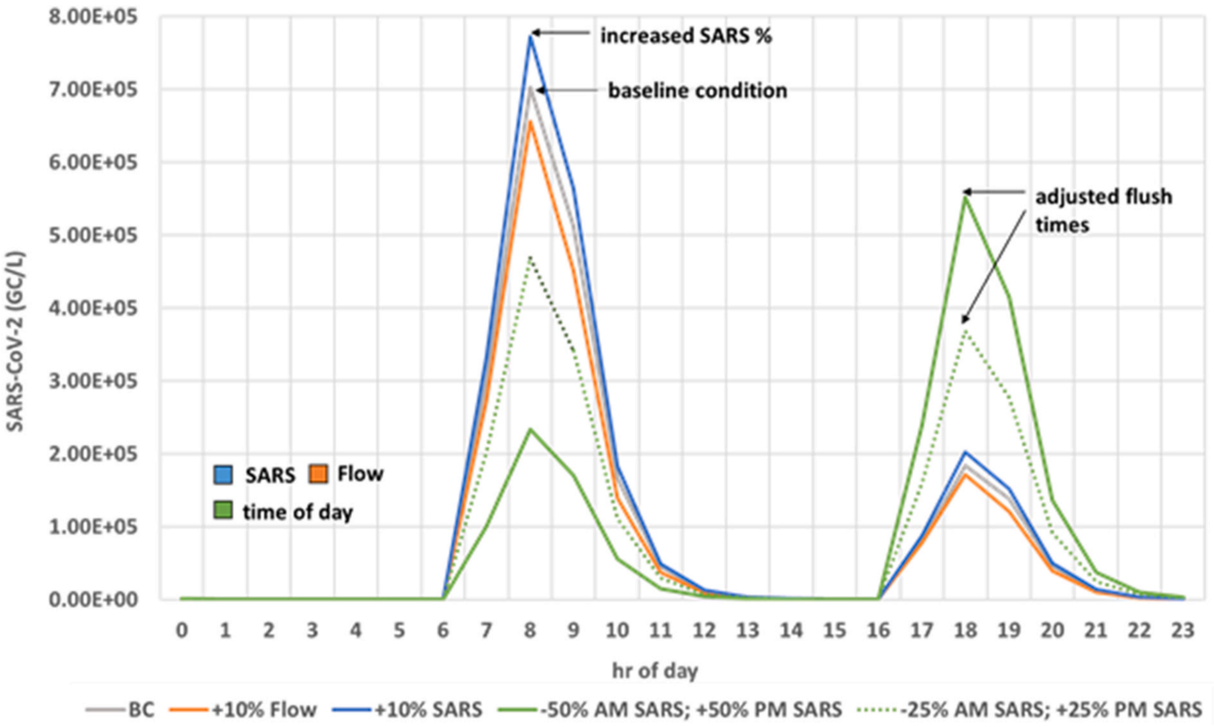

Fig. 3. A graph depicting the temporal change in sewershed SARS-CoV-2 concentration under selected model conditions.

elevated wave of infected individuals. Harris County is 600 mi from the study sewershed. Harris County does have some sewersheds slightly larger than the model sewershed in both population and wastewater flow rate, with a similar infection wave peak as Amarillo during the Omicron wave of early 2022. Using these inputs allowed us to create a model scenario consisting of a fictitious sewershed with mostly similar initial conditions as the initial model sewershed but with a higher population and higher wastewater flow rate.

The three scenarios that had the greatest impact on the SARS-CoV-2 wastewater signal during the sampling times were increased/decreased SARS-CoV-2, a heightened viral decay rate of 3.12 day^{-1}, and a change in the fecal shedding rate of infected individuals. An increase and decrease of SARS-CoV-2 (GC/L) based on the population's positivity rate directly impacts the viral quantity in the model. The baseline condition had a viral decay rate of approximately 0.134 day^{-1}, and so a 2300% increase in the decay constant produced a significant drop in observable viral concentration. These results in Fig. 4 indicate that change in temperatures in the sewer network and variances in the specific decay rate for SARS-CoV-2 have a minimal impact on the quantification of SARS-CoV-2 in the model (ability to detect and quantify as determined by exceedance above the field and lab PQLs shown). The model indicates that the virus is not in the sewer network long enough to degrade before the next day where potentially infected individuals input new virus into the sewer network. Instead, it is washed further downstream from the modeling region. Examples of environmental factors in the sewer network can potentially contribute to viral degradation include biofilm and bacteria ([48]).

The change in the fecal shedding rate of infected individuals also impacts the levels of concentration. The following equation (Eq. (4)) was used to calculate the fecal shedding rate C_f (GC/g wet)—

$$C_f = V_{con} * \left(\frac{1}{g_{cap}}\right) * \left(\frac{1}{n_{pop}}\right) * Q_{ave} * 3.78 \tag{4}$$

where V_{con} (GC/L) is the virus concentration, g_{cap} is the fecal generation rate (g wet/person-day), and Q_{ave}. is the average daily wastewater flow rate within the sewershed. This was computed using daily flow rates from the city of Amarillo (gal/day).

In this application, the fecal shedding rate adjustment reduced the viral load by a factor of 10. A reduction of fecal shedding by a factor of 10 results in a reduction in SARS-CoV-2 concentration in the sewershed by more than a factor of 10. The baseline condition had a fecal shedding rate of 103,170 GC/L. A decrease <10% in the fecal shedding rate greatly impacts the concentration of SARS-CoV-2 and thus impacts how the user can determine when and where to sample. In this model, this study was limited to using fecal shedding rate as a means of virus transport. It is understood that individuals also secrete fluids into the wastewater. However, Crank et al. (2022) concluded that it is more likely that stool than any other fluid contributes to identifying the spread of the virus within a community [49]. Additionally, the morning sampling time aligns with the designated flush times and results in a higher chance of the sampling containing a quantifiable amount of SARS-CoV-2 [48].

Based on the results, the afternoon sampling would not be as successful as the morning sampling times, which means that the model is valuable to help samplers determine which times of the day are best to sample, whether it be a grab sampling method or an

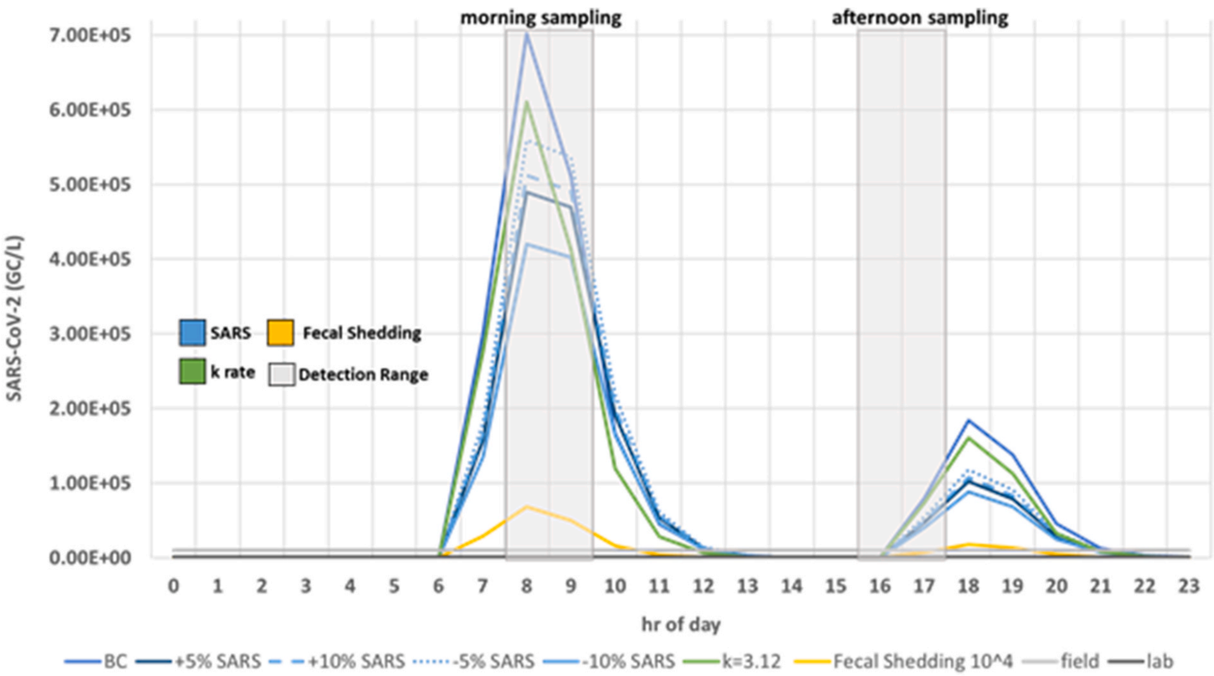

Fig. 4. A graph depicting the temporal change in sewershed SARS-CoV-2 concentration under selected model conditions during potential morning and afternoon sampling campaigns. This graph also highlights the limit of detection (LOD) for the field and lab qPCR instrumentation as a baseline concentration.

autosampler. To the degree that a WBE constituent load apportionment is noticeably higher (like the example of 75% morning and 25% afternoon), others should also find the morning concentrations to be higher and therefore have the best chance of quantification.

General monthly flow variability within the simulation period (Dec 2021–Mar 2022) was evaluated to determine how much and if the wastewater flows through this region of the sewer network is variable on a month-to-month basis and also to examine the diurnal flow pattern. The model confirmed flow variability between the original flow data and flow produced from the residence time distribution. Fig. 5 provides a statistical summary of the difference in flows in each of the four months of the simulation. These are the typical windows when morning or afternoon-evening grab wastewater samples would be collected. The variation generally indicates that flow in December is frequently much lower than in Jan–Mar 2022, and these lower flows are most pronounced in the early evening through early morning (18 6 h overlapping between days). It is also seen that most of the hours in the month of March have significantly wider variation than all of the other months as evidenced by the long whiskers indicating the end range or the outlier threshold at the 1.5 × interquartile region (IQR).

While there is a typical morning dip which hits a minimum at 05:00, the flow does not increase greatly until about 10:00. It then remains elevated. There is a small lull that occurs at 17–19 h relative to the middle of the day (12–13 h) and late evening (21–22 h). These flows are true to the local condition in the sewer near the upstream of the model domain as they have already been lagged by 2 h to account for the travel time between this segment of the sewer and the influent to the local wastewater treatment plant, the point from which these flows are based. Also noteworthy is that the two periods when this city is typically sampled, morning and evening, both exhibit local minimum flows. These minima may serve to concentrate the tracer and any SARS-CoV-2 shedding that occurs at these times making detection easier.

Fig. 6a–c presents an examination of residence time in the sewer network. The figure shows residence time distributions for just one of three days examined (January 10, 2022). Three different nodes in the model domain were chosen, each of which is a manhole in the actual sewer network. These are places where wastewater sampling for SARS-CoV-2 can occur. The release occurred as a tracer concentration which increased from 0 to 1000 mg/L over a period of 13 min (06:00 to 06:13) and then was reduced at the same rate from 1000 mg/L for another 13 min (06:13 to 06:26). Due to the flows which occurred in this 26-min interval, it was determined that the total amount of tracer released was 40.5 kg with an average mass rate during the release period of 1.56 kg/min. Thus, the release profile at this point was a triangular pulse. Using a $\Delta t = 26$ min, tracer mass recovery within ±10% by the end of the model domain was achieved. In this case, the recovery ranged 102–103%. The release of tracer began at 18:00 and lasted until 18:26 with a maximum release concentration of 1000 mg/L occurring at 18:13. The concentration profile was thus the same general shape as the morning (06:00) release with a 12-hr. time shift forward. The total release mass was 66.1 kg, a substantial increase from the morning release due to the higher wastewater flow in the afternoon during this short 26-min interval. The tracer mass recovery was comparable at 100–101%.

To summarize the tracer analysis, there are a few points which are most relevant to the understanding of residence time in the sewer network. First, this sanitary sewer line is relatively isolated from other parts of the city sewer network resulting in a relatively constant volume reactor space in the sewer network made up of lift stations, manhole junctions, and pipe conduits. Second, flow increases the mean velocity in the network in a way which is not offset by increases in depth in the lift stations or in conduits. Since storage volume does not increase appreciably with volumetric flow, the flow rate at the upstream of the network does decrease the travel time. This decrease is seen mostly clearly in the difference between tracers released in the evening versus the morning. In the evening, there is an increase in flow rate which lowers the residence time to most every point in the network by 5–20 min.

Third, as volumetric flow is diminished, RTs increase both at one point in time and throughout a wider range of times. The lowest flow observed on the March 9 tracer release date, a date that also has the greatest standard deviation and skewness. All three days (Jan 10, Feb 11, and Mar 09) have detailed spatial and temporal RT analysis in SI Figs. S5 and S6. The 99th percentile of residence time is

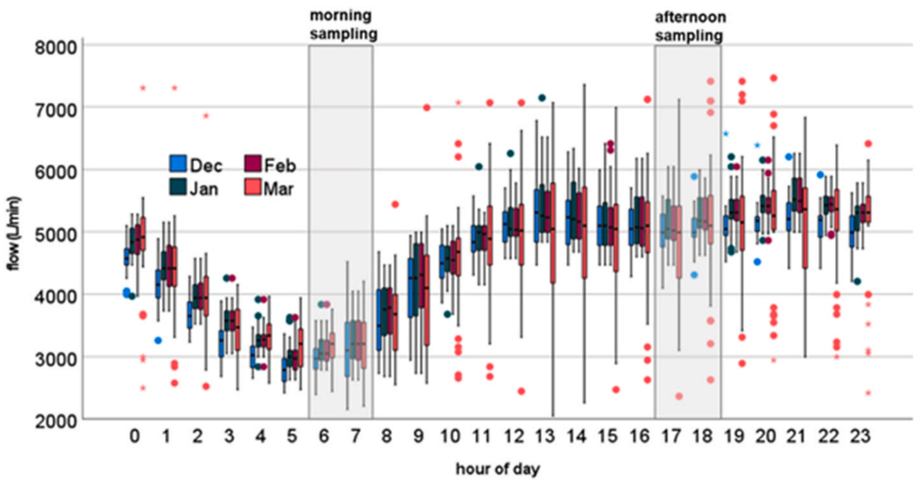

Fig. 5. A box plot depicting temporal change in flow rate throughout the residence time distribution tracer experiments. Flow rates during potential morning and afternoon sampling campaigns have been highlighted for emphasis.

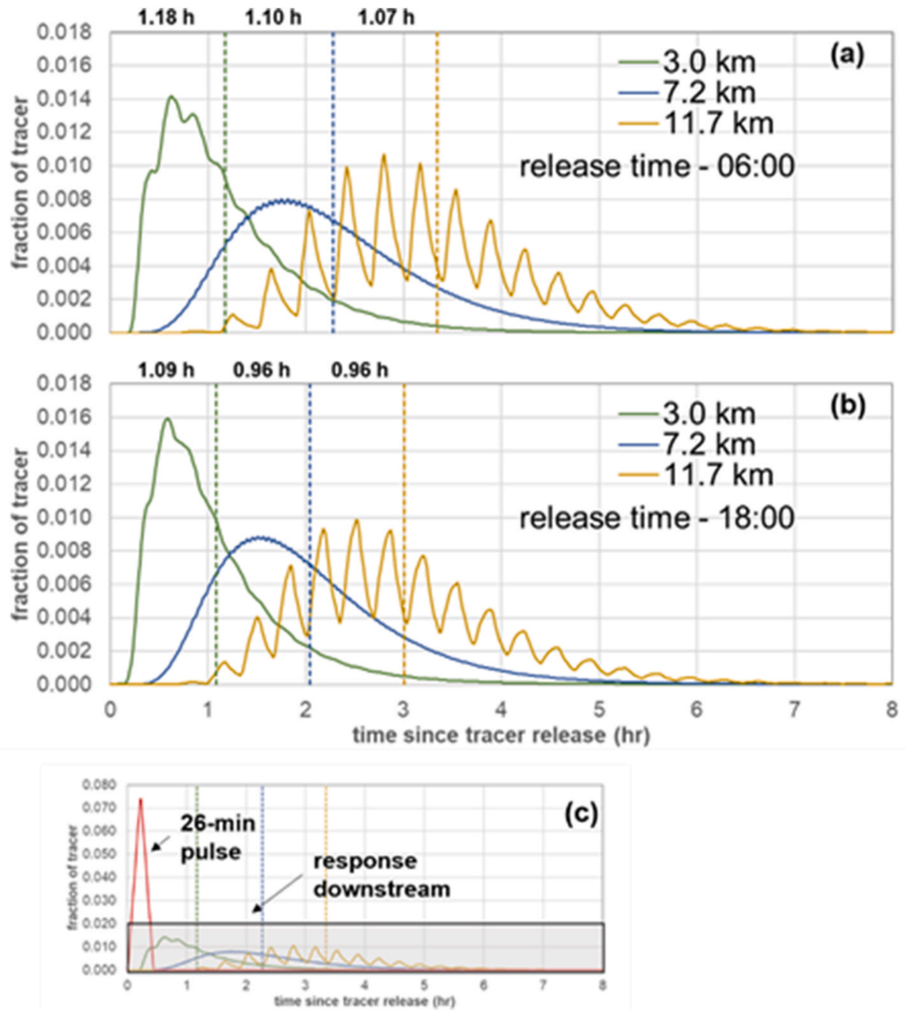

Fig. 6. A graph highlighting temporal changes in tracer presence from initial release throughout the residence time distribution tracer experiments.

7.25 h for a morning release on that day. Thus, the last bit of water which entered the sewer at 06:00 left at 13:25. This is well ahead of the time when an evening fecal shedding event is presumed to happen around 18:00. When a morning fecal event has completed in this section of the network, no water from that event remains to influence an evening event. Thus, the morning and the evening shedding events are completely separate from one another at even the most extreme residence times. The detailed RT analysis also reveals that there is more variation in RT the further from the upstream release point one moved. In practice, this will make catching higher concentrations in a sampling scheme increasingly difficult the further one is from the source area being examined.

4. Discussion

Sensitivity simulations were designed and ran to quantify the degree of uncertainty in the model simulations. Due to evolving environmental conditions in sanitary networks such as human health factors, biological or water conditions, population flux, etc. it is difficult to account for each factor. Additionally, specific factors such as flow, decay and infection rates, and flush trends can be predicted to a reasonable degree but will not perfectly reflect the true sanitary network conditions [50]. The baseline condition represents the input conditions that most accurately reflect the true sanitary network conditions within the designated rural region of Amarillo.

Based on the sensitivity analysis in this modeling case study, the input conditions utilized did not drastically change the prediction model's ability to inform the most effective times and location to sampling in the designated sampling campaign. The simulations concluded that the SARS-CoV-2 concentration was impacted when the input conditions differed from the baseline by at least 20%. Therefore, if there is confidence that the baseline conditions of the model are within a margin of 20%, the model can reasonably predict concentrations despite uncertainty.

The role of viral RNA decay is significant over the span of travel time and associated length in the sewer presented in this modeling case. Although there is a consistent flow rate that decreases travel time in the sewer and a short sewer length of less than 10 miles, there is a 50% drop in SARS-CoV-2 concentration within 2.5–3 h of the peak flush time where the viral concentration is introduced in the model. The viral concentration is still above both the field and lab detection limits; however, the viral load is significantly decreased and impacts the location of where to sample. For example, sampling too far downstream decreases the likelihood of a single time grab sample having quantifiable viral load. If the location and the timing were not flexible to change, a sampler would have greater success using a passive sampling method over a time period of a couple hours.

It is important to quantify the cost of SARS-CoV-2 RNA in a wastewater sample. One can compare the costs between the use of clinical data, a field method such as LuminUltra's GeneCount® Q-16 for measuring RNA in wastewater, and a professional commercial lab using traditional RT-qPCR to measure RNA in wastewater. A quick calculation can be made with the following assumptions for each method–clinical: assume $15.00/test [20] with 705,199 total tests completed over three years (Amarillo); field: assume $16,000 instrument cost, 80 tests per week at four locations in the sewershed, twice a day for five days, testing cost of $45.00/sample [51]; or commercial lab: similar test frequency as field, but $400.00/test (cost of test + shipping). If one were to conduct the field method and the commercial lab testing for three years to match the timeline of clinical testing, it can be determined that the annual costs to execute each of these programs in this scenario would be approximately $3.6 million USD for clinical testing, $200,000 USD for the field method, and about $1.7 million USD when using the professional lab. At first glance, costs greatly favor a community to strongly consider a turnkey device. However, one must consider the limit of detection when making such a decision. Daughton (2020) presents three possible options for detection. They are (1) affirming the presence of virus (presence/absence tests), (2) limited quantification (cannot detect during times of low prevalence), and (3) a higher level of certainty in quantification (can detect during times of low and high prevalence) [50]. Selection of the method employed by any community should consider not just the financial cost for the program, but also the value of the method in order to provide support in making decisions to help prevent spread within a community. The work from this study can help a community decide between a field or laboratory method by simply adjusting the SARS-CoV-2 concentrations to lower infection rates, reproduce outputs such as Fig. 4, and compare the likelihood of detection between two methods.

The reality of low infection rates is a significant potential problem for the use of WBE for any disease-related analyte of concern, SARS-CoV-2 or otherwise as has been observed in other studies previously [52–56]. The value of WBE for a particular situation depends on the purpose behind its use. If the purpose of its use is to be able to identify only when a public health problem is happening, then the general requirement in wastewater surveillance is that the combined sampling scheme and water quantification technique can tell with high confidence if a concentration threshold corresponding to a certain disease spread in a community is occurring. For example, if a positivity rate in a community of 3% is considered to be critical and the correlated wastewater concentration to 3% is 12, 000 GC/L, then the lower sensitivity field method described would likely be sufficient to warn a community that there was a public health event occurring. However, if the particulars of the sewershed (for example, greater dilution by commercial wastewater sources, higher wastewater temperature increasing the decay rate) made it such that the threshold warning concentration in wastewater was 8000 GC/L, then there is a very real possibility that the field method will not be sufficient to observe and provide early warning to a local epidemic in time for any advanced action on the part of public health authorities.

The modeling case study examined reveals some important ideas about the effect of the sewer network geometry on the design of a WBE sampling scheme and its interpretation. The major spatial designs for WBE sampling are sampling directly at the inflow to a WWTP (by far most common) and at select in-network points that are meant to provide greater understanding at the scale of individual sewersheds for public health information. Typically, these in-network points will be at locations that are already visited by wastewater staff for maintenance and sampling (manholes, lift stations). As shown, the residence time of any dissolved phase or small particle (not likely to settle) constituent can be greatly reduced by the presence of any kind of intermediate holding space which would especially include lift stations. The longer water is allowed to reside in these places, while it is convenient for sampling access, the greater a pathogen-based analyte like SARS-CoV-2 will decay. Thus, it is wise whenever possible to limit any instances of delay between the fecal generation source and the point of sampling.

Similarly, the sewer geography is important even if pathogen quantification is not a challenge. If the proposed sampling locations within a rural sewer network are in the vicinity of many tributary pipe confluences, then it will be difficult to discern the meaning of the wastewater signal since the alignment of the wastewater concentration with the number of households of individuals who may be infected is made more challenging. Making the choice of sampling location based only on the ease of traveling to the location or the ease of taking a sample at the depth of flow below ground level frequently will not be sufficient to achieve success.

This work in general reinforces the opportunities for instituting a WBE program in rural communities. While there are opportunities to institute WBE beyond COVID-19, there are several challenges that must be addressed. These challenges include– the appropriation of wastewater collection methods (composite, grab, or passive), sample time of day (morning or afternoon) and frequency. There is also a lack of uniform sample analysis and detection methods within the industry, thereby producing inconsistent results. Inconsistent results can hamper accuracy and impact on the best way to serve communities. Most importantly, there is a need for considering the properties of a pathogen and its degradation rate through the sewers, and for new modeling tools that are sensitive to uncertainties in factors such as fecal shedding rate. While challenges associated with the design of a WBE program within a community are well-documented and would avail for opportunities to refine what has been here in future studies, this work can provide a starting point in that discussion [50,57,58].

5. Conclusion

This work is a modeling study that used EPA SWMM to simulate the fate-and-transport of a rural, isolated sewer network in Amarillo, TX, USA, a network which is a sanitary (not combined with stormwater) only network. The purpose was to examine a real case which is similar to a rural sanitary sewer network in terms of size, network complexity, and population with a focus on WBE feasibility. The particular aspect of feasibility in view was the sensitivity of the quantification technique for SARS-CoV-2 RNA in a wastewater based on two readily available quantification techniques for wastewater sample analysis. There are four important outcomes from this modeling case study.

First, modeling constituents related to WBE will involve sensitivity to input parameters. The three greatest model parameters for sensitivity in this study were wastewater hourly flows, community infection rate, and how a daily viral load is apportioned or fractionated during release events. Second, there is a spike in constituent concentration around the time of a flush event, and a 10-fold sensitivity difference will make a difference in ability to recognize and quantify wastewater concentration increases if the method is not fully considered when choosing the time of collection. This becomes especially important during low-incident levels. Third, in a rural system, a pollutant could spend up to 7 h between an upstream and downstream monitoring point. While it is less likely that a blurring of SARS-CoV-2 signals from a morning flush event and an afternoon one exists, each system would have to be examined individually to know if this is always the case. Finally, field methods of quantification are inexpensive in comparison with laboratory or clinical testing. While the field method is certainly cost-effective, those conducting a WBE campaign need to know the exact goals to determine what is truly best.

A recommendation in future work is to examine if the role of decay for any WBE constituent of interest is better quantified in mixed modeling-monitoring studies. The values used here were taken exclusively from laboratory studies, and these decay constants can better be ascertained from a mixed approach. The decay constants are needed since they can exert a large influence on how WBE constituents are modeled and how WBE results in practice are interpreted. Also, more work should be done on the potential value of WBE with science and engineering researchers collaborating to identify sampling methods, locations for sampling, timing of sampling, quantification methods, and the potential to sample for multiple public health related constituents. Rural wastewater utilities will need more guidance about how to best use their limited resources from health and STEM professionals to appropriate the benefits of WBE in their communities. The model employed in this study has the potential for application in other rural communities. But this cannot be confirmed without proper study and examination within other contexts, something highly encouraged for other researchers to consider doing so.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Gabrielle Bognich: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nathan Howell:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Erick Butler:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

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