

Effectiveness of Wastewater-Based Epidemiology as an Early Warning Tool to Detect SARS-CoV-2 (COVID-19)

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Abstract

Medical diagnostic tests to detect Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) for individuals in the United States were initially limited to people who were traveling or symptomatic to track disease incidence due to the cost of providing testing for all people in a community on a routine basis. As an alternative to randomly sampling large groups of people to track disease incidence at significant cost, wastewater-based epidemiology (WBE) is a well-established and cost-effective technique to passively measure the prevalence of disease in communities without requiring invasive testing. WBE can also be used as a forecasting tool since the virus is shed in individuals prior to developing symptoms that might otherwise prompt testing. This study applied the WBE approach to understand its effectiveness as a possible forecasting tool by monitoring the SARS-CoV-2 levels in raw wastewater sampled from sewer lift stations at a large public university campus setting including dormitories, academic buildings, and athletic facilities. The WBE analysis was conducted by sampling from building-specific lift stations and enumerating target viral copies using RT-qPCR analysis. The WBE results were compared with the 7-day rolling averages of confirmed infected individuals for the following week after the wastewater sample analysis. In most cases, changes in the WBE outcomes were followed by similar trends in the clinical data. The positive predictive value of the applied WBE approach was 86% for the following week of the sample collection. In contrast, positive correlations between the two data with Spearman correlation (r_s) ranged from 0.16 to 0.36. A stronger correlation ($r_s = 0.18$ to 0.51) was observed when WBE results were compared with COVID-19 cases identified on the next day of the sampling events. The P value of 0.007 for Dorm A suggests high signif-

ificance, while moderate significance was observed for the other dormitories (B, C, and D). The outcomes of this investigation demonstrate that WBE can be a valuable tool to track the progression of diseases like COVID-19 seven days before diagnostic cases are confirmed, allowing authorities to take necessary measures in advance and also enable authorities to decide to reopen a facility after a quarantine.

Keywords

SARS-CoV-2, Wastewater Detection, Wastewater-Based Epidemiology (WBE)

1. Introduction

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is an enveloped single-stranded RNA virus belonging to the *Betacoronavirus* genus [1] [2]. Infections of SARS-CoV-2 result in the disease known as COVID-19, which was first identified in late 2019 and was declared a global pandemic on March 11, 2020, by the World Health Organization (WHO) [3]. COVID-19 can be spread by touching an object containing the virus and inhaling droplets or aerosols discharged from an infected person's mouth (via sneezing, coughing, or breathing) [4]. According to the United States Centers for Disease Control and Prevention [5], the primary symptoms of COVID-19 include fever, cough, shortness of breath, fatigue, muscle/body aches, headaches, congestion, sore throat, nausea or vomiting, diarrhea, and loss of taste or smell. The most commonly reported symptoms are fever, cough, and fatigue [6]. These symptoms can appear in an infected patient within 2 - 14 days of exposure, but it has been reported that up to 45% of patients are asymptomatic [7] [8] [9].

Although the identification method for viral presence in the human body was established rapidly, the limited production and availability of detection kits initially required testing to be restricted to symptomatic patients and those who recently visited foreign countries [3]. In addition, the tracking of positive tests, hospitalizations, or deaths are all lagging indicators (post-infection and symptoms) and are, therefore, less useful for predicting localized outbreaks and determining with precision where precautions should be enacted to prevent the further spread of the disease. As a result, the ability to employ a detection method for both symptomatic and asymptomatic patients is important to controlling communicability. Estimates based on European and North American data suggest that each person infected with SARS-CoV-2, including those who are asymptomatic, excretes billions of viral genomes daily, which translates to between 0.15 to 141.5 million viral genomes per liter of wastewater generated [10].

The wastewater-based epidemiology (WBE) technique was first conceptualized by Daughton in 2001 [11] and later implemented in 2005 for tracing drugs such as cocaine [12] and then oseltamivir during the 2009 influenza pandemic [13] [14]. Applications have grown since then to include illicit drug use (heroin, metham-

phetamines, opioids, etc.) [15], alcohol metabolites [16], psychoactive substances [17] [18], oxidative stress markers [19] [20], and other emerging contaminants of concern such as flame retardants [21], plasticizers [22], endocrine disruptors [23], carcinogens [24], mycotoxins [25], etc. [15]. With the success of chemical markers, attention has been placed on tracking DNA [26] or RNA [27]-[31] signatures of microbial pathogens.

The theory behind the WBE method for the surveillance of SARS-CoV-2 is the presence of potentially large amounts of viral RNA in human excreta and its survivability in wastewater [7] [32]. To detect the target substance, a grab or composite sample of wastewater or sludge is collected from the sewer network. Then, the sample is transported to the lab for extraction and analysis. The underlying assumption is that a substance such as viral RNA excreted by humans is stable in the wastewater matrix and can be traced to the population served by the sewershed that contributed the material to the collection point. Ahmed *et al.* [1] reported that SARS-CoV-2 RNA is likely to persist long enough in wastewater to facilitate detection, although higher temperatures negatively influence decay rates. Because only the presence of the viral RNA is relevant (as opposed to symptoms), WBE can be used to determine the prevalence of infection before the onset of community-wide impacts. Hence, if the virus is detected in wastewater, it means infected individuals are shedding the virus into the sewer collection system, providing a rapid early warning system and a cost-effective approach that does not require invasive testing [33]. The challenges to be considered include dilution of the signal and the complex nature of the wastewater matrix itself [33]. Another challenge is associated with the variable nature of human contributions to the sewershed [34]. For instance, tourists or commuters who use the facilities but are not native to the location where the material is disposed of. Another concern is temporal variability since it is not currently possible to trace the virus particle to a particular individual.

This WBE approach has successfully been implemented to provide predictive surveillance of pathogenic viruses such as Hepatitis A [27], Zika [29], and Norovirus [27] [35]. Put on a timeline, an individual is exposed to the virus and becomes infected. Then, it takes up to 2 weeks in some cases before an individual starts experiencing symptoms and seeks treatment or gets tested. Then, it takes up to 7 days or more for laboratory results to confirm the diagnosis. At some point after infection, the virus replicates rapidly, and the infected individual starts shedding the virus. Some researchers suggest that this incubation period of shedding can begin up to 5 - 7 days before the onset of symptoms [36] [37] and may reach a maximum at 3 days prior to symptom onset [38]. The duration of shedding has been measured from 6 - 17 days [39]. The kinetics of viral shedding has been reviewed by Puhach *et al.* [40] and was found to be influenced by the characteristics of the host, such as age, sex, preexisting conditions, vaccine history, obesity, incubation period, etc. From the work published by Peccia *et al.* [41] and others, testing sewage sludge samples at a centralized wastewater treatment plant can be a leading indicator of viral presence in the service area. In the

case of Peccia *et al.* [41], the treatment plant studied served about 200,000 people and was able to detect the virus in 1 case per 50,000, but sampling at the centralized treatment plant means that there is no way to pinpoint where in the collection system the virus came from, only that its presence can be detected within the broader service area.

During the COVID-19 pandemic, WBE was used in many communities to detect SARS-CoV-2 [42]-[45] because this technique is capable of detecting a single infected case, either symptomatic or asymptomatic, per 100 to 2,000,000 non-infected individuals [3] [10] [46]-[49]. By using raw wastewater collected from gravity sewers, lift station wet wells, or centralized treatment facilities [1] [3] [50] [51], WBE also reduces bias from testing [46]-[49]. Arora *et al.* [52] confirmed that WBE testing at centralized wastewater treatment plants in Jaipur City, India, correlated with publicly available health data, and Hasan *et al.* [53] collected samples from sewer pumping stations in different types of communities and confirmed that the virus was detected in 85% of centralized pumping stations of raw wastewater with simultaneous decreases in both the viral loads in wastewater and the number of reported infected people following precautionary measures taken by the government to limit the spread of the disease. WBE was also employed to monitor SARS-CoV-2 levels in aircraft lavatories at the Dubai Airport, UAE [54] to limit the further spread of the disease by travelers. McMahan *et al.* [55] developed a model based on the relationship between viral copies in sewersheds and estimated disease prevalence in the community to consider when to reopen Clemson University.

The study of Bivins *et al.* [56] revealed higher stability for nucleic acids of SARS-CoV-2 than the viable infectious virus itself in raw wastewater. The average time needed for a 90% reduction of the SARS-CoV-2 virus is around 1.5 days, while viral RNA reportedly can be persistent from 8 to 27.8 days depending on temperature, with decay being faster at higher temperatures [1] [56]. Peccia *et al.* [41] observed a time lag of 1-4 days between WBE detection and hospital admission and greater than 6 days for reported cases. Gonzalez *et al.* [3] found a similar trend for medical diagnostic test data results compared to WBE results, although it was initially inconsistent. The investigations of Kumar *et al.* [57], Arora *et al.* [52], Medema *et al.* [58], and Wurtzer *et al.* [59] revealed a more stable and similar trend. Galani *et al.* [60] observed the lag of positive WBE results from new cases, new hospitalizations, and ICU admissions by 5, 8, and 9 days, respectively. Randazzo *et al.* [61] employed the WBE approach in untreated wastewater, secondary, and tertiary effluent of six treatment plants in Murcia, Spain, and compared the outcomes with clinical data. The study found positive results from WBE testing one week before COVID-19 cases were declared by the authorities. The investigation conducted by Wurtzer *et al.* [59] also observed spikes in WBE results before the diagnostic confirmation during 49 days of the study accompanied by a rise of viral load in wastewater by 2.95×10^6 genome unit/L (March 5, 2020 to March 12, 2020) followed by an increase in the number of COVID-19 related hospitalizations and quarantines by 400 in the next week

(March 12 to March 19, 2020).

Sherchen *et al.* [51] found different outcomes where negative WBE results were observed even after declared positive COVID-19 cases. The investigation used CDC N1 and N2 primers and probes. The study was carried out for a four-month period (January to April, 2020) where it examined untreated wastewater, secondary effluent, and final effluent samples from two treatment plants located in Louisiana and compared the WBE outcomes with the diagnostic test results. Although CDC announced the first positive COVID-19 case in the representative communities in March 2020, the analysis of Sherchen *et al.* [51] obtained positive WBE results on April 8, 2020, when the number of COVID-19 infected persons was 6173 and 308 in the two surrogate communities, respectively. The study mentioned lower viral RNA levels in the tested wastewater as the reason for the negative WBE results.

The promising outlook from literature review led the research team to investigate a predictive means, replicating studies at the University of Arizona [62] and later in Boston [63]. A wastewater-based surveillance program in the collection system may be able to detect the virus even earlier than waiting for the signal to arrive at a centralized wastewater treatment plant. Thus, a smaller portion of the service area can be localized for tracking as a leading indicator that will predict well in advance of rapid diagnostic testing results at the individual level. Of benefit, in southeast Florida, the nature of the subsurface (groundwater is often less than 4 feet below the surface) is such that gravity sewers cannot be used to convey raw wastewater for very long distances, so the radius of infected patients is narrowed considerably compared to areas with topography. Thus, a large number of sewage pumping stations collect raw wastewater from relatively small localized contributing areas (sewersheds). In urban southeast Florida, sewersheds can be as small as individual buildings or relatively small single-family neighborhoods of 50 - 100 homes, for example.

Because the SARS-CoV-2 virus can be detected in wastewater, not just sludge, the rapid testing of raw wastewater samples via real-time polymerase chain reaction (RT-PCR) is possible. Testing wastewater can be a leading indicator to potentially identify hotspots before individuals begin showing symptoms and seek diagnostic testing. WBE allows non-invasive sampling without requiring individual testing. Sewer lift stations allow for targeted localized (neighborhood-level and even single building-level) testing and trend analysis with the goal of supporting the safe reopening of schools such as a large public university.

The current study focused on the surveillance of SARS-CoV-2 in three types of buildings: 1) dormitories, 2) academic buildings, and 3) athletic facilities located within a large public university campus by collecting wastewater samples from lift stations that concentrated raw wastewater generated from isolated populations. In addition, the study aimed to evaluate the efficacy of the WBE technique as an early warning prediction tool by comparing the WBE outcomes and the number of confirmed infected cases documented after seven days of

wastewater sampling.

2. Methodology

This study conducted a WBE approach to detect SARS-CoV-2 virus following the methodology depicted in **Figure 1**.

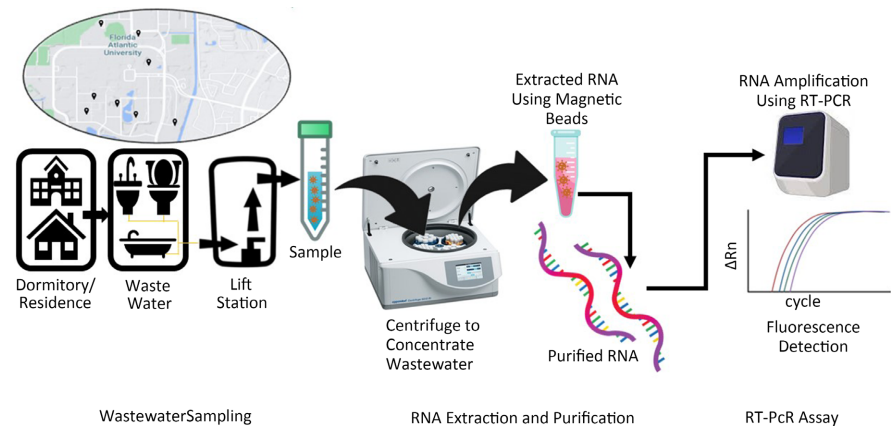


Figure 1. WBE approach flow chart for identifying COVID-19 in wastewater samples collected from sewer lift stations.

2.1. Sampling Locations and Sample Collection

In this study, raw wastewater samples were collected from several sources. Among these sources, four were dormitories (Dorm A-D), one was an athletic facility (AF), one was an academic building (AB), and one was taken from a closed loop cooling water blowdown station that did not have any possible human contact as a negative control (CT) site. The source of wastewater collected for Dorm A was a small manhole pump station located immediately adjacent to the sewer outlet of the building, while the sources of the other dormitories, along with the athletic facility and academic building, were small lift stations. The manhole and lift stations temporarily stored raw wastewater discharged from each of the isolated buildings.

The investigation started on October 13, 2020, and continued until September 14, 2021. During this time, each sample location was collected 41 times, generally on a weekly basis. The sample timing was collected on a Tuesday or Wednesday morning selected to maximize the capture of fresh contributions from dormitory residents, athletes, and students. Weekends and afternoons were avoided to limit any possible impact of 1) visitors who might create false positives or 2) wastewater pumping that purged the station to create false negatives. **Table 1** summarizes the sampling events conducted during the study period.

A total of 410 samples were collected during the study period. Sampling was conducted downstream first in order to avoid cross-contamination. **Table 2** summarizes the documented occupancy in each of the dormitories for each time period of the study. The number of residents in each dormitory facility varied by semester from none to up to 1171.

Table 1. Number of sampling events conducted during the study period.

Term	Total Number of Sampling Events	Sampling Campaign Start Date	Sampling Campaign End Date
Fall 2020	6	October 13, 2020	December 16, 2020
Break between Fall 2020 and Spring 2021	1	December 17, 2020	January 11, 2021
Spring 2021	14	January 11, 2021	April 27, 2021
Break between Spring 2021 and Summer 2021	2	April 28, 2021	May 18, 2021
Summer 2021	12	May 19, 2021	August 3, 2021
Break between Summer 2021 and Fall 2021	2	August 3, 2021	August 18, 2021
Fall 2021	4	August 19, 2021	September 14, 2021
Total	41	October 13, 2020	September 14, 2021

Table 2. Occupancy in each of the dormitories for each sampling event period.

Dormitory	Number of Residents			
	Fall 2020	Spring 2021	Summer 2021	Fall 2021
Dorm A	396	431	2	585
Dorm B	787	946	36	1171
Dorm C	301	390	0	408
Dorm D	473	535	0	598

All samples were grabbed in 250 mL sterile bottles (**Figure 2**). A 15-ft long sampling pole was used to collect the samples from the wastewater in the wet well of each station. The samples were transferred to a 250 mL presterilized sample bottle. The pole was washed with deionized water immediately after sampling in a location to avoid cross-contamination. All collected samples were stored at 4°C during the sampling events prior to analysis to minimize sample degradation. After collection, all wastewater specimens were brought to the laboratory for processing within 12 hours of collection.

**Figure 2.** Sample collection from a representative lift station.

2.2. Sample Preparation for Analysis

IDEXX magnetic beads-based nucleic acid extraction kits were used for the RNA recovery, and an IDEXX water SARS-CoV-2 RT-PCR kit was employed for the molecular analysis using RT-qPCR. The sample concentration step was conducted following the manufacturer's protocol. Briefly, a total of 105 mL of each wastewater specimen was subdivided into three centrifuge tubes (35 mL in each tube), which were then centrifuged at 4700G for 30 minutes at 4°C. This step served to separate suspended solids from the liquid samples. Then, the supernatants were dissolved in 3.5 ± 0.1 g of polyethylene glycol (PEG) 8000 and 0.788 ± 0.01 g of sodium chloride (NaCl), as per Colombet *et al.* [64]. The prepared solutions were centrifuged at 12,000G for 2 hours at 4°C followed by an additional centrifugation for 5 minutes at the same speed and temperature. The supernatant was discarded, and the pellets were resuspended in 400 µL of nuclease-free water. Therefore, 400 µL of concentrated samples were prepared for each of the representative wastewater specimens and stored at -20°C until RNA extraction.

2.3. Viral RNA Extraction

For the RNA extraction, IDEXX Magnetic Beads RNA extraction kits were used (Cat. No.: WCOV2MAG). The nucleic acid extraction was carried out according to the manufacturer's protocol. At first, a working solution was prepared by mixing 500 µL of binding buffer (BB), 50 µL of proteinase K (PK), and 20 µL of magnetic beads (MB) in a micro-centrifuge tube for each sample. Then, 200 µL of the concentrated wastewater specimen was added to the working solution. The final solution was incubated at 58°C for 10 minutes. Then, the magnetic beads were washed with 500 µL wash buffer-1 once and 500 µL of wash buffer-2 twice for each sample to remove inhibitors, proteins, and other contaminants. A magnetic rack was used to discard the liquid solution while keeping the beads in the micro-centrifuge tube. Finally, 100 µL of elution buffer was used to dissolve the purified viral RNA after incubating the washed magnetic beads at room temperature for 10 minutes. As a result, 100 µL of viral RNA solution was prepared for each raw wastewater sample. The extracted viral RNA was stored at -20°C until the PCR assay.

2.4. PCR Assay and Quality Control

IDEXX RT-PCR kits (Cat. No.: WCOV2PCR) targeting the N3 gene of the SARS-CoV-2 were employed for the molecular analysis. The PCR reporter used in this kit is FAM, while ROX is employed as the passive reference. The quencher used in the kit is BHQ[®]. The RT-PCR step was conducted following the manufacturer's instructions. At first, 10 µL each of the SARS-CoV-2 mix and RNA master mix were added, followed by the addition of 5 µL of representative recovered RNA to make the final volume of each reaction 25 µL. For the positive control (PC) and negative target control (NTC), 5 µL each of PC and PCR grade water were added respectively in place of the template. The experiments for each

of the samples were carried out in duplicate. Finally, the product was amplified following the thermal cycle described in **Table 3**.

Table 3. Thermal cycle conditions used for the RT-PCR analysis.

Step	Temperature (°C)	Time (sec)	Cycles
Reverse transcription (RT)	50	900	1
Denaturation	95	60	1
Amplification	95	15	45
	60	30	

After PCR amplification, C_q values were recorded for each reaction. The final C_q value for a specific wastewater RNA solution was measured by taking the average C_q values obtained from the duplicate reactions. To quantify the number of RNA copies as well as for quality assurance purposes, a four-point standard curve was prepared after obtaining the C_q values of serially diluted standard samples with known numbers of gene copies. The coefficient of determination (R^2) for the prepared curve was 0.9987, indicating a strong correlation. The equation obtained from the standard curve was used to evaluate the log copies for each of the tested RNA solutions. From the prepared curve, the detection limit for the approach was found to be 40 RNA copies/100 mL of sample. Finally, the number of viral RNA copies for each of the reactions was calculated using the following mathematical expression:

$$\frac{\text{Viral copies}}{100\text{mL}} = \log^{-1}(\log \text{copies per reaction})$$

$$\times \frac{\text{Extracted RNA}(\mu\text{L})}{\text{PCR RNA}(\mu\text{L})} \times \frac{\text{Prepared concentrate}(\mu\text{L})}{\text{Used concentrate}(\mu\text{L})} \times \frac{100}{\text{Initial sample}(\text{mL})}$$

Where the extracted RNA is the volume of recovered RNA (100 μL), PCR RNA refers to the volume of RNA solution used in each reaction (5 μL), prepared concentration is the total volume of concentrated samples for each wastewater specimen (400 μL), used concentrate is concentrated used for the RNA extraction (200 μL), and finally, initial sample refers to the wastewater specimens used for the concentration step (105 mL).

2.5. Clinical Data

In the current study, two types of clinical data were used. The number of infected individuals occupying Dorm A, Dorm B, Dorm C, and Dorm D were regularly monitored by university administration officials. Clinical diagnostic tests were conducted within the campus for symptomatic patients as well as for new students and student residents returning to campus from breaks. This data was tracked by the university. The infected number of residents/guests from the dormitories each day was taken into consideration for further analysis. Since the number of infected individuals was not routinely or systematically monitored for

the AF and AB facilities during the time of the study, the WBE outcomes for the sampling sites were also compared with the 7-day rolling averages of the broader infected population in Palm Beach County. The 7-day rolling averages of the number of infected cases in Palm Beach County were retrieved from USA Facts [65].

3. Results and Discussions

SARS-CoV-2 RNA in Raw Wastewater Samples

The time series data of the obtained WBE outcomes, along with the number of COVID-19 cases identified from the four isolated dormitories recorded from October 13, 2020, to September 14, 2021, are summarized in **Figure 3**. During fall 2020, the pandemic lockdown was lifted at the university, but classes were still being conducted in online format only. However, dormitories were reopened for students who chose to reside on campus. In the case of Dorm A, a sharp spike in viral signal was observed on October 20, 2020, with 275 viral RNA copies/100 mL. In the following week of this peak result (October 27, 2020), the 7-day rolling average of infected individuals was greater than one (1.1). As expected, immediately after returning from the winter break to start the spring 2021 term, spikes were observed for all dormitories. Classes were still only being offered in fully online format throughout the spring 2021 term. The maximum viral RNA concentration was seen on January 29, 2021 (291 copies/100 mL), while a 25% increase in the 7-day rolling average was observed (0.57 in January 27, 2021 to 0.71 in January 29, 2021). A similar phenomenon was also observed for Dorm B (0.29 to 0.85) and Dorm D (0.29 to 0.71) (**Figure 3b** and **Figure 3d**). This can be attributed to students and their parents/friends arriving, onboarding, moving in, and transitioning later than usual. In addition, the university was closed on January 18, 2021, in observance of the Martin Luther King Jr. federal holiday. During spring 2021, semester maximum viral concentrations were found on March 16, 2021, for Dorms B, C, and D with 10,680, 34,520, and 4,130 copies/100 mL, respectively. The 7-day rolling averages of COVID-19 cases were also found to be about 50% higher in the following week (March 23, 2021) compared to the previous one. This is attributed to the residents returning from mid-semester break (March 5 - 15, 2021) in which the opportunities for infection were greater due to vacation travel exposures. Later, vaccines became widely available for the residents from mid-April 2021 (being limited to age- and occupation-related restrictions prior to this date), and both the numbers of infected individuals and viral RNA concentrations were found to decrease thereafter.

During the break between the spring and summer terms, no spikes were observed in Dorms A, B, and D. In Dorm C, only one individual tested SARS-CoV-2 positive, while the number of viral RNA copies was 103 copies/100 mL for the wastewater sample collected on May 4, 2021 (**Figure 3c**). In the summer 2021 term, fewer numbers of residents remained in the dorms, and as expected many of the samples were below detection. The maximum viral RNA

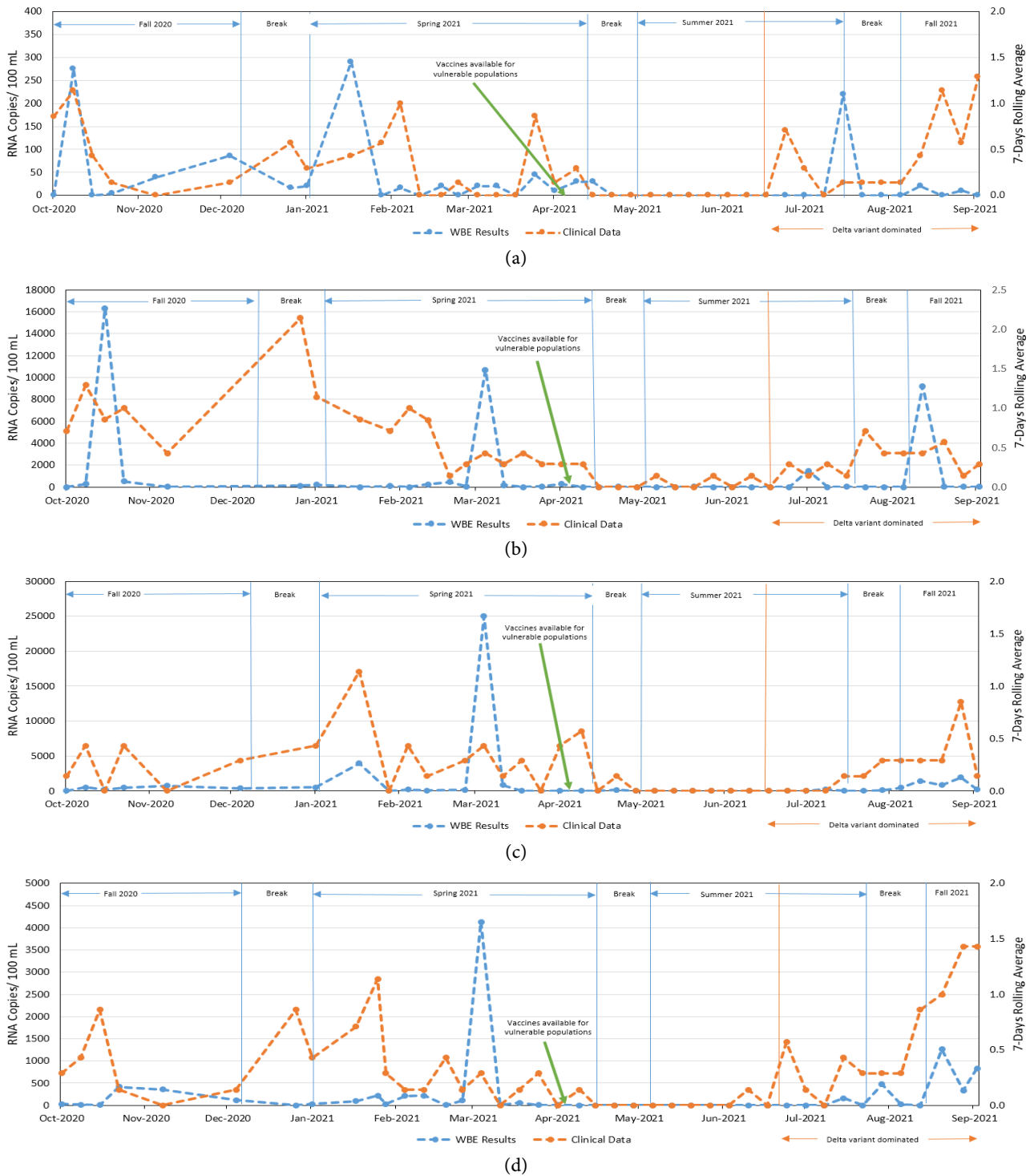


Figure 3. Time series for the WBE and clinical data for (a) Dorm A; (b) Dorm B; (c) Dorm C; and (d) Dorm D.

concentrations were determined as 220, 7340, 360, and 477 copies/100 mL for Dorm A, Dorm B, Dorm C, and Dorm D, respectively, during the summer 2021 term. Most of the positive WBE results were obtained during the second half of the summer term. This coincided with the new summer admits and freshman class arriving on campus in anticipation of the campus fully reopening to

in-person coursework. The 7-day rolling averages were below 0.2 cases during most of the term. In the meantime, the Delta Variant wave began to dominate in South Florida from the beginning of July 2021, and upward trends were observed for the number of infected students from the second week of the month. During fall 2021, coursework began to transition back to in-person (face-to-face) modalities. Although no correlation was observed between the number of viral RNA copies and 7-day rolling averages of infected individuals, the trends between viral copies and infection incidence remained similar, just as reported in studies conducted at the University of Arizona [62] and the University of Miami [66] using RT-PCR testing.

The current study also tested samples collected from the lift stations of the school's main athletic facility (AF) as well as from one isolated academic building (AB). **Figure 4** summarizes the outcomes of the WBE results for the two sampling locations along with the 7-day rolling average of the infected population in Palm Beach County. An athletic event took place on October 31, 2020, and the measured viral concentration for the following week (November 4, 2020) was found to be 52 copies/100 mL. In most of the WBE results in fall 2020, the athletic facility sample was observed as SARS-CoV-2 positive, as the athletic teams had a busy training and game schedule during that time.

The graduation ceremony for the spring 2021 term took place on April 29, 2021, outdoors in the athletic facility, where almost 3200 degrees were awarded in two in-person commencement ceremonies. In addition, graduates were provided with up to 5 tickets for family members to attend, and several hundred volunteers, security personnel, paramedics, faculty, and staff were also in attendance. As expected, there was a discernible uptick on May 4, 2021, with an elevated RNA concentration of 80 copies/100 mL compared to the several weeks prior when the facility was not being utilized as heavily. Spikes were also observed at the AB for the fall 2020, spring 2021, and fall 2021, especially during the beginning of the terms. The rise in the WBE results for the AB may have happened due to the fact that faculty members and students used offices and lab facilities of the building throughout the semesters, although no in-person classes were offered in fall 2020 and spring 2021. During the semester breaks, no spike was observed for the AB, likely because the occupancy of the building was low during the holidays.

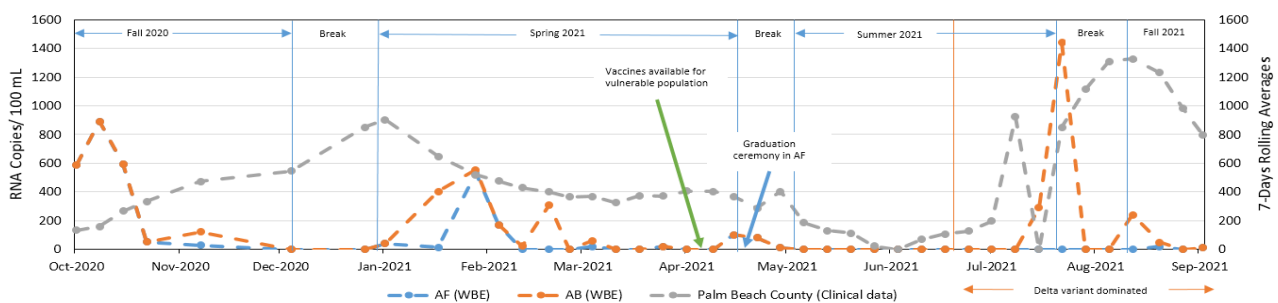


Figure 4. Timelines for WBE results of the AF and AB with clinical data for Palm Beach County.

The current study collected a total of 41 weekly wastewater samples from each of the four student dormitories from October 13, 2020, to September 14, 2021. The RT-PCR results were compared with the diagnostic test results. A summary of the comparison between the clinical data and positive/negative outcomes is presented in **Table 4** and **Table 5**, respectively. From **Table 4**, the samples collected from Dorm C demonstrated maximum positive results (68.3%), while Dorm A had the minimum (43.9%). In most cases, the number of infections for a specific dormitory followed the WBE outcomes for the previous week. In the case of Dorm C, the WBE approach provided correct prediction nearly 80% of the time for the positive and negative cases, one week before the diagnostic test results. There were only a few sporadic incidents where positive cases occurred in the following weeks after a non-detect WBE result for the studied dormitories – 12, 11, 5, and 8 times for Dorm A, Dorm B, Dorm C and Dorm D, respectively. These incidents decreased when the data was compared with the infected cases confirmed in the two days following sample collection from each dormitory (4, 6, 2, and 3 times for Dorm A, Dorm B, Dorm C, and Dorm D, respectively). The samples for Dorm A were collected from a manhole, where the duration of wastewater storage was relatively lower when compared to the residence time in the other lift stations, which during the shutdown, could have been days for the AB since few people were using the facilities.

Table 4. Comparison of positive WBE and diagnostic test results.

Dormitories	No. of samples collected and tested	No. of positive results confirmed by WBE	No. of at least one positive case in the following week of the positive WBE results	No. of zero positive cases in the following week of the positive WBE results
Dorm A	41	18	14 (77.8%)	4 (22.2%)
Dorm B	41	24	23 (95.8%)	1 (4.2%)
Dorm C	41	28	23 (82.1%)	5 (17.9%)
Dorm D	41	23	20 (87.0%)	3 (13.0%)
Control Sample	41	0	0	0

Table 5. Comparison of the negative WBE and diagnostic test results.

Sample location	No. of samples collected and tested	No. of negative results confirmed by WBE	No. of zero positive cases in the week following a negative WBE result	No. of at least one positive case after a negative WBE result						
				Following day	Following two days	Following three days	Following four days	Following five days	Following six days	Following seven days
Dorm A	41	23	11	4	9	11	11	11	11	12
Dorm B	41	17	6	6	9	10	10	10	11	11
Dorm C	41	13	8	2	4	4	4	4	5	5
Dorm D	41	18	10	3	4	5	7	6	8	8
Control Sample	41	41	41	0	0	0	0	0	0	0

In contrast, in 52% of the instances where the WBE result was below detection, there was at least one infection in Dorm A. In the case of Dorm B, 11 out of the 17 below detection WBE results were followed with at least one positive case the next week, and 6 of them occurred in the following few days. However, in nearly all (5 of these 6) scenarios, the number of infected individuals was only one. It is reasonable to suppose that a portion of infected individuals might not shed the virus, or could have lower virus shedding, or might have shed the virus while in another location, or the viral copies might have been transported downstream before sampling [62]. In all three other occurrences where positive clinical cases were observed within 2 days after collection, the number of infected individuals was one. On the other hand, below detection results for Dorm C and D provided accurate predictions in nearly all cases.

Overall, the positive predictive value of the WBE approach conducted in the current study was 86% considering the number infected individuals in the following one week after sample collection. In Betancourt *et al.* [62], sensitivity and specificity were 76% and 90.7%, respectively, considering the diagnostic test results obtained within 4 days of the wastewater sample collection. The approach enabled early detection of 85% of COVID-19 cases in the study of Karthikeyan *et al.* (2021) carried out at the University of California, while the study of Betancourt *et al.* [62] successfully predicted 82% and 88% of the positive and negative cases respectively after four days of the sampling. In the case of the control sample used in this study, none of the collected specimens showed positive results, as expected.

The Spearman correlation coefficient was used to evaluate the relationship strength of the number of RNA copies/100 mL results from the WBE analysis and the collected data of the 7-day rolling average of the infected individuals for each of the dormitories. The current study also observed the Spearman correlation coefficient after comparing the data with the documented COVID-19 cases on the next day of sampling. The relationships are summarized in **Table 6**, which describes a moderate correlation for Dorm B, C, and D and a weak relationship for Dorm A (which was located furthest upstream and had the smallest wastewater storage volume capacity of any of the sampling locations). For comparison, a study conducted at the University of Miami [65] found that the value of r_s was 0.39, while comparing the results obtained by qPCR (WBE approach) with the 7-day rolling average for the same week.

Positive r_s values for each dormitory location suggest that an increase in the RNA copies was generally followed by a subsequent rise of the 7-day rolling average for the following week. The other three dorms tested showed relatively stronger correlations between the WBE outcomes and COVID-19 cases for the following week. The statistical analysis of the WBE outputs and clinical cases of the next days of the sampling events showed a stronger relationship with r_s values of 0.18, 0.51, 0.51, and 0.48 for Dorm A, Dorm B, Dorm C, and Dorm D, respectively. **Table 6** also lists the P-values, which demonstrate the significance of

Table 6. Statistical analysis for the dormitory locations considering the number of infected individuals confirmed on the following day and one week after sample collection

Dormitories	Spearman correlation coefficient, r_s		P values	
	Next day of samples	Next week of samples	Next day of samples	Next week of samples
Dorm A	0.18	0.16	0.06	0.01
Dorm B	0.51	0.36	0.03	0.03
Dorm C	0.51	0.36	0.07	0.07
Dorm D	0.48	0.35	0.02	0.03

the relationships. The P values of 0.06 and 0.007 for the following day and the following week of the sampling events, respectively, represent high significance of the data obtained for Dorm A. The maximum P values were observed for Dorm C (0.07 for both the next day and the next week of the sampling), indicating the moderate significance of the outcomes.

The probability of obtaining a positive WBE result after comparing it to the number of infected individuals in the following 7-day period after the sampling is summarized in **Table 7**. For Dorm A, there were 19 occurrences when the number of infected individuals was confirmed to be between 1 and 4 in the week following sample collection, with positive WBE results found eight times during these incidents.

Table 7. Probabilities of getting positive WBE results compared with the number of infected cases in the week immediately following the sampling event.

Probability of positive WBE results when	Dorm A	Dorm B	Dorm C	Dorm D
Infected individuals are 1 - 4	(n = 19) 42.1%	(n = 20) 55.0%	(n = 24) 83.0%	(n = 20) 65.0%
Infected individuals are 5 or more	(n = 6) 83.3%	(n = 14) 85.7%	(n = 3) 67.0%	(n = 7) 85.7%

4. Conclusions

This study detected the relationships between the WBE results from weekly surveillance monitoring in sewer lift stations and the clinical data of the infected population identified after wastewater sampling. The WBE results generally followed the same trend as the 7-day rolling averages of the infected individuals. In particular, spikes were observed both in the WBE outcomes as well as in the clinical data during early January 2021, which coincided with the start of the spring 2021 term. Vaccination was made available to the general public in mid-April 2021, which was followed by a noticeable reduction in the number of confirmed infections and viral RNA copies detected in the sewer network. The impact of the new delta variant initiated a new wave of infections beginning in July 2021, with the predictive trend from the WBE process continuing to be present.

Throughout the study period, a correlation was observed between the WBE

results and the 7-day rolling average of infected individuals for the week following the sampling, which was expected as the shedding of SARS-CoV-2 in the upper respiratory tract and stool specimens are not expected to be similar. The WBE peaks were followed by an increase in the number of infected individuals in most cases. The positive predictive value of this study was found to be 86% for the WBE approach as a 7-day early forecasting tool. The probability of having positive results in the medical diagnostic tests in the following week after sampling based on the positive WBE result was also investigated. The probabilities of positive clinical results were 67% - 86% when the number of infected individuals was higher than four. The Spearman correlation coefficients suggest a moderate relationship between the WBE results and the clinical data for one week after sewer lift station sampling events, especially for Dorm B, C, and D. Stronger relationships were observed when the WBE results were compared with the clinical data obtained for the following day after sampling.

A couple of caveats should be noted. The detection limit of the WBE approach used in this study was calculated as 40 viral RNA copies/100 mL, and thus, there is a chance that some of the samples were false negatives. Dorm A was collected from the manhole immediately adjacent to the building where the wastewater storage duration is much lower than the other sewer lift stations sampled during this study. The manhole collection site was more likely to have sample results below detection because of its low storage volume as opposed to the longer holding times in the other lift station wet wells on campus. Also, run times for lift station pumps or data from pump alarms were not readily available, as this information was manually collected only on a sporadic basis. Presumably, if pump runtime data is recorded and available, the timing of sampling events can be coordinated to maximize the surveillance value. Normalization of SARS-CoV-2 levels against human waste indicators was not conducted, and the interval of sample collection was not always possible to maintain due to access to the lift stations. These factors will provide a better understanding of the true correlation between WBE surveillance testing results and the clinical data for the following week after sample collection.

These results show that the WBE approach used here can be a useful tool to forecast COVID-19 infections one week prior to clinical onset of symptoms or hospitalization. This type of WBE surveillance can be helpful when attempting to determine when it will be safe to reopen facilities after quarantine when universal diagnostic testing is not possible, and where there is a need to protect vulnerable populations. Furthermore, it is likely that trailing indicators like hospitalizations and deaths underestimate the extent of infection in a community. Using WBE would appear to be an indirect, non-invasive means to reduce transmission and deaths resulting from infection simply by pinpointing viral load hotspots in the sewer network. The goal of this would be to allow authorities to better direct resources to protect vulnerable populations while easing restrictions in virus-free communities to minimize economic disruption.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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