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Tracking SARS-CoV-2 Levels in Wastewater During College Football Events Using Cell Phone Data for Population Dynamics

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Abstract: Coronavirus disease 2019 (COVID-19) can be tracked through wastewater, enabling the prediction of cases by wastewater-based epidemiology (WBE). An issue that complicates WBE is that humans are not static, moving in and out of sewer drainage areas throughout the day. During large-scale events (i.e., sports, music, culture), large populations move during a small time frame in certain areas, with some individuals carrying along the virus. To track such human movement anonymously, cell phone location data (using StreetLight®) were used to monitor the flow of populations in and out of the sewershed during football games at the University of Oklahoma for two consecutive seasons (2020–2021). Hourly wastewater samples were taken during gamedays (Saturday to Sunday mornings) and on one control Saturday (no game) for each season, along with controls in the form of composite samples for days surrounding the events. Hourly population data during gamedays allowed for the calculation of viral load per capita, which increased for most games, indicating the existence of incoming infected individuals in the region. This case study aims to help decision makers understand how hosting large-scale events during this and potential future disease outbreaks may impact public health.

Keywords: wastewater-based epidemiology; COVID-19; large events; human mobility

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1. Introduction

Coronavirus disease 2019 (COVID-19) is the disease caused by Severe Acute Respiratory Syndrome Corona-Virus 2 (SARS-CoV-2) [1]. What began as an outbreak of SARS-CoV-2 in Wuhan, China, in December 2019 was classified as a pandemic by the World Health Organization (WHO) in March 2020 and was described as the "most consequential infectious disease since the 1918 influenza pandemic" [2]. A virus that predominantly impacts the respiratory system, SARS-CoV-2 is spread mostly through the inhalation of droplets from an infected person [3,4]. Symptoms of this disease are typically fever, cough, chest discomfort, fatigue, headache, and diarrhea, among others [5]. However, a sizable portion of those infected will have mild symptoms or may even be asymptomatic, making the clinical confirmation of cases difficult [2]. It is possible for asymptomatic persons to spread COVID-19 to healthy individuals, making spread from asymptomatic persons a problem [6]. Obtaining an accurate count of infections using traditional clinical testing in a timely manner is also difficult due to the volume of cases around the world, shortages of supplies, and a lack of understanding of the disease by medical and academic experts [7]. The virus's high transmissibility, lack of symptoms in some cases, and difficulty in detection makes large-scale public events—where attendees from different regions concentrate in small areas—a potential hazard to public health [8,9].

1.1. Wastewater-Based Epidimiology for COVID-19

To account for the difficulty in tracking outbreaks using traditional testing methods, researchers have turned to using wastewater-based epidemiology (WBE) to track COVID-19. WBE is the process of using wastewater to track diseases and other human health indicators through the sewage system. This method has been used as early as the polio eradication program in the twentieth century [2] but was named in 2001 when it was used to study pharmaceutical concentrations in wastewater [7]. WBE relies on the fact that as long as a particle/substance excreted by humans is stable in wastewater, it can be tracked [7]. Researchers realized early on during the pandemic that WBE could be used to track COVID-19 in an area [1,2,10,11], with approximately 43% of people who actively have COVID-19 shedding viral RNA particles in their feces [12]. The exact concentration of SARS-CoV-2 shed per infected person is subject to investigation but could vary geographically [13]. This difference in shedding in feces could also be impacted by variants, manifestations or symptoms of the disease, or differences in the populations infected. Because SARS-CoV-2 (like all other viruses) does not replicate outside of the human body, WBE has proven to be an effective way to track the spread of disease.

Collection of samples from a sanitary sewer system that represents shedding from the population of interest is a vital part of WBE. However, several human and environmental factors such as the time and frequency of restroom use, sewage residence time in the wastewater system, variable shedding rates of infected persons, and variable proportions of black and gray water present challenges to obtaining and interpreting representative samples [2]. Different collection methods can be used, such as grab samples or time-weighted and flow-weighted composites. Grab samples can provide a snapshot of the time interval in which they are taken [14]. Moreover, successive grab samples collected at certain time intervals can capture the temporal variation in the pathogen in wastewater. Time-weighted composite samples are a series of grab samples taken at a certain time intervals and combined, resulting in one sample representing the entire sampling period. Time-weighted composites are most representative of the contributing population if the flow rate and ratio of feces to other water in the sewer is consistent throughout the day. Flow-weighted composite samples are a series of samples taken after a certain amount of water has passed through the pipe and combined. The frequency of sampling is therefore dictated by flow rate, resulting in one sample normalized for flow across the entire period. Flow-weighted samples are often considered the best choice for representing a given population over a given period of time [14]. However, both time-weighted and flow-weighted composites have drawbacks. It is time consuming and costly to run an autosampler for long periods of time to achieve long-term composites, with mechanical and other failures preventing the extraction of continuous samples [14]. Also, unlike grab samples taken throughout the period, composite samples cannot show variability in the amount of a pathogen throughout the period.

1.2. Background and Research Gaps on WBE for Large-Scale Events

WBE is scalable and can be used to monitor certain analytes during specific events. To date, most event-based wastewater monitoring experiments have been focused on tobacco, alcohol, and illicit drug use during sporting events and concerts [15–17]. In Italy, researchers observed an increase in alcohol consumption during sporting events by collecting samples on an hourly basis [18]. In Florida, researchers at the University of Florida tracked the concentration of illicit drugs in the wastewater leaving a football stadium during a game by sampling wastewater on a 30 min basis beginning one hour before the game and ending approximately 30 min after the end of the game [17].

While there are not yet data available on the temporal variation in SARS-CoV-2 in wastewater at large sporting events, there is existing research about the use of WBE at sporting events to track other public health phenomena. Montgomery et al. [19] monitored for illicit drugs during basketball games. Sassano et al. [20] speculated that European soccer matches were associated with the spread of SARS-CoV-2 in Italy and Spain. However, regarding sporting events, much of the existing research looked at the spread of the virus among team members rather than among incoming spectators. For instance, researchers studied whether SARS-CoV-2 is spread by players during professional rugby matches [21], European professional soccer games [22], and even American high school football games [23].

In this manuscript, the focus was shifted to the large traveling populations that move within a region for 24–48 h to attend a large-scale event such as a football game, participating in different indoor and outdoor activities related to the main event (including indoor/outdoor gatherings before, during, and after the game). Among such large populations, the underlying assumption is that asymptomatic individuals will be carrying and transmitting the virus during their stay within a certain region, something that WBE can capture accurately.

Monitoring large-scale events, such as football games, is crucial for public health, since these events may act as focal points for the spread of infectious diseases such as COVID-19 or similar viruses. Such gatherings tend to bring together diverse populations from various regions, creating an ideal environment for pathogens to spread. Close contact, shared facilities and crowd density increase the potential for disease transmission. The information that this research aims to provide can be used to guide policy and inform decision makers in the face of our next public health crisis. Specifically, it can elucidate how an incoming population may affect local viral loads, potentially leading to a local outbreak and the exposure of many people to the virus, which may lead to strain on healthcare systems.

1.3. Background and Research Gaps on WBE for Dynamic Population Movement

One of the most difficult aspects of WBE is correlating the number of positive cases with the values detected in wastewater. The first step in this process is to obtain an accurate count of the population in the sampling area. However, because humans are not static, it can be difficult to accurately estimate such a population, particularly during large-scale events. There has long been a need for devices that provide the high-resolution data that cell phones now offer in the study of epidemiology.

Using smartphones and their Global Positioning System (GPS) capabilities allows researchers to track the movement of people at fine temporal and spatial resolutions [24–26]. This practice is also beneficial because it does not require that participants self-report movement and activities [25], but still provides a timely estimate of the population and its geographical flux [24,27]. Specifically, as Chaix [25] points out, with high-resolution movement-tracking data, researchers can place travel into context, such as determining the reason for a person to be in a certain place at a certain time. Mobile device data have been used to estimate park visitation in Orange County, California [28]. In Oslo, Norway, researchers used WBE combined with mobile device population data to monitor for illicit drug use [29] or have combined mobile phone data with census data to estimate populations in large cities [30]. So far, such an investigation for COVID-19 for large-scale events has not been conducted to estimate the impacts of dynamic population movement on the viral load of a certain region due to the event itself and its related activities.

1.4. Background on COVID-19 Response in Study Region

The COVID-19 response in the U.S. varied significantly by state, city, and even academic institution. The public policies, mandates, and recommendations in place had a direct impact on the transmission of the virus at both temporal and spatial scales. Thus, a brief summary is provided herein focusing on the state of Oklahoma and specifically on the University of Oklahoma's Norman campus to better describe the conditions under which each football season was held. Norman is the third most populous city in the state, is located only 20 miles away from Oklahoma City, and has a population of approximately 128,000 (2020 Census Data). As the hometown of the University of Oklahoma (OU), the largest university in the state, with nearly 32,000 students, it is also a popular destination owing to its longstanding college football tradition. The Gaylord Family Oklahoma Memorial Stadium, home of the Sooners, has a capacity of a little below 84,000 spectators, and during football gamedays, the population around campus and the city of Norman nearly doubles due to people that attend the event in person, watch the game in restaurants, or even tailgate around campus.

In 2020, the State of Oklahoma recommended the use of masks in "red or orange" counties for individuals aged 11 yrs and older at work, in public spaces, and in restaurants [31]. A county was classified as orange by the Oklahoma State Department of Health when it exceeded 14.29 new cases per 100,000 people per day, and Cleveland County, the home of the City of Norman and the University of Oklahoma, was classified as orange from at least 24 September 2020 to the end of 2020 (State of Oklahoma Department of Health, n.d.) [32]. Following state guidance, along with mask mandates in place at both the university and in the City of Norman, the University of Oklahoma allowed for 25% occupancy at home football games during the 2020 season, resulting in 22,700 people in attendance at each of five games in total that were held in the Gaylord Family Oklahoma Memorial Stadium in Norman [33]. Masks were required at these events, though there were limits to the enforcement of mask usage.

In 2021, the state legislature in Oklahoma passed Senate Bill 658, which banned schools from mandating in-classroom mask usage [34]. Because of this and other circumstances, the fall 2021 football season was treated much differently than the prior season, with a return to full stadium capacity and on-campus tailgating. Notably, there were no masking or vaccination requirements to attend games [35].

At the same time, new SARS-CoV-2 variants arose during 2021 that were generally more infective, believed to be due to changes in the structure of the virus, particularly the spike protein [36]. These variants often had a geographic signature [3], typically originating in areas with high community circulation and low vaccination coverage [37]. The primary variants of concern during this study were the alpha and delta variants. The alpha variant was first identified in the UK, and some estimate that it was 50% more transmissible than the original virus and was associated with an estimated 50% increase in mortality [36]. This variant was the most dominant variant in the state during the 2020 football season. The major variant that was at its peak in the fall of 2021 across the globe was the delta variant. The delta variant was also the major variant circulating in Oklahoma from June through approximately December 2021 and was associated with another wave of COVID-19 outbreaks. The delta variant was replaced by the omicron variant as the dominant variant of concern in the state of Oklahoma in January 2022 [38]. The delta variant demonstrated a household transmission risk approximately 60% greater than that of the alpha variant, which was already more transmissible than the original version of SARS-CoV-2.

Another factor to consider regarding the fall of 2021 compared to that of 2020 was the existence of SARS-CoV-2 vaccines. By 15 October 2021, the percentage of adults in Oklahoma who had received at least one dose of a vaccine was 71%, while 60% of adults in the state received both doses [39]. While vaccination does reduce the risk of hospitalization and severe disease from the delta variant, experts recommended that other measures such as mask usage and social distancing should be continued to effectively reduce the spread and prevent further mutations of the virus [40].

1.5. Case Study Goals—Paper Organization

The overarching goal of this study was to determine if WBE can be used to measure temporal changes in SARS-CoV-2 in wastewater in order provide some preliminary results on the ways in which large-scale events may affect the local region when it comes to infectious diseases such as COVID-19 due to the dynamic effects that incoming traveling populations might bring to the region. WBE is ideal for such an investigation, since there is a high likelihood that any infected individuals might be asymptomatic but still able to transmit the virus to other attendees or locals. Secondary goals were set with respect to the quantification of the viral change due to travel into the City of Norman for the football game and whether data from tracking mobility platforms such as StreetLight® can be used to measure changes in population for an entire city.

The methods employed (Section 2) and results achieved (Section 3) are described for the surveillance of an important infectious disease during large-scale events, combined with the dynamic traffic flow of the population into and out of the region of interest. Sections 4 and 5 provide a discussion of the results and conclusions for this study, stressing the effects of population monitoring and other factors that influence the recorded in this study trends.

2. Methods

Based on the background discussed in the introduction, grab samples and time-weighted composites were leveraged for both football seasons to capture the desired temporal variations during the game and to establish comparisons with daily averages from days before and after the games.

2.1. Sampling Process

For the 2020 football season, five weekends were selected for sampling based on the football schedule, while seven weekends were selected for the 2021 football season. The complete information regarding the two seasons, game list, opponents, and results can be found in [33,41]. Table 1 shows specific information about each gameday and the control day for each season. The control days were selected on an away gamedays when only residents would be expected to be present in the sewershed. The 24 h time-weighted composite samples for Friday (day before the game), Saturday (day of the game), and Tuesday (when everyone who visited for the game has left) for each sampling event were collected from the treatment facility and analyzed. All the samples were collected at the City of Norman Water Reclamation Facility (NWRF) after initial grit screening, which removed large solids, but before any water treatment. The NWRF is an activated sludge treatment facility that serves a population of approximately 87,779 with an inflow rate of 43 MLD, including the OU campus and the stadium, which is at a distance of approximately 4 km north of the facility [42]. Hourly samples were collected for the gamedays and control Saturdays, except for cases where the autosampler clogged due to its extended presence in the sewage. Samples were collected using Teledyne ISCO (Lincoln, NE, USA) Avalanche refrigerated autosamplers set to collect 700 mL samples every hour for 24 h beginning Saturday morning. Two sets of fourteen 950 mL plastic ISCO sample bottles were cleaned using a 10% percent bleach solution, rinsed with tap water, washed with 2% detergent (Citranox), rinsed with DI water, and finally treated with sodium thiosulfate and rinsed again with DI water [43]. It should be noted that the method employed here closely follows the sampling and analysis procedures that are detailed in [44].

Table 1. Home game information and control dates for the two sampled seasons.

2020 Season			
Date	**Opponent**	**Start Time**	**Attendance**
12 September	Missouri State	6:00 PM	22,700
7 November	Kansas	2:30 PM	22,700
14 November	Control (no game)	-	-
21 November	Oklahoma State	6:30 PM	22,700
5 December	Baylor	7:00 PM	22,700
2021 Season			
Date	**Opponent**	**Start Time**	**Attendance**
4 September	Tulane	11:00 AM	42,206
11 September	Western Carolina	6:00 PM	83,538
18 September	Nebraska	11:00 AM	84,659
25 September	West Virginia	6:30 PM	84,353
16 October	Texas Christian	6:30 PM	84,391
13 November	Control (no game)	-	-
20 November	Iowa State	11:00 AM	82,685

2.2. SARS-CoV-2 Analysis

Samples were maintained between 4 and 6 °C until they could be processed (typically within 24 h of receipt), as described in [44] and described briefly here. The samples were strained, divided into three technical replicates, mixed with polyethylene glycol (PEG 8000), and incubated overnight at 6 °C. After incubation (10–16 h later), samples were centrifuged at 14,600 RCF for 45 min at 4 °C, and the supernatant was decanted. Pellets were resuspended in a guanidine thiocyanate lysis buffer. Total nucleic acids, including viral RNA, were precipitated with an equal volume of 100% isopropanol, bound to carboxylated magnetic beads, and eluted in DEPC water following several washes in 80% ethanol. The concentration of SARS-CoV-2 RNA in the extracted nucleic acid was estimated using quantitative reverse transcription polymerase chain reaction (RT-qPCR) [44]. The geometric mean of the replicates was used in this analysis to normalize non-detections. Non-detections were assigned a value of 312 copies/L, approximately half of the detection level [45]. The microbiological analyses were performed at the University of Oklahoma's Department of Microbiology and Plant Biology. Because these samples represent an entire sewershed, infections in individual persons could not be inferred or determined.

2.3. Cell Phone Data

Data on the number of mobile devices entering and leaving the Norman sewershed were obtained from the City of Norman's sewer boundary shapefile from StreetLight Data, San Francisco, CA, USA (streetlightdata.com-accessed on 15 January 2022) (SL), a data analytics company that deidentifies and organizes location information from mobile technologies (e.g., phones, communication towers, and GPS-enabled devices) [46,47]. SL was originally designed to provide information about traffic movement for mobility and transportation planning, but mobility analytics platforms like SL are growing in popularity, with numerous investigations examining traffic patterns, vehicle volume metrics, and other applications that require the mapping of user behavior or an accurate headcount of populations within a region of interest [48–51]. Such an ability to track human mobility at an individual level has, as expected, raised significant concerns with respect to privacy and sharing policies, with extensive literature on this matter [52,53]. It should be noted though that most such platforms, including SL, have strong policies in place that deidentify the data and focus on measurable quantities (like travel distance and location positioning), protecting the cell phone users at an individual level [46,47]. Like every method, SL has some inherent limitations regarding the provided data related to (i) inaccuracies in estimates due to data aggregation and anonymization that can obscure individual travel patterns and reduce the precision necessary for certain analyses, (ii) the uneven representation of populations that might not have access to smartphones or connected vehicles [26], and (iii) GPS and cellular coverage for the region of interest. Some of these limitations are explicitly examined in Section 4 of the manuscript to quantify the effect that they might have had on the recorded results.

Using SL, here, community mobility pattern estimates were obtained along roadway corridors and between specific locations. Origin-Destination (OD) analysis was performed via the SL platform to aggregate trip count data using algorithmically transformed location point data and was validated using embedded in vivo road network sensors and traffic counters [47]. Origin-Destination identifiers (OD-IDs) were generated into and out of the Norman sewershed every hour starting on Friday at 12:00 a.m. CST and ending at 11:59 p.m. CST on Sunday for each sampling weekend. In addition to trip counts, SL also reports trip attributes, including average trip duration and average trip length. These data were tabulated using STATA 16.1. Hourly population estimates within the Norman sewershed were calculated using 2019 census tract estimates from the American Community Survey (ACS) data. Census tract geographies (n = 26) were clipped to the Norman sewershed boundary, and aerial the apportionment of census tract populations was performed to estimate the total population serviced within the sewershed. The total estimated population was 87,779. The US Census Bureau, which manages the ACS, tries to include college students in population estimates, aiming to count "people where they live and sleep most of the time" [54]. This baseline total population is pinned to the 12:00–1:00 a.m. time slot. SL hourly traffic counts into and out of the sewershed were then iteratively added and subtracted from the estimated baseline total population to generate hourly estimates of the population within the sewershed area. This enabled a more accurate quantification of the viral load per capita, as described in the next section, aiming to remove the population number from the viral concentration that is recorded.

2.4. Viral Load Metrics and Statistical Analysis

Viral load per capita was the major metric used to determine the amount of SARS-CoV-2 in the wastewater in this study and was calculated according to Equation (1) presented below. Q_{avg} is the average flow for the approximate hour that the sample represents; for instance, if the sample was taken at 8:30 a.m., the flow was averaged from 8:00 a.m. to 8:59 a.m. and so forth. Flow data were provided by the NWRF at 15 min intervals. The viral load per capita was calculated based on the sample and the generated triplicates as follows:

$$Viral\ Load = \frac{C_n \cdot Q_{avg}}{P} \tag{1}$$

where $C_n = \sqrt[3]{C_1 \cdot C_2 \cdot C_3}$ is the concentration geometric mean of the three triplicates, Q_{avg} is the average flow for the hour, and P is the population present in the sewershed in that hour.

Statistical analyses were performed in SPSS 28 and Microsoft Excel to test the hypotheses of this project by testing the following variables: flow, population, concentration, and viral load per capita. The analyses were performed for each game, across games, and across seasons to test various hypotheses on the existence of differences at all these temporal scales. Two approaches were used to compare these variables between games. The first one examined the variability of each variable for each day compared to each other, and a one-way Analysis of Variance (ANOVA) test [55] was used. For the concentration and viral load per capita, there were fewer than 30 values for each day, and thus a Kruskal-Wallis H-test (or a nonparametric one-way ANOVA) [56,57] was used. It is also worth examining whether the mean value for each variable for the control (non-game) is statistically different from the mean value of the same variable for all the weekends with a game. To address the uneven sample size resulting from comparing variables on one day to variables on multiple days, a Welch's *t*-test was used. A *t*-test was used to compare these variables between two games.

Within-day variability was analyzed by breaking the day into subgroups consisting of before the game, during the game, after the game, and a final group called late night. The before-game group consisted of the 4 h directly before kickoff. The during-game subgroup, consisted of the 4 h in which the game was played. All games lasted between 3 and 4 h for both seasons [58]. The after-game group represented the 4 h window directly after the end of the game; however, this category was only valid for some games because this defined 4 h window directly after evening games ran into the late-night category. The late-night category represented the hours between 11:00 p.m. and 3:00 a.m. For the comparison of these groups against each other, given their small sample sizes, a nonparametric test of means was performed in SPSS, and the software was allowed to select the most appropriate test given the data. This resulted in the use of a Mann-Whitney U test [59] to compare the values. Due to the significantly small sample size (only 8 samples were available to be compared to each other), the results of these comparisons were not further analyzed and presented here to avoid overinterpretation. Across all games, it is of interest to know if there are time groups that are different from the others. To determine this, for instance, the flow, population, concentration, and viral load per capita of the before group of all games were compared to the during-game group values for those variables for all games. This analysis was performed within seasons as well as across seasons. The statistical tests for this analysis utilized *t*-tests to compare means.

Finally, the concentration of the gameday samples was also compared to the concentration of samples from the NWRF collected on other days of the week to assess whether there was a change in the concentration associated with the football games. Two methods were used to test this: the days temporally near a gameday were compared to the gameday (using a Mann-Whitney nonparametric test given the small and uneven samples sizes), and for within- and across-season comparisons, a similar analysis was performed using Welch's *t*-test to compare the two groups.

3. Results

To better interpret the study results, multiple factors need to be accounted for, including federal, statewide and institutional health policies, mandates, availability of vaccines, along with trends in COVID-19 cases and student presence during the investigated seasons. Looking into the trends of reported cases of COVID-19 in Oklahoma during the 2020 and 2021 football seasons, major differences can be identified. The highest reported cases for the 2020 season occurred later in the season, while the greatest number of cases for the 2021 season occurred at the start of the season. For the purposes of this manuscript, the 2020 football season was defined as 1 August–5 December 2020, which was the final home game of the season, while the 2021 football season was defined as 1 August–1 December 2021. Figure 1 displays the number of new cases per day as reported by the United States Centers for Disease Control and Prevention (CDC) for each season. The peak for the 2020 season occurred on 24 November 2020, while the 2021 peak occurred on 25 August 2021. The COVID-19 pandemic produced new cases throughout the autumn of 2020 for many reasons. There is an element of seasonality associated with COVID-19 prevalence, where transmission is reduced during warmer weather [60]. Throughout the 2020 football season, the temperature in Oklahoma decreased heading toward winter season. Another reason for the peak in cases at the end of the 2020 football season was due to travel for the holidays, particularly Thanksgiving [61]. Though the University of Oklahoma held classes entirely online after the Thanksgiving vacation [62], the number of cases throughout the state was still increasing, likely due in part to the holiday. For the 2021 season, more infective variants were present, and strict mask usage policies were no longer in place, but the number of vaccinated individuals within the state of Oklahoma significantly increased toward the end of 2021. These factors could explain to a degree the smaller effect that dropping temperatures and the congregation of individuals in closed spaces without capacity or mask constraints had as the 2021 fall season progressed. Other factors, such as weather conditions during each game and the team's performance within the season were not explicitly quantified or correlated with certain trends during this case study.

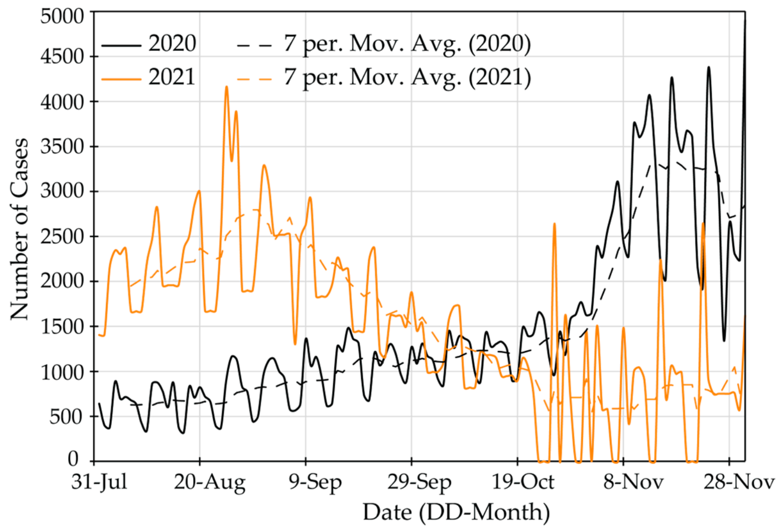

Figure 1. New cases per day in Oklahoma during the football seasons of 2020 (black line for 1 August–5 December) and 2021 (orange line for 1 August–1 December), along with their 7-day moving averages (dashed lines of respective colors for each season) [63].

Samples were collected over the 24 h period from Saturday morning to Sunday morning for game days, and time-weighted composite samples were additionally acquired on Friday, Saturday, and Tuesday for each sampling event. Each season was analyzed individually and was also compared against the other. Analyses consisted of determining relationships between flow, population, concentration, and viral load per capita over time.

3.1. Population Estimates

The SL data were used to produce an estimate of the number of people present in the sewershed on an hourly basis for each weekend that was sampled. Figure 2 contains the estimated population present in the Norman sewershed during each sampling period. As mentioned earlier, samples were collected from approximately 8:00 a.m. Saturday to approximately 8:00 a.m. the following Sunday. It should be noted that the population on campus was decreasing as the semester approached December for a couple of reasons. First, many classes had limited or no in-person meetings throughout the fall of 2020. University policy was that classes of more than 30 people were to be held online. Second, after the Thanksgiving holiday, from November 23 to November 27, students who traveled home were asked to stay there for the remainder of the semester, as all classes were moved online [62]. This likely led to a gradual migration of students away from campus as the semester went on. However, there is no way to gather an accurate count of who left the sewershed across the entire semester. This means that the estimated baseline population (black line in Figure 2a) for the 2020 season is likely incorrect for the football games that occurred later in the semester. The potential impact of this population migration on the overall results is presented in the Section 4.

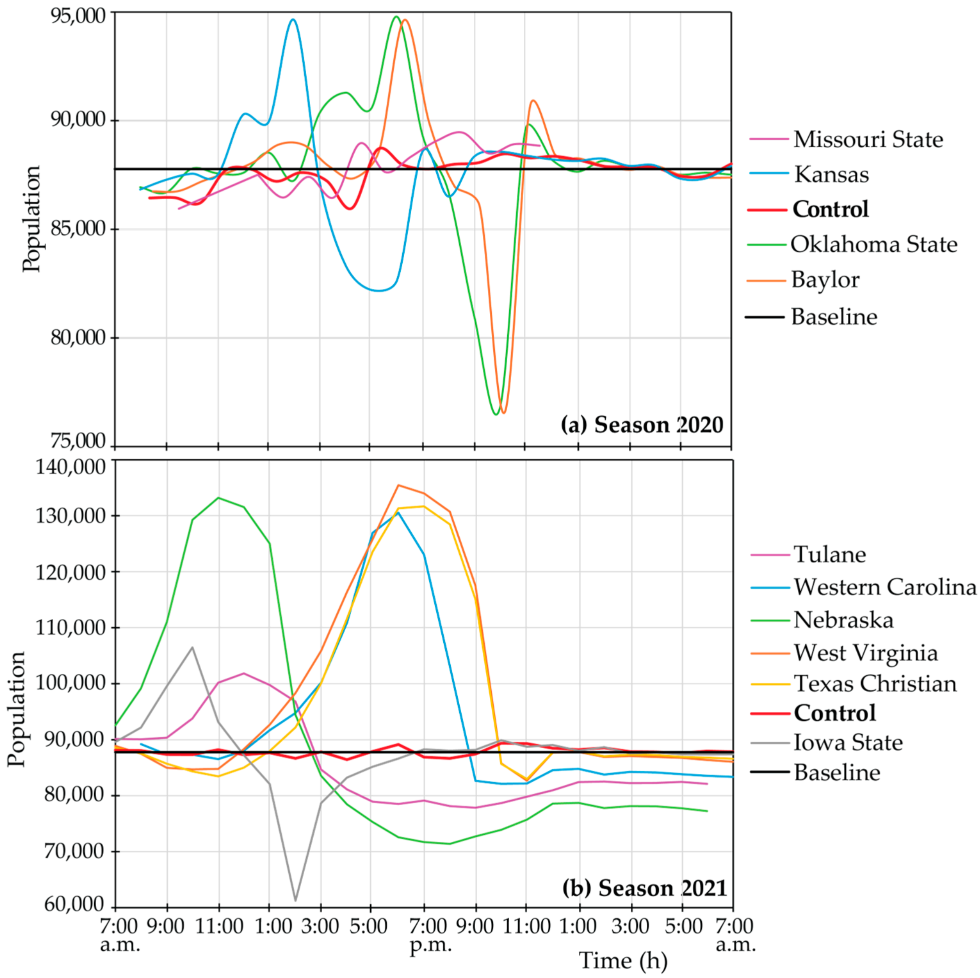

Figure 2. Estimated population over time for all sampling periods on football gamedays for (**a**) the 2020 season; (**b**) the 2021 season. The games in the legends are listed in chronological order for every season following the same color pattern.

2020 Season: Compared to that of the control weekend, the population for each gameday in 2020 was significantly greater according to *t*-tests ($p < 0.01$, respectively). The population for the control day was never greater than the baseline population in 2020. For all days that were sampled, the before-game group was the same as the during-game group ($p = 0.14$). The difference between the before-game group and the late-night group was significant ($p = 0.01$). The during-game group was also significantly different from the late-night group when all sampling days were analyzed together ($p < 0.01$), which indicates the dynamic effect of the population moving into a specific region to follow the game.

2021 Season: Moving to the 2021 season, the estimated population for each gameday was found to be significantly greater than the control ($p < 0.01$ for Tulane, Western Carolina, Nebraska, West Virginia, and Texas Christian; $p = 0.01$ for Iowa State). When compared to one another using an ANOVA, the population for each day is significantly different ($p < 0.01$). For all games in the 2021 season combined, the population in the before-game category was not significantly different from that in the during-game category ($p = 0.87$). The difference in population was also not significant when the after-after category was compared to that in the late-night category ($p = 0.40$). The population in the before-game group was significantly different from that of the after-game ($p < 0.01$) and late-night categories ($p < 0.01$). The population in the during-game group was significantly different from the after-game and late-night categories ($p < 0.01$ for both).

Overall, the population was greatest just before or during the game for all days that had a football game, as seen in Figure 2. This affirms the assumption that people traveled into the city for the football games.

3.2. Concentration Comparisons

Figure 3 presents daily viral concentrations during each season separately, establishing a common hourly time axis. Here, comparisons were made across games within each season, across seasons, and against the control samples, as well as with days surrounding the sampling day (either actual gameday or control). Looking first at within-gameday SARS-CoV-2 concentration variability (defined in Section 2.4), it should be noted that statistically significant comparisons could not be performed because for each time category (before, during, after, and late night), there were only four samples available.

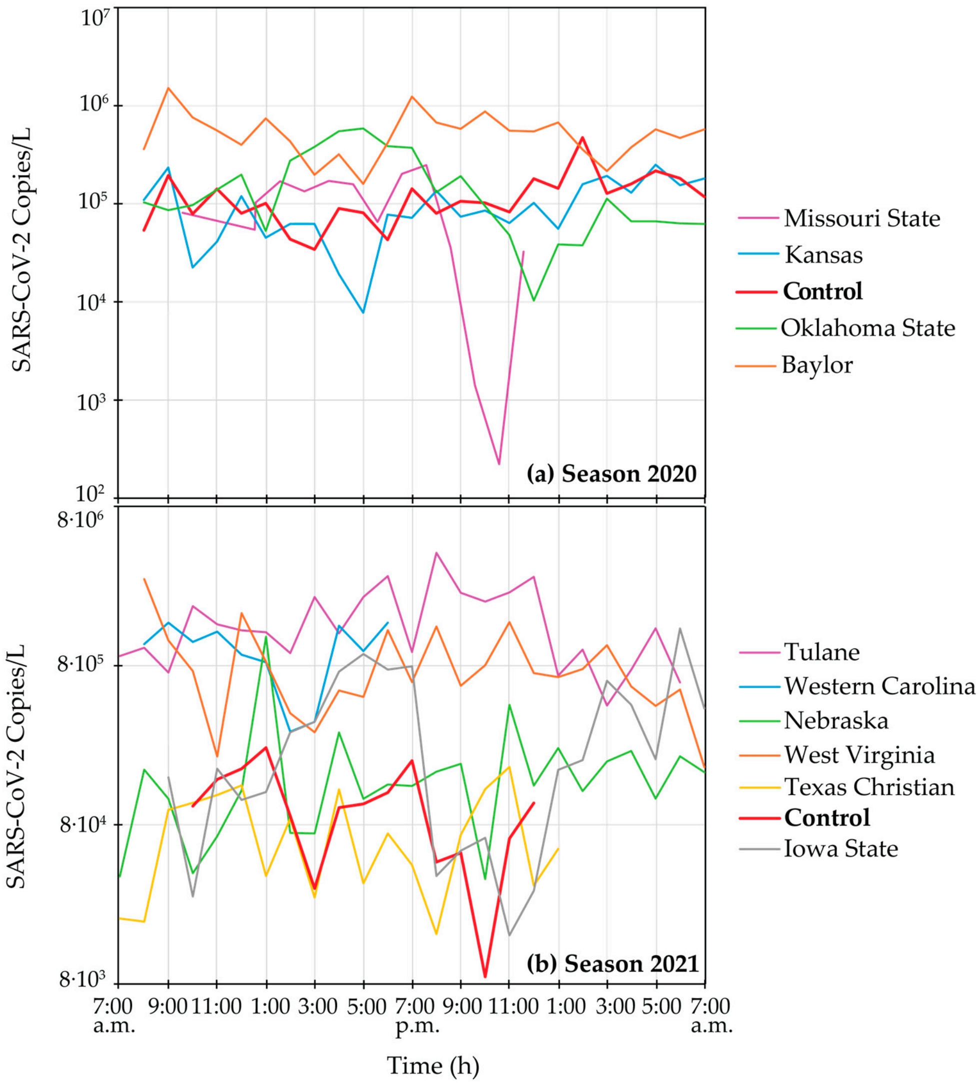

Figure 3. SARS-CoV-2 concentration for all sampling periods on football gamedays for (**a**) the 2020 season; (**b**) the 2021 season. When time series appear to be shorter in duration, this was due to mechanical errors in the autosampler that prevented further sample collection. The games in the legends are listed in chronological order for every season following the same color pattern.

2020 Season: For 2020, the viral concentrations during the Missouri State, Kansas, and Oklahoma State games were not significantly different from those of the control (p = 0.50, 0.25, and 0.95, respectively). However, the concentration from the Baylor game (last game of the 2020 season) samples compared to that of the control day samples was significantly different ($p < 0.01$). Looking at within-gameday time categories, the SARS-CoV-2 concentration was the greatest before the game for the Missouri State, Kansas, and Oklahoma State games. The concentration was greatest for the during-game time category for the Baylor game.

2021 Season: For the 2021 season, the difference in concentration from the control day was significant for the Tulane game ($p < 0.01$), the Western Carolina game ($p < 0.01$), the Nebraska game ($p = 0.04$), and the Iowa State game ($p = 0.02$). The only game with a concentration that was not significantly different from the control was that with Texas Christian ($p = 0.18$). Within the gameday time categories for 2021, the concentration was greatest during the game for the Western Carolina and Nebraska games, was greatest after the game for the Tulane and Iowa State games, and was greatest in the late-night category for the West Virginia and Texas Christian games. The only time groups with significantly different concentrations from each other were the before and after categories when all games were compared together ($p = 0.03$).

To determine if there was an increase in SARS-CoV-2 in wastewater due to football games, gameday samples were compared to composite samples collected from the NWRF on days surrounding the game (Thursday, Friday, and Tuesday). Figure 4 shows the mean concentration of the hourly gameday samples (orange squares) compared to that of the NWRF time-weighted composite samples (blue dots) during the 2020 and 2021 football seasons, respectively.

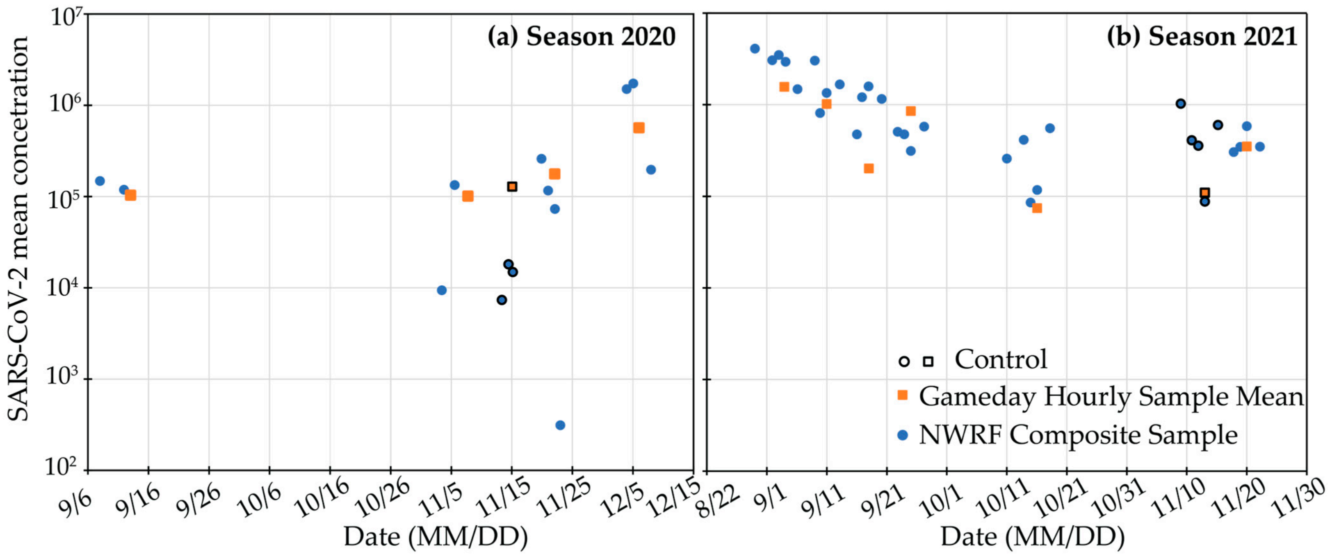

Figure 4. Gameday and control mean SARS-CoV-2 concentrations (blue dots) compared to the concentration from the City of Norman, Oklahoma (NWRF) (orange squares), over the course of the (**a**) 2020 season; (**b**) 2021 season. The control dates are distinguished by a black frame.

2020 Season: For 2020, as the season progressed, the viral concentrations increased, which aligns with the trends in confirmed cases at the same time (refer to Figure 1). The difference in concentration between the NWRF time-weighted composite samples in the surrounding days and the mean of the hourly samples on gameday was not significant for any of the four gamedays in 2020: Missouri State ($p = 0.33$), Kansas ($p = 0.62$), Oklahoma State ($p = 0.68$), and Baylor ($p = 0.38$). However, there was a significant difference between concentrations in the surrounding-day composite samples and the mean concentrations of the hourly samples for the control, non-gameday Saturday ($p < 0.01$).

2021 Season: For the across-day comparison in 2021, it was found that the difference in the concentrations between the surrounding-day NWRF composite samples and the mean of the hourly gameday samples was significant for the Tulane ($p< 0.01$), Nebraska ($p < 0.01$), and Texas Christian ($p = 0.01$) gamedays and was significant during the control non-game Saturday ($p = 0.02$), but was not significant for the Western Carolina ($p = 0.23$), West Virginia ($p = 0.07$), or Iowa State gamedays ($p = 0.41$).

Average gameday concentrations were compared directly to the control Saturday concentration when there was no football game during each season. With all games combined for each season, the concentration during the 2021 season was significantly greater than that during the 2020 season ($p < 0.01$). Overall, the gameday daily average concentrations of SAR-CoV-2 were not significantly different between any monitored gamedays in 2020 and were significantly different between gamedays for half of the games in 2021.

3.3. Viral Load Comparisons

Cell phone and flow data were used with the concentration data to calculate the viral load per capita within the sewershed during each time period. Figure 5 shows the SARS-CoV-2 viral load per capita over time for each sampling day. After normalizing the viral concentration and flow data with the measured population, the differences between each day were more pronounced.

2020 Season: The viral load per capita decreased for all games in the 2020 football season. When compared to each other, the difference in viral load per capita for all games is significantly different ($p < 0.01$). Furthermore, when compared individually to the control day value, the viral load per capita of each gameday was significantly greater ($p < 0.01$ for all tests). Looking next at the temporal variations within the predefined time windows for each game, the mean viral load per capita was greatest in the during-game group. It can also be observed that the viral load per capita had an increasing tendency over time for all games except the Missouri State game, which was the first game of the season. Further investigations were performed to reveal whether strong correlations existed for the viral load per capita and the concentration and population variables independently. For both cases, only a weak correlation was identified with the available data.

2021 Season: For the 2021 season, the viral load per capita was significantly different for the Tulane ($p < 0.01$), Western Carolina ($p < 0.01$), West Virginia ($p < 0.01$), and Iowa State ($p = 0.02$) games. The difference in viral load per person was not significant between the control and Nebraska ($p =0.05$) or Texas Christian ($p = 0.73$) games. These games were combined to analyze the viral load per person for time groups (before, during, after, and late night). The difference in viral load per person for the before group was not significant when compared to that for the during-game category ($p = 0.06$) or the late-night category ($p = 0.70$), but the difference was significant when compared to the after-game category ($p = 0.01$). The viral load per person for the during-game group was not significantly different from that for the after-game ($p = 0.11$) or the late-night categories ($p = 0.52$). The after-game category was not significantly different from the late-night group ($p = 0.41$). Finally, the viral load per capita before the game increased leading up to kickoff for the Tulane, Western Carolina, and West Virginia games and decreased for the Nebraska, Texas Christian, and Iowa State games. The viral load per person at the during-game time scale increased for the Nebraska, West Virginia, and Texas Christian games, but decreased for the Tulane game.

The results of this analysis for the 2021 season are more varied than those for the 2020 season (Figure 5). When compared individually to that of the control day, the viral load per capita of each gameday during the 2020 season was significantly greater ($p < 0.01$ for all tests). For 2021, the viral load per capita for the Tulane, Western Carolina, West Virginia, and Iowa State games were significantly greater than the control value at the $p < 0.03$ level. The viral load per capita for the Nebraska and Texas Christian games were greater than the control value, but not significantly. Overall, the average viral load per capita for the 2021 season was significantly greater than the calculated one for the 2020 season ($p < 0.01$). This variability in 2021 is likely a result of changing COVID-19 activity during the season combined with larger attendance at the games during 2021 compared to that in 2020.

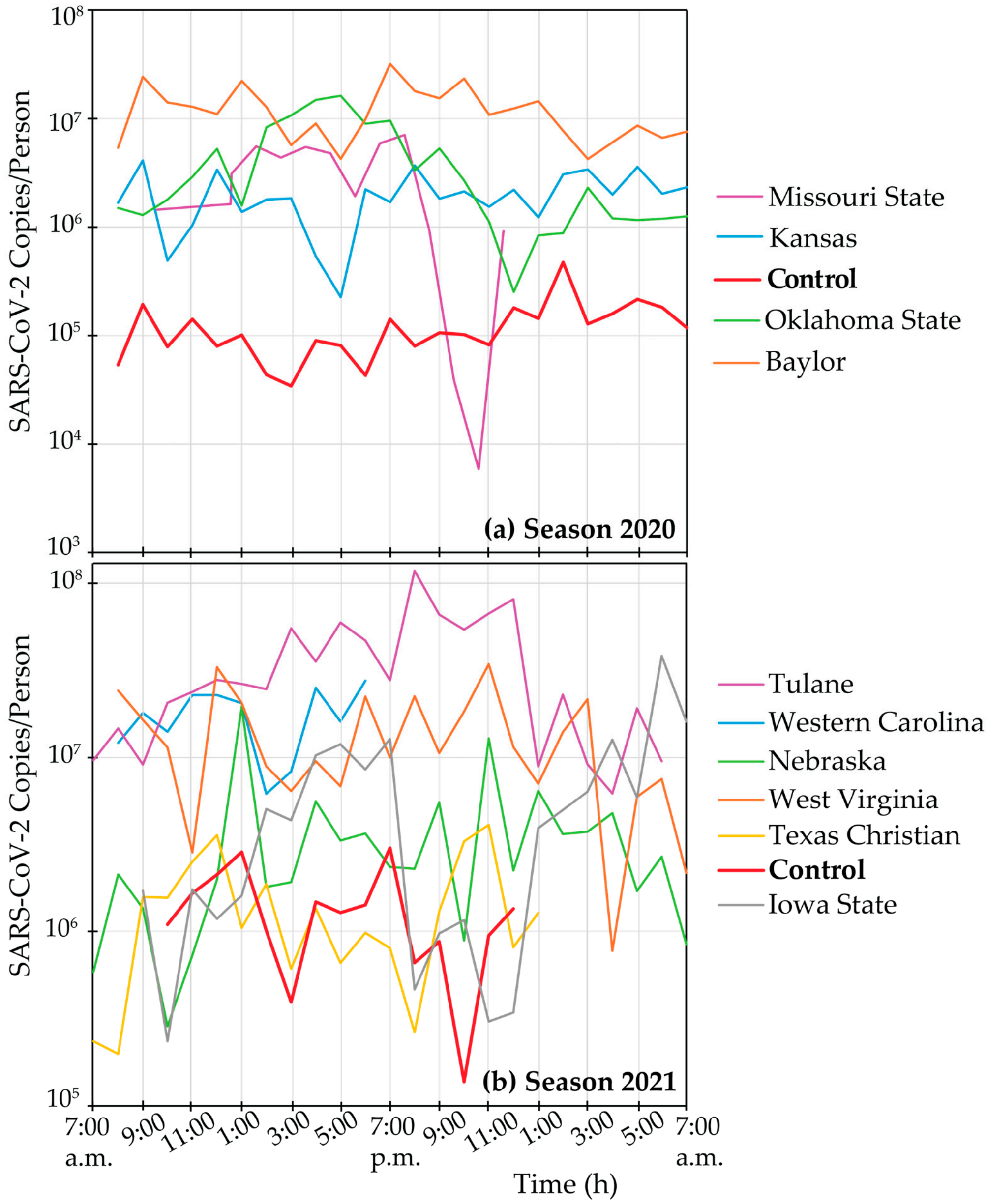

Figure 5. Gameday SARS-CoV-2 viral load per capita for all sampling periods on football gamedays for (**a**) the 2020 season; (**b**) the 2021 season. The games in the legends are listed in chronological order for every season following the same color pattern.

3.4. Season-to-Season Comparisons

When comparing both seasons, 2021 season gamedays had an average population that was significantly greater than that of the 2020 season gamedays, as expected with the university resuming regular operations and holding most classes in person, as well as policies loosening capacity restrictions for large-scale events. Regarding the estimated population, the 2021 football season was significantly greater than the 2020 season ($p < 0.01$). As for the time category levels defined in Section 2.4 (before, during, after, and late night), for both seasons together, the before-game group was not significantly different from the during-game group ($p = 0.27$), nor was the after-game category significantly different from the late-night group ($p = 0.32$). The differences in population were significant when the before-game group was compared to the after-game ($p = 0.04$) and late-night categories ($p < 0.01$), as well as when the during-game category was compared to the after-game ($p = 0.01$) and late-night categories ($p < 0.01$).

The wastewater flow for the 2020 football season was greater than that for the 2021 football season ($p < 0.01$). For both seasons aggregated, the difference in flow was significant when the before-game group was compared to the during-game ($p = 0.02$), after-game ($p < 0.01$), and late-night ($p < 0.01$) groups. The during-game group did not have a significantly different flow compared to that of the after-game category ($p = 0.13$). The flow recorded in the late-night group was significantly different from that of the during- ($p < 0.01$) and after-game categories ($p < 0.01$).

The virus's concentration in the 2021 football season was significantly greater than that in the prior season ($p < 0.01$), as mentioned in Section 3.2. When both seasons were combined, the concentration of the before-game category was not significantly different from that of the during-game ($p = 0.314$) or late-night groups ($p = 0.42$). The concentration in the before-game category was significantly different from that in the after-game category ($p = 0.04$). This clearly indicates that the longer the traveling population resides in the area following an event, the more the concentration of SARS-CoV-2 increases. The differences were not significant for the during-game category compared to the after-game ($p = 0.22$) or late-night category ($p = 0.74$).

The viral load per capita for the 2021 season was significantly greater than that for the 2020 football season ($p < 0.01$). Following the same trends as the concentration, it can be observed that the viral load per capita was able to capture the impact of the traveling population on the recorded viral load: The viral load per person of the before category was not significantly different from that of the during-game ($p = 0.06$) or late-night categories ($p = 0.16$) but was significantly different from that of the after-game category ($p = 0.02$). The viral load per person for the during-game group was not significantly different from that of the after-game ($p = 0.19$) or late-night groups ($p = 0.90$). The viral load per person in the after-game category is not different from that in the late-night category ($p = 0.54$). Table 2 summarizes the means for the flow (CFS), population, concentration (copies/L), and viral load per capita (copies/capita) for each football season.

Table 2. Mean flow (CFS), population, concentration (copies/L), and viral load per capita (copies/capita) for the 2020 and 2021 football seasons.

Season	Flow (CFS)	Population	Concentration (Copies/L)	Viral Load Per Capita (Copies/Capita)
2020	19.72	83,525	2.14×10^5	4.41×10^6
2021	16.26	90,832	5.99×10^5	1.13×10^7

Finally, determining if there were strong relationships between variables such as flow and concentration, population and concentration, flow and viral load per capita, and population and viral load per capita was of great importance. The correlations between the flow and concentration for the 2020 and 2021 football seasons were calculated, with the 2021 season demonstrating a positive trend, but overall, the relationship between these two variables appeared to be slightly negative. For the concentration versus the population, the correlation between these variables was very weak for both seasons, especially when the seasons were combined. The viral load per capita and flow appeared to be uncorrelated in both per-season and aggregated explorations. The 2021 season had a stronger correlation between the two variables than the 2020 season. Finally, for the correlations between the viral load per capita and the population, a slightly stronger relationship than that of the concentration with the population was recorded. The 2020 football season had the weakest correlation between the two variables, though it was positive. While the correlations for the 2021 season and both seasons combined were stronger, they were negative.

4. Discussion

4.1. SARS-CoV-2 Trends

Notably, the amount of SARS-CoV-2 in the wastewater aligns with the trends in cases throughout the seasons (Figure 1). For instance, the Delta variant was at a peak at the start of the 2021 football season, as reflected by the case data at the time, and corresponded to the greatest amount of SARS-CoV-2 in wastewater at the time. A similar phenomenon occurred at the end of the 2020 football season, where confirmed cases increased after Thanksgiving and going into the December holidays. To better capture this, the mean value of the hourly viral load per capita for each game was compared to the number of new cases in Oklahoma during the week leading up to the corresponding game to determine if there was a correlation. There was a positive relationship between the variables, indicating that there appeared to be more SARS-CoV-2 in the wastewater when the weekly cases were greater; however, this relationship was not significant at the 95% confidence level. The NWRF composite samples taken on days surrounding the gameday from the wastewater treatment plant also indicate that the SARS-CoV-2 concentration in wastewater followed the trends of the confirmed cases. The concentration of SARS-CoV-2 in wastewater increased over time for both gameday and NWRF composite samples in 2020. For the 2021 football season, the concentration decreased over time for both the NWRF and gameday samples. Again, the correlation was slightly stronger for the gameday samples (R^2 = 0.48) than for the NWRF samples (R^2 = 0.44). An interesting result from the comparison of the gameday concentration to the NWRF concentration is that for the 2021 football season, only the West Virginia game value was greater than the NWRF value, though the difference was not significant.

Despite these trends aligning with the confirmed cases, the amount of SARS-CoV-2 found in the wastewater was greater in 2021, when there were no restrictions about tailgating or attendance and Oklahoma was experiencing the "Delta surge". In fact, the population, concentration, and viral load per capita were all greater in the 2021 football season than they were in the 2020 football season (the difference was significant for all metrics except relative viral load per capita). This could suggest that at least some of the variability in SARS-CoV-2 was likely due to football game attendance.

Table 3 shows the gameday compared to the control non-game Saturday for that season for the concentration, viral load per capita, population, and flow for every game. Red boxes with an upward-facing arrow indicate that the gameday value was significantly greater than the control value, while yellow boxes with side-to-side arrows indicate that the difference between the gameday and control values was not significant. There were more games that had significantly greater values than the control for any metric of measuring SARS-CoV-2; this was more evident for the 2021 season. When the concentration was used as the SARS-CoV-2 metric for the 2020 season, most of the games did not have a significant difference.

Table 3. Comparison of the SARS-CoV-2 concentration, viral load per capita, and flow for each game compared to the control values for that season *.

Compared to Control	**2020**			**2021**						
	Missouri State	**Kansas**	**Oklahoma State**	**Baylor**	**Tulane**	**Western Carolina**	**Nebraska**	**West Virginia**	**Texas Christian**	**Iowa State**
Concentration	↔	↔	↔	↑	↑	↑	↑	↑	↔	↑
Viral load per person	↑	↑	↑	↑	↑	↑	↔	↑	↔	↑
Population	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
Flow	↑	↔	↑	↔	↑	↔	↔	↔	↔	↔

* Notation: Red boxes with upward arrows indicate that the game value was significantly greater than the control value. Yellow boxes with side-to-side arrows indicate that the game value was not significantly different from the control value.

Across seasons, analysis was improved when the viral load per capita was used as the SARS-CoV-2 metric, revealing more games in the 2021 football season that had within-day variability. The benefit of this metric is that it is not dependent on the population or size of a drainage area and could be compared to anywhere in the world that can also accurately calculate the number of copies per capita.

4.2. Impacts of Population Estimation Errors

Population comparisons between gamedays and non-gamedays for both seasons revealed that the gameday population was significantly greater than the control Saturday population in each case, indicating that more people traveled to Norman for gamedays than left. The baseline population was not dynamically estimated throughout the season, something that might have resulted in inaccurate estimates of viral load per capita. The influence of this is examined next.

The population estimated by SL likely did not accurately estimate the number of people in the sewershed because of potential student migration out of Norman as the season progressed, especially during the latter part of the 2020 football season. However, it does provide a useful estimate for comparison purposes. Table 4 shows the mean and relative standard deviation for the concentration (calculated from triplicate analysis), population change estimates (taken from [64]), and a range of assumed errors in the population base estimate to determine the relative contribution of population base estimates to the overall error of the estimate of the viral load per capita for each game. Other sources of errors, i.e., in the measurement of flow, are considered negligible for this exercise, as specific data were not available for the City of Norman's WRF flow measurements.

Table 4. Estimation of the percentage of error associated with viral load per capita estimates based on assumed percentages of population migration due to students moving out of town.

	Game	Mean Concentration Relative Standard Error (%)	Flow Relative Standard Error (%)	Cell Phone Data Median Error (%)	Total Error (%) from Assumed Standard Error of Baseline Population			
					Migration (%)			
					1%	5%	10%	25%
2020	Missouri State (12 September)	43	3	3	2	9	17	34
	Kansas (7 November)	22	3	3	3	15	26	47
	Control (14 November)	26	3	3	3	14	24	44
	Oklahoma State (21 November)	29	3	3	3	13	22	42
	Baylor (5 December)	17	3	3	4	18	31	53
2021	Tulane (4 September)	14	3	3	5	20	33	56
	Western Carolina (11 September)	17	3	3	4	18	30	52
	Nebraska (18 September)	24	3	3	3	14	25	45
	West Virginia (25 September)	13	3	3	5	21	35	57
	Texas Christian (17 October)	24	3	3	3	14	25	45
	Control (13 November)	24	3	3	3	14	25	45
	Iowa State (20 November)	22	3	3	4	15	27	48

Table 4 demonstrates that the percentage of total error in estimating viral load per capita was less than 20% for most games when there was less than 5% migration in the baseline population. However, for the latter part of the 2020 season, there was a noticeable amount of undocumented migration from campus as COVID-19 cases increased and classes went online after Thanksgiving. If the student movement off campus resulted in a decrease of 25% in the Norman population moving out of town by the time the 2020 Baylor game occurred, these errors could have contributed to a much larger percentage error (56%) corresponding to the population estimates utilizing a static baseline for our calculations. This game also happened to correspond to one of the lower concentration standard error estimates. While these potential biases are not quantified in our analysis, they should be considered when comparing event data that are obtained over a period of time.

4.3. Case Study Limitations/Biases

This case study, beyond the potential baseline population estimation error that was quantified explicitly above, could potentially have some further limitations and biases. As mentioned earlier, the way that SL counts and aggregates individuals within the region of interest by detecting cell phone GPS signals excludes individuals without a smartphone, and its accuracy is dependent on mobile service in the region of interest. Due to the result de-identification that SL performs, it is impossible to know whether all the recorded individuals were moving into the region of interest for the gameday. However, given the college-town character of the City of Norman, the size of gameday populations in the stadium, and the lack of any other major events in the region, any bias introduced by random individuals would have been minor. Other uncontrolled factors, such as the potential correlations between gameday attendance and the team's success that season, gameday weather, and tailgating tickets were not investigated as additional factors that might have created within-season variations. Funding constraints limited the sampling of more control days per season or in more than one location, but it is worth noting that the same site, sampling equipment, and analysis were used across both seasons. Finally, some samples were not successfully obtained due to autosampler issues or did not provide conclusive results after analysis, preventing more robust and consistent comparisons for a small percentage of the gamedays. For wastewater analysis, there is also an inherent uncertainty in the concentrations that were quantified through the triplicate analysis of each sample. However, this uncertainty is generally consistent over time and is not expected to contribute to bias in the analysis. Finally, each hour during the game was represented by a grab sample that was individually sampled and analyzed, so some variability between sampling times may have been missed on this time scale.

5. Conclusions

The impact of major sporting events (college football games) on the concentration and load per capita of SARS-CoV-2 was determined during football weekends for the University of Oklahoma in Norman during the 2020 and 2021 football seasons. Cell phone data were utilized to quantify changes in population over these weekends and for one control (non-football) Saturday during each season. Multiple comparisons were established at various temporal scales (in hourly grouped windows, daily, and seasonally) between the control, non-football days and the gamedays independently, as well as between the seasons. The results indicated that (a) the amount of SARS-CoV-2 in the wastewater was generally greater on gamedays than on the control day, although the time-dependent change in the concentration of SARS-CoV-2 in the wastewater during the gameday was not always significant, and (b) significantly greater amounts of SARS-CoV-2 in the wastewater were detected in the 2021 football season than in the 2020 season regarding the average concentration and viral load per capita, which coincides with the emergence of a more transmissible variant along with the relaxation of statewide and university policies.

This study demonstrated the potential of an hourly sampling method to effectively capture temporal variation in the SARS-CoV-2 concentration during large events. It also showed that by using the flow and population, a viral load per capita can be calculated, which is a useful way to normalize viral concentration data. Such a metric also provided a way to standardize the data to compare days with different populations and circumstances. Finally, this study leveraged the use of mobile device counting technology such as SL to estimate the population for an entire sewershed, a long-desired ability for WBE research.

For public policy makers, this case study demonstrated how hosting large-scale events for COVID-19 and other potential future disease outbreaks may impact public health by introducing the local community to a higher viral load, especially through asymptomatic patients who may unknowingly attend the event or come to the area to participate in event-related activities. In response, we suppose that new patients may emerge proportionally with the introduced viral load, but an increase in cases could certainly be recorded as a result of the gathering and the transmissibility of the virus. Authorities should be aware of such trends, simply because dynamically moving populations cannot be sourced or controlled to avoid viral hotspots in the region, nor can travelers be excluded from attending an event simply because of their home origin.

Future research in this direction should involve a more formal design of experiments around the football season or other events, including more control days, backup samplers, and multiple sampling locations to better isolate and capture the event-day effects on the recorded viral load. In addition to this, sampling should continue in the region of interest for at least a week beyond the gameday weekend to capture new emerging cases, especially in control communities like university dorms, that may have risen due to the gameday population movement. Finally, research should aim to achieve faster turn-around times on analysis, which could enable viral results to be estimated in near real time so that they can be communicated during the event duration to make attendees aware of the increased risk and allow them to act accordingly. Additionally, newer, more sensitive methods for detecting viral load could be used, like droplet digital PCR (ddPCR) [65], to identify time-dependent variations in the viral load with less uncertainty.

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