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Detection of SARS-CoV-2 in urban stormwater: An environmental reservoir and potential interface between human and animal sources

Détection du SARS-CoV-2 dans les eaux pluviales urbaines : un réservoir environnemental et une interface potentielle entre les sources humaines et animales

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RÉSUMÉ

Le SRAS-CoV-2 a été détecté dans les eaux usées, mais il est mal compris dans les eaux pluviales. En raison de la contamination fécale d'origine humaine par les débordements d'égouts sanitaires, les déchets animaux non humains et les tuyaux qui fuient, le virus peut être détectable dans les échantillons d'eaux pluviales. Des voies de contamination comme celles-ci indiquent la possibilité que les eaux pluviales puissent agir comme un réservoir environnemental pour le SRAS-CoV-2 et pourraient être une voie de transmission. Dans cette étude, nous avons déterminé si le SRAS-CoV-2 pouvait être détecté dans les eaux pluviales par PCR numérique à transcription inverse, en utilisant le suivi des sources microbiennes pour quantifier la contamination fécale spécifique à l'homme et étudier la relation entre les caractéristiques des précipitations et les concentrations de flacons. Nous avons surveillé 10 émissaires d'égouts pluviaux dans 3 villes de l'Ohio, chacune drainant une seule utilisation dominante des terres. 25 échantillons ont été prélevés sur ces sites en 2020. 88 % contenaient au moins un gène cible du SRAS-CoV-2 (N2 [US-CDC et CN-CDC] et E). La période sèche antérieure et US-CDC N2 étaient significativement corrélées. Au niveau de la ville, deux relations significatives ont émergé montrant que les villes avaient différents niveaux du gène E. Des corrélations significatives ont confirmé le nombre de cas de COVID-19 et les concentrations de gènes dans le comté et le niveau de la COVID-19 n'ont pas été détectées. Cette recherche a confirmé que la contamination fécale humaine et le matériel génétique du SRAS-CoV-2 sont présents dans les eaux pluviales. On ignore actuellement si les humains peuvent contracter la COVID-19 à partir des eaux pluviales et quels facteurs affectent la longévité et la transmission.

ABSTRACT

Wastewater-based epidemiology has been used to track outbreaks of infectious diseases, including the recent spread of the SARS-CoV-2 virus. Due to human-originated fecal contamination from sanitary sewer overflows, non-human animal waste, and leaky pipes, it was surmised that SARS-CoV-2 may also be detectable in stormwater samples. Contamination pathways indicate that stormwater may act as an environmental reservoir for SARS-CoV-2 and could be a transmission pathway. In this study, we determined whether SARS-CoV-2 could be detected in stormwater samples via reverse transcription-digital droplet PCR, using microbial source tracking to quantify human-specific fecal contamination and study the relationship between rainfall characteristics and viral concentrations. We monitored 10 storm sewer outfalls in 3 Ohio cities each draining a single, dominant land use. 25 samples were collected from these watersheds in 2020. 88% of samples contained at least one SARS-CoV-2 target gene (N2 [US-CDC and CN-CDC] and E). Antecedent dry period and US-CDC N2 were significantly correlated. At the city level, two significant relationships emerged showing cities had different levels of the E gene. County-level COVID-19 case numbers and genes concentrations were not significantly correlated. This research confirmed that human fecal contamination and the genetic material of SARS-CoV-2 are present in stormwater. It is currently unknown whether humans can contract COVID-19 from stormwater

and what factors affect longevity and transmission.

KEYWORDS

Coronavirus, COVID-19, Microbial source tracking, One Health, urban runoff

1 INTRODUCTION

The presence and detection of SARS-CoV-2 has been studied extensively in wastewater, but little is known about presence and transmission of the virus in stormwater. SARS-CoV-2 is shed in fecal matter of infected individuals (Ahmed et al., 2020). Because the virus can be intact and viable in water under specific conditions, stormwater must be investigated as a potential source of infection of SARS-CoV-2 (Sayess et al., 2020). Published studies currently show that SARS-CoV-2 is detectable and viable for up to two weeks in sewage, and thus could cause COVID-19 infections (Zaneti et al., 2020). Studies found that SARS-CoV-2 was shed and detectable in human stool multiple weeks past the cessation of COVID-19 symptoms (Kitajima et al., 2020; Zheng et al., 2020). Even less is known about the ability of SARS-CoV-2 to persist in stormwater.

Through animal feces, leaks between separate sanitary and storm sewers, or other accidental cross-connection, stormwater may contain fecal matter contaminated with SARS-CoV-2 (Figure 1). Stormwater is herein defined as runoff discharging from separated storm sewers into open bodies of water. Positive correlations between population density and higher transmission rates of SARS-CoV-2 have been confirmed, making urban environments more likely hotspots for the virus (Liu, 2020). The magnitude of risk associated with SARS-CoV-2 in surface waters is unclear, especially at varied dilutions.

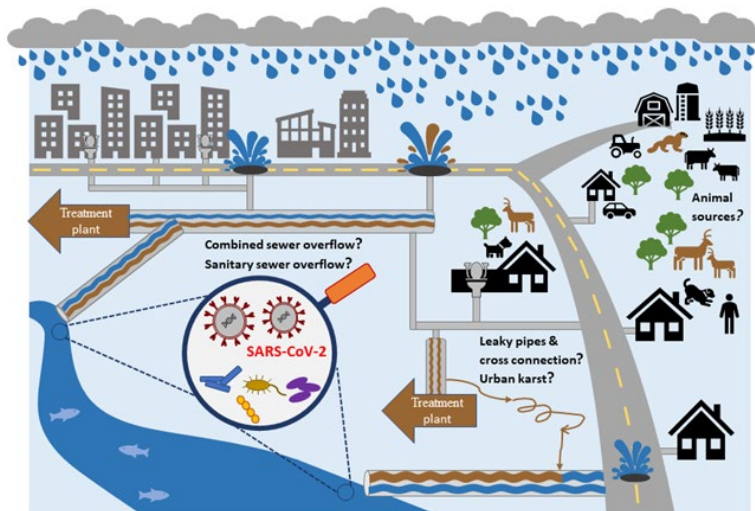


Figure 1. Visual overview of stormwater sample collection, contamination with wastewater, and potential interactions resulting in non-point source fecal contamination

In this study, we set out to determine whether target SARS-CoV-2 genes are detectable in urban stormwater. If so, the magnitude of SARS-CoV-2 genes and human and animal fecal contamination in stormwater would be measured to assess potential risks posed by stormwater-borne SARS-CoV-2. Finally, the relationships between SARS-CoV-2 gene concentrations, rainfall characteristics, and population were assessed to highlight the potential drivers of elevated SARS-CoV-2 gene concentrations in stormwater.

2 METHODS

Stormwater runoff from ten sewersheds in three Ohio counties was monitored from May 10th to July 24th of 2020 for SARS-CoV-2 (i.e., during the peak of the pandemic). Each sewershed, either urban or suburban, was monitored for rainfall and runoff characteristics and characterized by a single, dominant land use. Automated samplers were used to sample wet weather flows. 25 total samples were collected from the sewersheds and grouped by land use and county for analysis; these samples were each representative of the event mean concentrations (EMC). COVID-19 case data was obtained from the Ohio Department of Health COVID-19 dashboard (Ohio Department of Health, 2021).

AllPrep PowerViral DNA/RNA kits (Cat. No. 28000-50; QIAGEN, Germantown, MD, USA) were used to extract cDNA for SARS-CoV-2 gene quantification. The envelope (E) and nucleocapsid (N) SARS-CoV-2 genes were targeted for gene quantification with ddPCR assays. Samples taken from Franklin County were also analyzed for *E. coli* concentration.

The Shapiro-Wilk test was used to assess normality for all data sets. Non-parametric data were analyzed using the Spearman correlation to determine relationships between indicator bacteria, SARS-CoV-2 genes, and rainfall patterns. Sewershed data were grouped by county and correlated to normalized county-level infection data. All analyses discussed in this section were performed using R statistical software. Significant results were determined at $p \leq 0.05$.

3 RESULTS

Twenty-two of 25 stormwater samples (88%) had detectable levels of at least one SARS-CoV-2 gene. Of these 25 samples, 14 were analyzed for and had detectable levels of *E. coli*. Human-specific fecal markers were more than 20 times more concentrated than ruminant-specific fecal markers. Analyses showed significant correlations between the E gene and HF183 concentrations, *E. coli* and the US-CDC N2 gene concentrations. Further, the CN-CDC N2 gene and E gene were also significantly correlated. N gene concentrations were highest in light industrial (LI) and commercial (Comm) sewersheds as shown in Figure 2. Single family residence (SFR) sewersheds had the highest concentration of the E gene with SARS-CoV-2 detected in 94% of SFR samples.

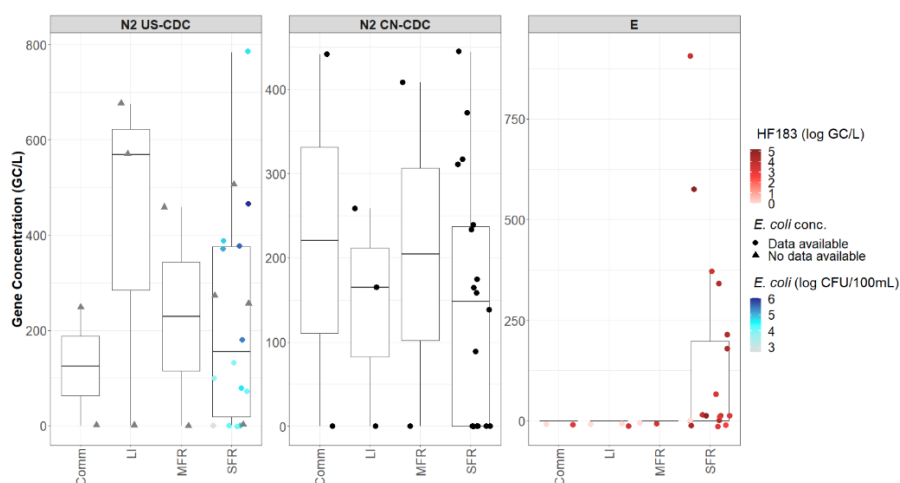


Figure 2. Land use groupings of SARS-CoV-2 target gene concentrations

County-level infection data was not significantly correlated to any of the SARS-CoV-2 genes. Groupings of Columbus, Xenia, and Springboro as well as Columbus and Dayton were found to have significantly different levels of the E gene in stormwater samples, with Dunn and Wilcoxon post-hoc tests showing that Columbus had a significantly higher concentrations than Dayton, Xenia, and Springboro.

4 DISCUSSION

Elevated concentrations of HF183 compared to Rum2Bac and significant correlation between *E. coli* and HF183 strongly suggests most of the fecal matter was human-originated. Rum2Bac was detectable in only 2 of the 25 samples analyzed for SARS-CoV-2. HF183 was detectable in 21 samples. From this, we concluded that human-associated fecal bacteria were most prevalent. Recent studies have found that approximately 33% of white-tailed deer populations in the Midwest have detectable levels of SARS-CoV-2 antibodies in their serum, indicating that deer may also be an environmental reservoir for the virus, though data from this study did not support this conclusion (Hale et al., 2022).

Most rainfall characteristics including duration, depth, and intensity did not correlate with any SARS-CoV-2 genes, possibly due to the negative protein spike charge on the SARS-CoV-2 envelope repelling the negative charges on suspended sediment surfaces. Build-up and wash off processes explain the very strong correlation between antecedent dry period and the SARS-CoV-2 E gene. Low viral stormwater concentrations, a high limit of detection, and the small sample size resulted in the lack of significant correlation observed in this study.

5 CONCLUSIONS

This study confirms that it is possible to utilize wastewater-based viral concentration methods to detect SARS-CoV-2 in other water types, such as stormwater. Though the sensitivity of this study was low, with sufficient improvement in detection technology, these methods could be used to monitor other pathogenic hazards in open waters. Stormwater was found to contain SARS-CoV-2 genetic material in almost all cases. Potential hazards may arise when human fecal contamination is present in stormwater, suggesting future investigation on the threat of viral outbreaks via surface waters where fecal contamination may have occurred. Future studies should investigate whether humans are able to contract SARS-CoV-2 from surface waters and the factors that may affect viral longevity and transmission.

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