Surveillance of SARS-CoV-2 in wastewater of Ohio: Consolidation of SARS-CoV-2 testing from academic, commercial and government labs to Ohio Department of Health Laboratory

J. Saxena*, J. Stiverson, J. Scott, P. Chalasani, S. Anders and E. Leasure.

Ohio Department of Health Laboratory

Ohio Department of Health

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BACKGROUND

Wastewater-based epidemiology (WBE) can be used as a complementary surveillance tool for the early detection of SARS-CoV-2 in wastewater (WW) before an outbreak may be able to be clinically detected [1,2]. Besides, WBE can provide real-time data that utilizes a non-invasive and unbiased (symptomatic, asymptomatic, access to testing) sampling of the whole community [1-3]. In May 2020, *"Ohio Coronavirus Wastewater Monitoring Network"* was started at the Governor's request to provide communities information on SARS-CoV-2 in wastewater to proactively prevent outbreaks (Figure 1) [4]. Routine WW monitoring of SARS-CoV-2 in Ohio started in July 2020. Seventy-seven WW treatment facilities participated in the network and 8 academic, commercial and government (US EPA) labs performed the SARS-CoV-2 testing via different methodologies (liquid supernatant or solid fraction of WW; RT-PCR, digital PCR, and digital droplet PCR). This project was undertaken to consolidate all WW testing sites from different testing labs to Ohio Department of Health Laboratories (ODHL) to utilize a standard method for SARS-CoV-2 testing, which can be used for an optimal comparison of WW data across different sites in Ohio. GT-Molecular Wastewater Surveillance guide and QIAGEN methods for RNA extraction and digital polymerase chain reaction (dPCR) were utilized. By July 2022, 72 WW sites (few sites dropped) were being tested at ODHL.

METHODS

Sites: Seventy-two WW treatment plant sites in Ohio representing a wide spectrum of demographics including rural, suburban, and urban locations were tested for the presence of SARS-CoV-2 (Figure 2).

Sample Collection: Untreated, 24h-composite wastewater samples (1 L) were collected twice per week for 6 months (July 2022 to December 2022, and stored at 4°C until processing. The samples (40 ml) were processed within 2-3 days of sample collection. Each sample was processed, extracted, and analyzed in duplicate.

GT-Molecular method from GT-Digital Wastewater Surveillance Guide was employed for virus filtration, concentration, RNA extraction and SARS-CoV-2 quantification.

Bovine Coronavirus (BCoV) was used as a process/ matrix recovery control.

Innovaprep Concentrating Pipette Select (ICP) was used to concentrate the virus from WW samples.

Nanotrap® Microbiome Particles (Ceres Nanosciences Inc.) were used to concentrate the virus from WW samples.

NOTE: <u>*With the increased number of WW sites (n=72) in this study, the initially used ICP method (hollow fiber tips) to concentrate viruses was replaced by a high throughput method from Ceres Nanociences (magnetic nano beads).*</u>

QIAGEN AllPrep PowerViral DNA/RNA Kit was used to extract RNA from the viral concentrates.

The N2 region of the SARS-CoV-2 nucleocapsid gene was quantified to monitor SARS-CoV-2 in WW via **digital PCR (dPCR)** using **GT-Digital SARS-CoV-2 Wastewater Surveillance Assay Kit** and **QIAcuity One-Step Viral RT-PCR Kit.**

For More Information:

Ohio Coronavirus Wastewater Monitoring Network
https://coronavirus.ohio.gov/dashboards/other-resources/wastewater

Ohio Water Resources Center
https://wrc.osu.edu/wastewater-sars-cov-2/inside-emerging-science-wastewater-surveillance

*Jyotisna.Saxena@odh.ohio.gov

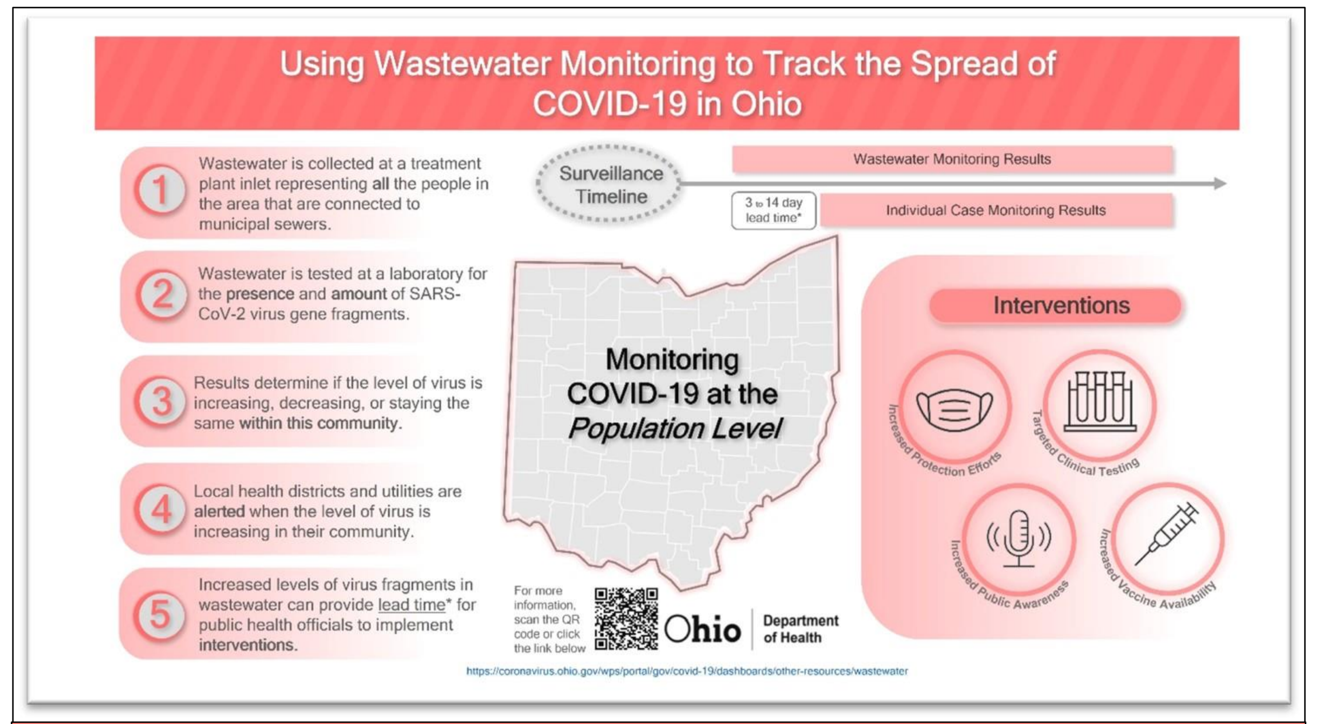

Figure 1. State of Ohio – Wastewater Monitoring of SARS-CoV-2 to track the spread of COVID-19.

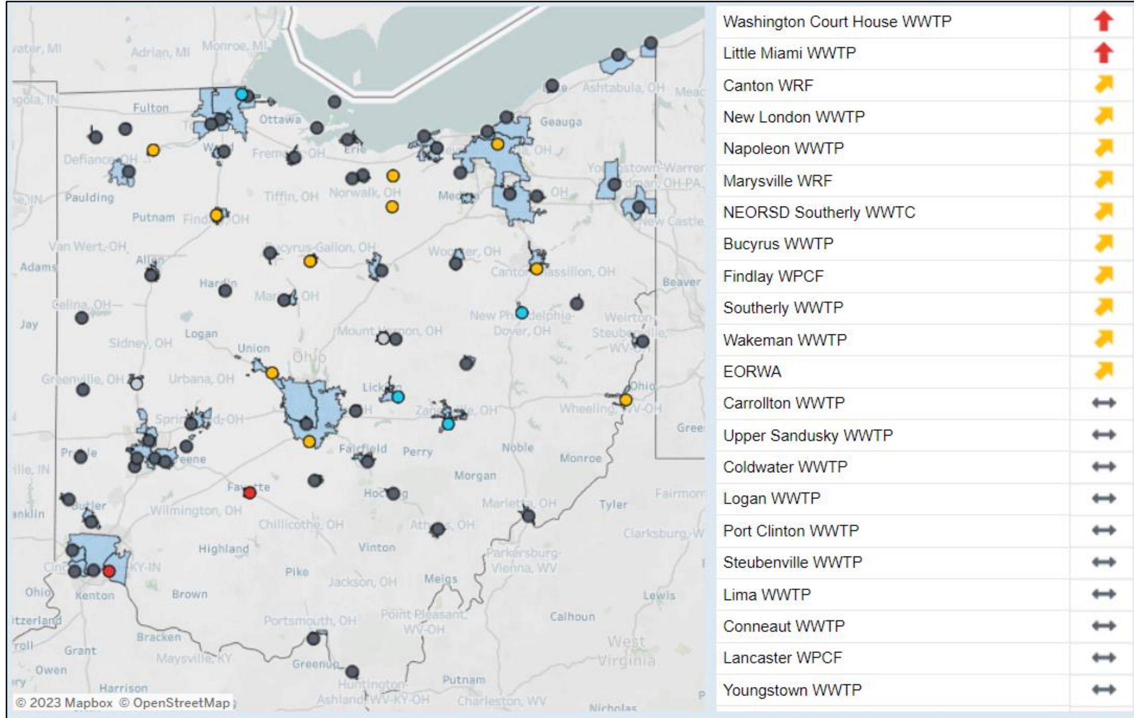

Figure 2. State of Ohio – COVID-19 Wastewater Treatment Plant locations and boundaries.

RESULTS

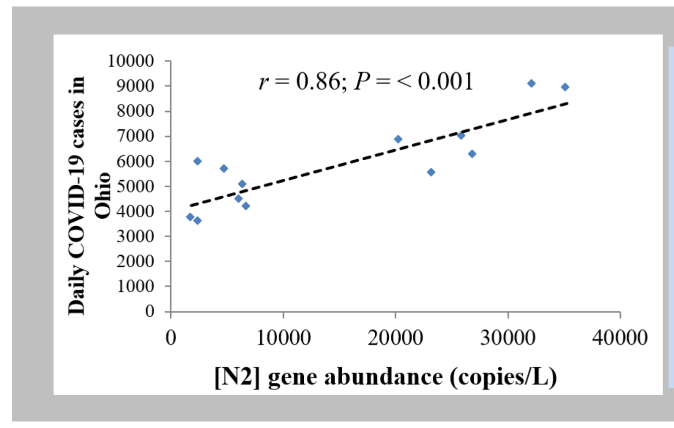

Figure 3. Pearson Correlation between [N2] gene abundances in four WW treatment plants (Jackson Pike, Newark, Lancaster and Zanesville WWTP) and daily COVID-19 cases in Ohio (APHL 2022 poster # 86). *[Note: ICP method was used for virus concentration]*

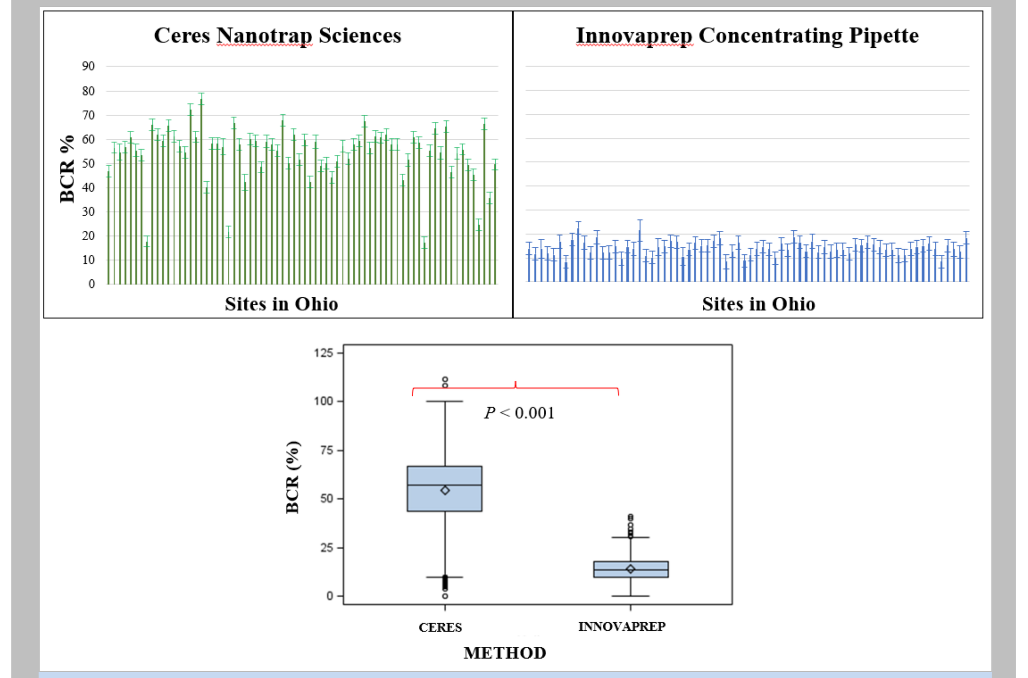

Figure 4: Bovine coronavirus recovery percent (BCR%; ± standard errors) by Ceres Nanotrap Sciences and Innovaprep Concentrating Pipette methods in 72 WW sites of Ohio (upper panels) and ANOVA result comparing two methods (lower panel).

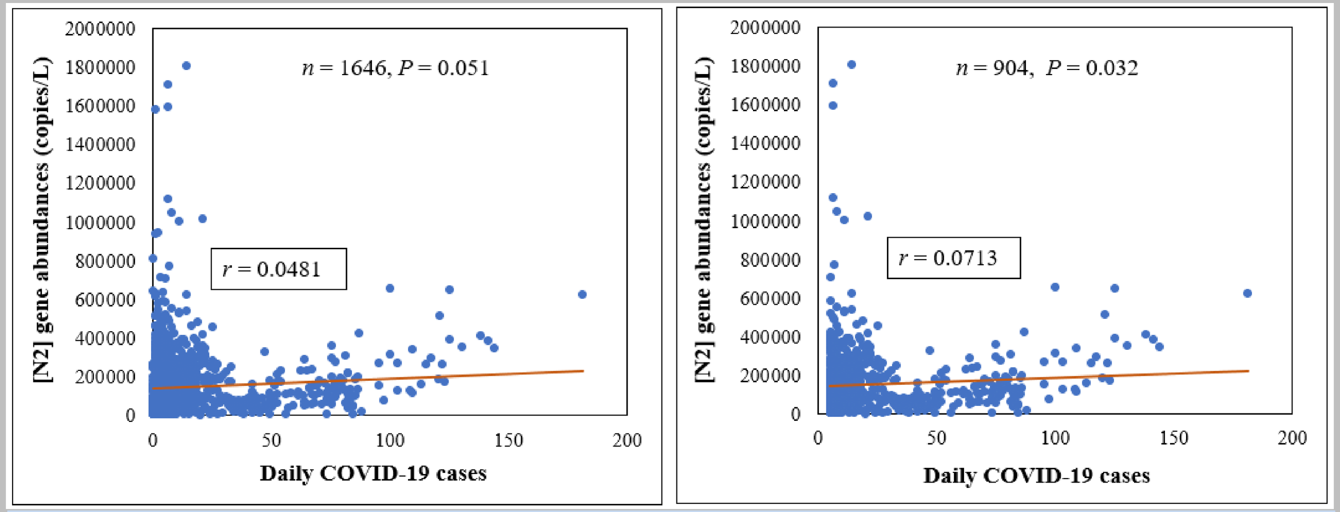

Figure 5: Pearson correlations between number of COVID-19 cases and [N2] copy number of SARS-Cov2 in WW. Left panel shows correlations using all the data and right panel with number of COVID-19 cases > 5. *[Note: Ceres Nanotrap Sciences method used for Virus concentration].*

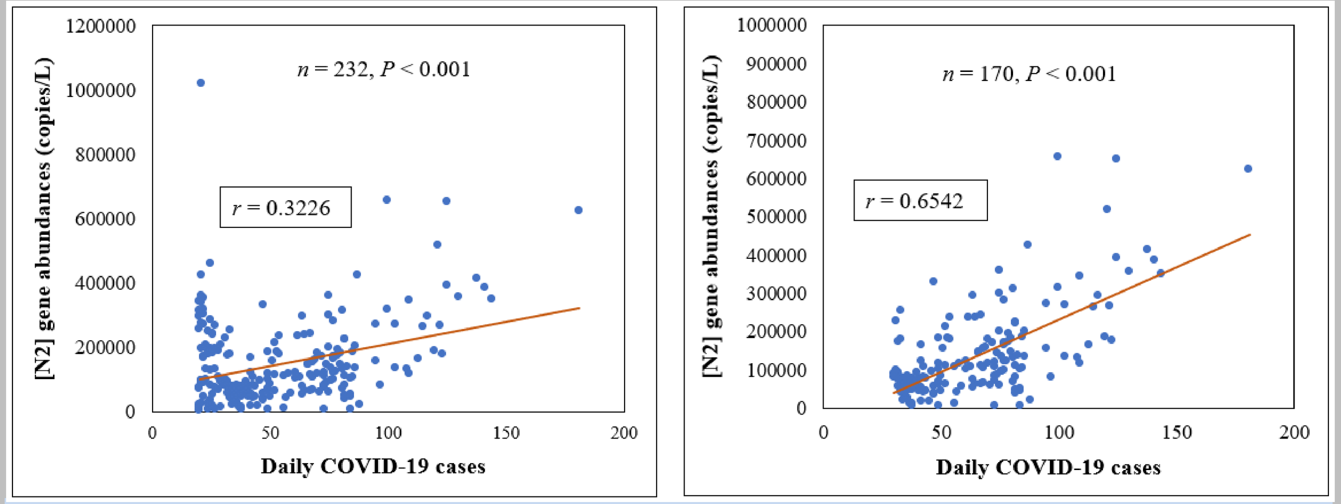

Figure 6: Pearson correlations between number of COVID-19 cases and [N2] copy number of SARS-Cov2 in WW. Left panel shows correlations with the number of COVID-19 cases >20 and right panel with number of cases > 30. *[Note: Ceres Nanotrap Sciences method used for Virus concentration].*

Acknowledgements / Sources

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RESULTS and CONCLUSIONS

- In our pilot study project (***APHL 2022 poster #86:*** SARS-CoV-2 tested at ODHL from 4 WW sites in Ohio from October 2021 to December 2021), the ICP concentration method showed a significant correlation (r = 0.86; P <0.001) between [N2] gene abundances and COVID-19 clinical cases *[**Note:** COVID-19 cases (>3000/day) were on rise during this period]* (Figure 3).
- Recovery of BCR (%) data in WW samples (n = 3058) from 72 sites from July 2022 to December 2022 were subjected to ANOVA analysis. There was a significant effect of viral concentrating method (average BCR from all sites was 54.4% and 14.0% for Ceres and ICP, respectively; P < 0.001) for BCR (%) recovery (Figure 4). SARS-CoV-2 is reported to be present in both liquid and solid fractions of WW [5]. The higher BCR recovery from Ceres method could be due to the use of unfiltered WW sample, whereas ICP method uses the filtrate from 0.2 µm filter.
- The correlation between [N2] gene abundance determined using Ceres method and COVID-19 cases tended to be weakly significant (r = 0.048, P = 0.051 and n = 1646; Figure 5), which was likely due to very low number of cases (12.4 average cases per site) in this period [6]. With an increase in access to home testing kits, the reported cases could be an underrepresentation of true COVID-19 cases [7]. However, there was a significant low correlation (r = 0.071, P = 0.032 and n = 904) when number of COVID-19 cases <5 at any sites were removed (Figure 5). With increasing COVID-19 cases, correlations increased gradually; for example, r = 0.32, P < 0.001 for cases > 20 to r = 0.65, P < 0.001 for cases > 30 (Figure 6).
- In conclusion, ODHL successfully consolidated the 72 WW testing sites from 8 different labs using Ceres Nanosciences concentration method, GT-Molecular Wastewater Surveillance guide and QIAGEN methods for RNA extraction and digital polymerase chain reaction (dPCR) to monitor COVID-19 cases in Ohio, which correlated with [N2] gene abundance of SARS-CoV-2 virus.

NOTE: <u>*Data for the incidences of clinical cases of COVID-19 in Ohio were obtained from ODH COVID-19 dashboard [6].*</u>

CURRENT & FUTURE WORK

Currently ODHL testing SARS-CoV-2 from:

- WW treatment plants – 81 sites
- Ohio Department of Rehabilitation and Correction (Manholes) – 36 sites
- City of Cincinnati sub sewer sheds- 16 sites

Future work: CDC's proposed expansion of National Wastewater Surveillance System (NWSS) testing panel:

- Antibiotic resistance genes
- Pathogen targets (Norovirus, Influenza virus, *Candida auris,* Respiratory Syncytial Virus (RSV), Shiga toxin-producing *E. coli, Campylobacter* sp., *Salmonella* sp., *Cyclospora*)

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