Correlation between Viral Wastewater Concentration and Respiratory Tests, Oregon, USA

Noah Lininger,[1] Rebecca Falender, Paul Cieslak, Arilene Novak, M. Andraya Hendrick, Devrim Kaya, Casey Kanalos, Oumaima Hachimi, David Mickle, Christine Kelly, Tyler Radniecki, Melissa Sutton

Author affiliations: Oregon Health Authority, Portland, Oregon (N. Lininger, R. Falender, P. Cieslak, A. Novak, M.A. Hendrick, M. Sutton); Oregon State University, Corvallis, Oregon, USA (D. Kaya, C. Kanalos, O. Hachimi, D. Mickle, C. Kelly, T. Radniecki).

We evaluated the association between wastewater concentration and weekly percent positivity of patient testing for SARS-CoV-2, influenza, and respiratory syncytial virus in Oregon, USA. We found strong, positive correlations for SARS-CoV-2 (ρ = 0.84, p<0.001), influenza (ρ = 0.73, p<0.001) and respiratory syncytial virus (ρ = 0.69, p<0.001).

Since the 1940s, wastewater surveillance has been used to track pathogens that are shed in feces (1). Testing wastewater for respiratory viral pathogens avoids potential biases found in case-based surveillance methods. Wastewater surveillance captures information from persons who are asymptomatic or not ill enough to seek care, who reside in rural areas or underserved communities with limited access to care, or who test at home and do not report their results (2).

Community transmission of SARS-CoV-2, influenza, and respiratory syncytial virus (RSV) are primarily monitored through test percent positivity. Previous studies have found positive regional correlations between wastewater viral concentration and percent positivity for COVID-19, influenza, and RSV (3–5). In this study, we compared weekly SARS-CoV-2, influenza, and RSV wastewater concentrations with patient test positivity during September 6, 2020–May 11, 2023, in Oregon, USA.

Our study included wastewater-treatment facilities that submitted >1 sample to Oregon's surveillance system during September 6, 2020–May 11, 2023. Sites located on sovereign tribal territories were excluded. Wastewater testing for SARS-CoV-2 was performed year-round. Influenza and RSV wastewater

testing was conducted during the influenza and RSV respiratory seasons (September 15, 2021–June 24, 2022, and August 28, 2022–April 30, 2023, for influenza; August 22, 2022–April 30, 2023, for RSV). We collected 24-hour composite samples from wastewater treatment facility influents 1–2 times weekly. We quantified SARS-CoV-2, influenza, and RSV RNA concentrations by using reverse transcription droplet digital PCR, as described previously (6). We derived primers and probes for SARS-CoV-2 testing from the 2019-nCoV CDC droplet digital PCR triplex probe assay (BioRad Laboratories, https://www.bio-rad.com) and those for influenza testing from the Center for Disease Control and Prevision influenza SARS-CoV-2 multiplex assay (Integrated DNA Technology, https://idtdna.com); we adopted those for RSV testing from previous studies (7). We calculated statewide weekly wastewater viral concentrations by averaging log gene copies per person per day normalized for population and flow across all participating sites. For sites that submitted >1 sample per week, the average concentration of the 2 samples was used.

Human test positivity is the count of positive patient tests divided by the count of tests performed. The National Respiratory and Enteric Virus Surveillance System (NREVSS) is a sentinel laboratory surveillance system that collects aggregate patient test results for 7 viral pathogens including SARS-CoV-2, influenza, and RSV (8). A total of 24 sentinel laboratories in Oregon are registered in NREVSS.

We used pairwise correlation to assess the relationship between the statewide weekly wastewater concentration and human test positivity for each pathogen. We assessed the normality of continuous variables with a visual inspection of histograms and quantile-quantile plots and the Shapiro-Wilk test (α = 0.05). The assumption of normality was not met, so we used the Spearman rank correlation (ρ) for all analyses. We used SAS 9.4 (SAS Institute Inc., https://www.sas.com) for the analyses.

A total of 48 wastewater treatment plants, serving 62.3% of the population of Oregon, submitted >1 sample during the study period. Of 7,185 wastewater samples tested for SARS-CoV-2, a total of 6,910 (96.2%) were positive; of 4,081 wastewater samples tested for influenza, 767 (18.8%) tested positive; and of 1,689 wastewater samples tested for RSV, 473 (28.0%) tested positive. We paired human test positivity with statewide viral concentrations, by week, over 140 weeks for SARS-CoV-2, over 76 weeks for influenza, and over 36 weeks for RSV. We found strong positive correlations between wastewater
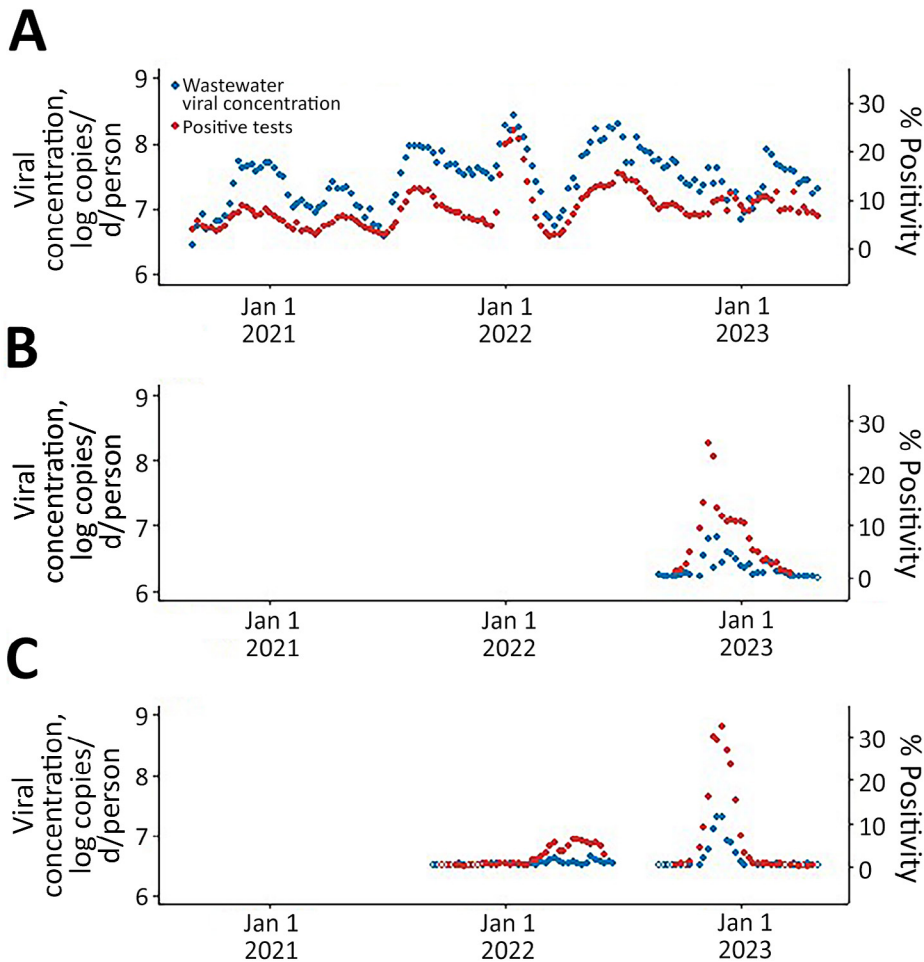
A

B

C

Figure. Statewide weekly wastewater viral concentration (log copies/d/person) and clinical human test positivity over time for respiratory pathogens, Oregon, USA, September 6, 2020–May 11, 2023. A) SARS-CoV-2; B) respiratory syncytial virus; C) influenza. Nondetect values were assigned the mean 1/2 limit of detection for each pathogen. Open circles indicate no detections for that week.

concentration and human test positivity for SARS-CoV-2 (ρ = 0.84, p<0.0001), influenza (0.73, p<0.0001), and RSV (ρ = 0.69, p<0.0001) (Figure).

A limitation of this study is that wastewater surveillance excludes people without access to municipal sewer service. Septic tanks are more prevalent in rural communities than in urban communities, which might have biased our results if people outside of sewersheds are infected at different rates than those within. In addition, whereas SARS-CoV-2 remained a reportable disease throughout the study period, influenza and RSV did not. Fewer influenza and RSV cases might have been reported (outside of NREVSS) during the study period, and statewide human test positivity metrics might not be fully representative of the 3 pathogens, weakening their associations with statewide wastewater data.

Our study found positive correlations between wastewater viral concentration and human test percent positivity for SARS-CoV-2, influenza, and RSV. The strength of association observed suggests wastewater surveillance acts as an indicator of community transmission for those pathogens. Wastewater data are not affected by healthcare-seeking behavior or testing biases, can be analyzed nearly in real-time from pooled community samples, and can be localized to the sewershed level, informing local public health decisions. Our results demonstrate how wastewater surveillance can strengthen our understanding of SARS-CoV-2, influenza, and RSV community transmission.

Acknowledgments

About the Author

At the time of this study, Mr. Lininger was an epidemiologist with the Acute and Communicable Disease Prevention Section at the Oregon Health Authority. His research interests include wastewater surveillance of respiratory viral pathogens and COVID-19 genomic surveillance.

References

1. Schmidt C. Watcher in the wastewater. Nat Biotechnol. 2020;38:917–20. https://doi.org/10.1038/s41587-020-0620-2
2. Larsen DA, Wigginton KR. Tracking COVID-19 with wastewater. Nat Biotechnol. 2020;38:1151–3. https://doi.org/10.1038/s41587-020-0690-1
3. Hopkins L, Persse D, Caton K, Ensor K, Schneider R, McCall C, et al. Citywide wastewater SARS-CoV-2 levels strongly correlated with multiple disease surveillance indicators and outcomes over three COVID-19 waves. Sci Total Environ. 2023;855:158967. https://doi.org/10.1016/j.scitotenv.2022.158967
4. Acosta N, Bautista MA, Waddell BJ, McCalder J, Beaudet AB, Man L, et al. Longitudinal SARS-CoV-2 RNA wastewater monitoring across a range of scales correlates with total and regional COVID-19 burden in a well-defined urban population. Water Res. 2022;220:118611. https://doi.org/10.1016/j.watres.2022.118611
5. Boehm AB, Hughes B, Duong D, Chan-Herur V, Buchman A, Wolfe MK, et al. Wastewater concentrations of human influenza, metapneumovirus, parainfluenza, respiratory syncytial virus, rhinovirus, and seasonal coronavirus nucleic-acids during the COVID-19 pandemic: a surveillance study. Lancet Microbe. 2023;4:e340–8. https://doi.org/10.1016/S2666-5247(22)00386-X
6. Sutton M, Radniecki TS, Kaya D, Alegre D, Geniza M, Girard A-M, et al. Detection of SARS-CoV-2 B.1.351 (Beta) variant through wastewater surveillance before case detection in a community, Oregon, USA. Emerg Infect Dis. 2022;28:1101–9. https://doi.org/10.3201/eid2806.211821
7. Sanghavi SK, Bullotta A, Husain S, Rinaldo CR. Clinical evaluation of multiplex real-time PCR panels for rapid detection of respiratory viral infections. J Med Virol. 2012;84:162–9. https://doi.org/10.1002/jmv.22186
8. Centers for Disease Control and Prevention. The national respiratory and enteric virus surveillance system (NREVSS) [cited 2024 Mar 12]. https://www.cdc.gov/surveillance/nrevss/index.html

Address for correspondence: Noah Lininger, Oregon State University, 116 Johnson Hall, 105 SW 26th St, Corvallis, OR 97331, USA; email: nlininger@gmail.com

Spatiotemporal Epidemiology of Oropouche Fever, Brazil, 2015–2024

Paulo Ricardo Martins-Filho, Thialla Andrade Carvalho, Cliomar Alves dos Santos

Author affiliations: Federal University of Sergipe, Aracaju, Brazil (P.R. Martins-Filho, T.A. Carvalho); Government of Sergipe State, Aracaju (C.A. dos Santos)

We assessed the spatiotemporal dynamics of Oropouche fever in Brazil during 2015–2024. We found the number of cases substantially increased during that period, particularly in the Amazon region. Our findings underscore the need for improved surveillance and public health measures in response to the disease's potential spread beyond endemic areas.

Oropouche fever is an emerging arboviral disease caused by Oropouche virus (OROV) and primarily transmitted by *Culicoides paraensis* biting midges. OROV is endemic to the Americas, predominantly the Amazon region of Brazil; estimates show ≈5 million persons live in areas at high risk for OROV transmission (*1*). Despite potential widespread transmission, Oropouche fever has been neglected, and limited data complicate implementation of effective disease control measures. In Brazil, OROV infection has caused numerous outbreaks, particularly in the Amazon region (*2*), where the climate and forest environment lead to vector proliferation. In 2024, the Pan American Health Organization and World Health Organization issued alerts of increased cases outside the Amazon (*3*) and possible vertical transmission events (*4*). Geographic spread affecting both rural areas and densely populated urban centers in non–Amazon region states underscores the virus' adaptability to varied environments and highlights the urgent need for intensified surveillance and proactive prevention strategies. We assessed the spatiotemporal dynamics of Oropouche fever in Brazil during January 2015–March 2024.

We used anonymized data from the General Coordination of Arbovirus Surveillance of the Ministry of Health (protocol no. 25072.020334/2024-62) and included cases confirmed by reverse transcription PCR or enzyme immunoassay. We extracted information on sex, age, symptom onset, sample collection date, diagnostic method, and location of case notification. We mapped case distributions and calculated cumulative incidence rates per 100,000 inhabitants by using 2022 population census data. We identified high-risk clusters through retrospective spatiotemporal scanning by using SaTScan version 10.1.3 (https://www.satscan.org), QGIS version 3.36.3 (https://qgis.org), and the discrete Poisson model adjusted for population size. For temporal analysis, we used sample collection dates as reference points, given their enhanced precision and reliability within our dataset. We ran Monte Carlo simulations for significance testing and applied the annual percentage change technique by using Joinpoint Regression Program version 5.0.2 (https://surveillance.cancer.gov/joinpoint) to analyze disease incidence trends. We considered p≤0.05 statistically significant in all analyses.