



# Making waves: The benefits and challenges of responsibly implementing wastewater-based surveillance for rural communities

Alasdair Cohen<sup>a,b,\*</sup>, Peter Vikesland<sup>b</sup>, Amy Pruden<sup>b</sup>, Leigh-Anne Krometis<sup>c</sup>, Lisa M. Lee<sup>a,d</sup>, Amanda Darling<sup>a,b</sup>, Michelle Yancey<sup>e</sup>, Meagan Helmick<sup>f</sup>, Rekha Singh<sup>e,g</sup>, Raul Gonzalez<sup>h</sup>, Michael Meit<sup>i</sup>, Marcia Degen<sup>e</sup>, Mami Taniuchi<sup>j,k,l</sup>

<sup>a</sup> Department of Population Health Sciences, Virginia Tech, Blacksburg, VA 24061, USA

<sup>b</sup> Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, VA 24061, USA

<sup>c</sup> Department of Biological Systems Engineering, Virginia Tech, Blacksburg, VA 24061, USA

<sup>d</sup> Division of Scholarly Integrity and Research Compliance, Virginia Tech, Blacksburg, VA 24061, USA

<sup>e</sup> Virginia Department of Health, Office of Environmental Health Services, Richmond, VA 23219, USA

<sup>f</sup> Virginia Department of Health, Mount Rogers Health District, Marion, VA 24354, USA

<sup>g</sup> Department of Civil and Environmental Engineering, Old Dominion University, Norfolk, VA 23529, USA

<sup>h</sup> Hampton Roads Sanitation District, Virginia Beach, VA 23455, USA

<sup>i</sup> Center for Rural Health Research, East Tennessee State University, Johnson City, TN 37614, USA

<sup>j</sup> Department of Biomedical Engineering, University of Virginia, Charlottesville, VA 22908, USA

<sup>k</sup> Department of Civil and Environmental Engineering, University of Virginia, Charlottesville, VA 22908, USA

<sup>l</sup> Department of Medicine, Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, VA 22908, USA

## ARTICLE INFO

### Keywords:

Wastewater-based surveillance  
Wastewater-based epidemiology  
Wastewater-based testing  
Environmental health  
Rural health  
Public Health

## ABSTRACT

The sampling and analysis of sewage for pathogens and other biomarkers offers a powerful tool for monitoring and understanding community health trends and potentially predicting disease outbreaks. Since the early months of the COVID-19 pandemic, the use of wastewater-based testing for public health surveillance has increased markedly. However, these efforts have focused on urban and peri-urban areas. In most rural regions of the world, healthcare service access is more limited than in urban areas, and rural public health agencies typically have less disease outcome surveillance data than their urban counterparts. The potential public health benefits of wastewater-based surveillance for rural communities are therefore substantial – though so too are the methodological and ethical challenges. For many rural communities, population dynamics and insufficient, aging, and inadequately maintained wastewater collection and treatment infrastructure present obstacles to the reliable and responsible implementation of wastewater-based surveillance. Practitioner observations and research findings indicate that for many rural systems, typical implementation approaches for wastewater-based surveillance will not yield sufficiently reliable or actionable results. We discuss key challenges and potential strategies to address them. However, to support and expand the implementation of responsible, reliable, and ethical wastewater-based surveillance for rural communities, best practice guidelines and standards are needed.

## 1. Introduction

The systematic collection and analysis of wastewater samples from wastewater treatment plants, sanitary sewers, and other sewage conveyance systems to inform public health action – an approach often referred to as wastewater-based surveillance (WBS) – offers a powerful tool for monitoring and understanding community health trends and the transmission of infectious diseases (Lee and Thacker, 2011; CDC,

2023a). The potential and promise of WBS is particularly great in regions with limited or no public health surveillance infrastructure and data, and where additional data are needed to supplement clinical surveillance systems, as has been done for diseases such as typhoid fever and polio since as early as the 1920s (Wilson, 1928; Metcalf et al., 1995). If linked case outcome and other related data are available at sufficient spatial, temporal, and quantitative resolutions, WBS can be expanded to wastewater-based epidemiology (WBE) to potentially predict disease

\* Corresponding author at: Department of Population Health Sciences, Virginia Tech, 205 Duck Pond Dr., Blacksburg, VA 24061, USA.

E-mail address: [alasdair.cohen@linacre.oxon.org](mailto:alasdair.cohen@linacre.oxon.org) (A. Cohen).

<https://doi.org/10.1016/j.watres.2023.121095>

Received 27 August 2023; Received in revised form 8 December 2023; Accepted 29 December 2023

Available online 30 December 2023

0043-1354/© 2024 Elsevier Ltd. All rights reserved.

outbreaks in advance of clinical diagnoses. Early in the COVID-19 Pandemic, WBS was used to monitor trends for SARS-CoV-2, the virus that causes COVID-19 (Medema et al., 2020; Zhang et al., 2021). However, the pandemic-driven increase in the use of WBS globally that followed has, to date, focused on urban and peri-urban areas (Medina et al., 2022). As we argue here, the potential public health benefits of expanded WBS for rural communities around the world are substantial - though so too are the associated methodological challenges and ethical hazards.

## 2. Rural health disparities and the potential benefits of rural wastewater-based surveillance

Historically, the vast majority of the global population lived in rural areas. In recent decades, this dynamic shifted and, as of 2018, ~55 % of the global population lived in urban areas (UN, 2018). However, the majority (~70 %) of the world's poorest people reside in rural areas (IFAD, 2010). Globally, 2.5 × more people living in rural areas lack healthcare coverage (~56 %), compared with those in urban areas, and the global deficit in the number of needed healthcare workers in rural areas (~7 million) is more than 2 × that of urban areas (~3 million) (Scheil-Adlung, 2015). Taken together, compared to typical urban settings, on average people in rural areas have less access to healthcare services, rural healthcare providers have less clinical capacity, and health outcome surveillance data are likewise less available in many rural areas.

Substantial rural-urban disparities in healthcare coverage and access are not limited to low- and middle-income countries; disparities continue to persist in some high-income countries as well, including Australia, Canada, and the USA (MacKinnon et al., 2023). While access to healthcare coverage in the USA expanded following passage of the Patient Protection and Affordable Care Act in 2010, rural-urban health inequities endure (Douthitt et al., 2015), and overall rates of uninsured people remain higher in rural areas (12.3 %) relative to urban ones (10.1 %) (USCB, 2019). With regard to disparities in health outcomes, from 2010 to 2017, mortality rates in the USA from cancer, chronic lower respiratory disease, heart disease, and stroke were higher in rural areas (Garcia et al., 2019), and in 2019 mortality rates for the top 10 leading causes of death were all higher in rural vs. urban areas (Curtin and Spencer, 2021). Many smaller rural health district offices in the USA also lack the capacity or staff to conduct public health surveillance, or to offer prevention-based healthcare services (Leider et al., 2020).

Given limitations associated with the availability of clinical surveillance and reportable disease data for many rural and lower-income rural regions, where feasible, the implementation of WBS and WBE could offer substantial informational support for public health surveillance systems and resource allocation decision-making.

## 3. The methodological challenges of implementing wastewater-based surveillance in rural areas

The use of wastewater-based testing for public health surveillance in any context is challenging. Shedding dynamics vary by pathogen type, variant, and infected individual characteristics, and wastewater constituents can inhibit pathogen gene amplification, resulting in false negatives or concentration underestimation (Hrudey and Conant, 2021; Kumblathan et al., 2021; Sims and Kasprzyk-Hordern, 2020; Bertels et al., 2022; Chen and Bibby, 2023). Due to variation in wastewater flows, the timing, frequency, and methods of sample collection can also impact pathogen detection, as can weather and seasonality (Ciesielski et al., 2021; Hamouda et al., 2021; Augusto et al., 2022). Extending WBS to WBE is more challenging still, and requires case outcome and other data (e.g., for population normalization) at appropriate temporal and spatial resolutions.

The size of a sewershed or sub-sewershed, and the size of the catchment population upstream of a sampling point, also impact

pathogen concentrations and the potential for detection (Wilder et al., 2021; Wu et al., 2021). All things being equal, pathogen signals from samples from smaller populations will exhibit more variation compared to those from larger populations. In addition, mobility-related characteristics of upstream populations can impact pathogen loads and detection (Gudra et al., 2022; Wiesner-Friedman et al., 2023), as, for example, in small systems or subsewershed nodes where schools, offices, or commercial areas are situated upstream of sampling points. In samples collected from particularly small populations, aside from periods of widespread infection, on average pathogen concentrations may be more likely to approach analytic limits of detection, given lower likelihoods that individuals in a catchment are infected at any given time; however, this is also dependent on individual shedding rates by pathogen, which could at times serve to increase the likelihood of detection in small catchments. For pathogens with lower shedding rates, such as some arboviral targets, the use of larger wastewater sample volumes (≥500 mL) may be needed to achieve reliable detection (Lee et al., 2022); an approach that may also be helpful for some smaller systems with considerable wastewater dilution (discussed below). In short, more research is needed to better understand indicators of minimum catchment size for WBS.

Many additional challenges arise in low-income and rural settings, where large proportions of the population are often not connected to centralized sanitary sewer collection and treatment systems (Calabria de Araujo et al., 2021). Where rural communities are connected to centralized wastewater treatment, lower population densities mean there is far more sewage infrastructure per capita in rural areas compared with urban areas. For rural systems spread over areas with relatively low population densities, wastewater travel times (i.e., wastewater age) can be relatively long. Available evidence indicates that pathogen decay tends to increase with longer wastewater travel times, and decay is also impacted by physicochemical factors such as temperature (Guo et al., 2022; Wiesner-Friedman et al., 2023).

More miles or kilometers of sewer lines per person in rural areas can also translate to relatively higher operational and capital costs for rural utilities, as compared with their urban counterparts. Because many rural utilities typically do not cover all of their operating and capital expenses from user fees alone (Hughes et al., 2005), and additional funding support is often likewise insufficient, rural utilities often have limited resources available for the identification and repair of broken, obstructed, or otherwise deficient sewage pipes and collection infrastructure. In rural areas facing continued population decline, the ability to cover costs based on user fees becomes increasingly challenging, and in such settings when design and actual flow rates become too divergent, additional operational challenges can also arise.

As a result of these factors and challenges, many older and poorly maintained rural systems are substantially impacted by water inflow and infiltration into wastewater collection infrastructure. Inflow and infiltration (often referred to as I&I) occurs when excess water flows into cracked and broken sewer pipes from groundwater, surface water, or stormwater runoff. Although all large sanitary sewer systems have some degree of inflow and infiltration, it can be more common and more pronounced in older and less-maintained systems. Hydraulic overload from inflow and infiltration can negatively impact sewer system collection and treatment effectiveness, can increase operational costs, and can result in unintentional sanitary sewer overflows (Ellis, 2001; Ellis and Bertrand-Krajewski, 2010; Hey et al., 2016; Karpf and Krebs, 2011; Rezaee and Tabesh, 2022). Untreated wastewater discharges can in turn contaminate downstream recreational and drinking water sources and reservoirs with pathogens, hazardous organic compounds, heavy metals, and other pollutants (Singh et al., 2004; Deblonde et al., 2011; Fewtrell and Kay, 2015).

Simulation studies indicate that inflow, infiltration, temperature, and pH differentials in sanitary sewer systems can impact pathogen decay (Guo et al., 2022; Parra-Arroyo et al., 2023). Thus, in many rural settings, if wastewater samples are collected only at the wastewater

treatment plant inlet – where WBS sampling is typically focused – measurements might not provide an accurate or reliable indicator of pathogen trends across the sewershed.

Initial findings from a 12 month sub-sewershed wastewater sampling and characterization research project by our Virginia Tech team in rural Virginia (USA) (Darling and Cohen, 2022), informed by our previous sub-sewershed WBS and WBE research (Cohen et al., 2022; McQuade et al., 2023), illustrate how mixing and dilution from inflow and infiltration can impact pathogen signals across a sewershed and at the wastewater treatment plant. For example, in this setting – a small town in rural Virginia where the wastewater treatment plant serves a population of <3000 – we observed how measures from repeated sampling across the sewershed for various parameters, such as Chemical Oxygen Demand (Fig. 1, panel A), fecal contamination indicators, such as CrAssphage (Fig. 1, panel B), and specific pathogens (data not shown), are impacted by inflow, infiltration, and other factors, resulting in concentration and gene copy measurements at the wastewater treatment plant inlet (hexagon “A” in Fig. 1) that do not reliably reflect population-adjusted averages for the catchment.

The challenges for successful WBS implementation outlined above, coupled with observations from our WBS work and associated research, help to contextualize likely causes of difficulties public health agencies, such as the Virginia Department of Health, have had when interpreting weekly WBS data from some smaller rural systems participating in statewide Wastewater Surveillance Sentinel Monitoring programs (VDH, 2023). Taken together, practitioner observations and findings from our research indicate that in many smaller, aging, rural systems, WBS based exclusively on sample collection from wastewater treatment plant influent – the norm for existing surveillance programs – will not provide sufficiently accurate, consistent, or actionable data on pathogen trends, and is therefore of limited use for public health surveillance, let alone WBE and outbreak prediction.

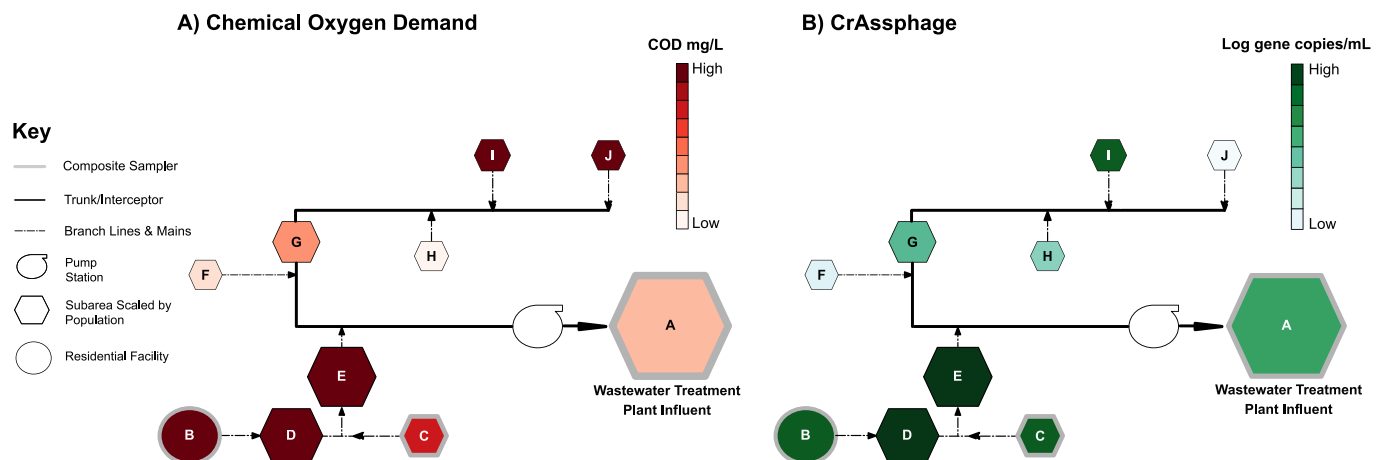
#### 4. Pathways toward the implementation of reliable rural wastewater-based surveillance

While the issues and observations discussed above highlight challenges, they also help elucidate some potential pathways toward more reliable application of WBS in rural settings (Table 1). However, to advance methods development and best-practice guidelines, we believe more rural-focused WBS research is needed, in both low- and middle-income countries, as well as in high-income countries such as the USA.

As we have done for our ongoing research in this domain (Darling and Cohen, 2022), one early step in such a process is collaboration with utilities and other relevant stakeholders to map and characterize key elements of a sewershed. Once sub-sewershed collection nodes, trunk lines, branch lines, and other key components are identified and mapped, targeted sub-sewershed sampling can allow for identification and characterization of the extent of factors such as inflow and infiltration (assessed via measurement of physicochemical parameters, flow, precipitation, etc.). A number of factors may be considered as part of such a process, including: the size of the population served (as well as demographic and socioeconomic data); system size and population density related indicators (e.g., sewer pipe length per capita); wastewater contributors and constituents (e.g., potential inhibitors and differences from commercial, residential, and other nodes); system design; system materials; average system age; soil types and corrosion indicators; minimum flow estimates at various points in the system; number and locations of pump stations and other infrastructure, as applicable; surface water and groundwater characteristics and proximity; and, importantly, indicators of inflow and infiltration.

Once rural systems are sufficiently characterized, and once various sampling approaches have been explored, validated, and implemented, then evidence-based judgements can be made as to whether a system may be suitable for a more conventional WBS approach, or whether alternate approaches are needed to collect and prepare sufficiently representative wastewater samples. Importantly, such data can also help determine if WBS can be implemented in a cost-effective manner and without placing an undue burden on utility staff. For some sites this might entail increased sampling frequency, for others an increase in the number of sampling sites/nodes, an increase in the volumes sampled, or the use of different sampling approaches (e.g., grab samples, or time-weighted or flow-weighted composite samples), or various combinations of such approaches, may be appropriate. Whether or not a given community's system is a candidate for some form of WBS, undertakings such as these also have the benefit of supporting utility efforts to identify and understand infrastructural issues and system deficiencies.

Taking a step back, it is important also to note that in many, if not most, rural settings, sufficient and sustained investment in the improvement, expansion, and maintenance of rural wastewater infrastructure would greatly expand opportunities for the reliable implementation of WBS.



**Fig. 1.** Simplified schematic map showing selected preliminary descriptive results for COD (A) and CrAssphage (B) (enumerated using digital droplet PCR) from repeated samples collected as part of a sub-sewershed wastewater characterization research study in a rural town in Virginia, USA. Shapes represent key sampling points for different nodes of the sewage collection system, with shape size approximately proportional to estimated upstream population size, and arbitrary labeling from A to J (with A representing the wastewater treatment plant inlet sampling point). Due to ethical considerations, and in accordance with our pre-registered protocols and data management plan (Darling and Cohen, 2022), we are not reporting the specific location where this research was conducted, and spatial relationships and distances in this figure have been re-configured for illustrative purposes. Data analysis and figure creation by Amanda Darling (Virginia Tech).

**Table 1**

Selected factors and associated challenges for implementing wastewater-based surveillance in smaller rural systems, and potential strategies for addressing them.

| Factors                              | Associated Challenges                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Potential Strategies                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|--------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Inflow and infiltration              | <ul style="list-style-type: none"> <li>-Understanding the impacts of precipitation, runoff, surface water, and/or groundwater on inflow and infiltration (I&amp;I).</li> <li>-Wastewater mixing and dilution due to I&amp;I can impact pathogen loads, decay rates, and associated detection potential.</li> <li>-Precipitation events can increase variability in pathogen signals and associated measurements.</li> </ul>                                                                                                                                | <ul style="list-style-type: none"> <li>-Model/estimate the impacts of relevant precipitation frequencies and durations on I&amp;I induced dilution (with appropriate adjustment for antecedent precipitation, groundwater recharge, and other context-specific factors), and adjust measured pathogen concentrations (and associated uncertainty estimates) accordingly.</li> <li>-Reduce I&amp;I via repair and/or replacement of sewage collection pipes and infrastructure.</li> </ul>                                                                                     |
| System size and population density   | <ul style="list-style-type: none"> <li>-Increased variation or pathogen signal loss may be higher for systems serving relatively dispersed (lower population density) and/or smaller populations, particularly for pathogens with relatively low shedding rates.</li> <li>-Longer wastewater travel time/age is likely associated with increased pathogen decay and attendant loss of pathogen signal.</li> <li>-Variability in detection of some human fecal indicator organisms (used for normalization) may be higher in smaller sewersheds.</li> </ul> | <ul style="list-style-type: none"> <li>-Increase the number of sampling points based on estimated populations contributing to different nodes in the sewershed, as well as estimates of wastewater travel time/age, and other factors such as I&amp;I.</li> <li>-Measure multiple fecal organism indicators to determine which indicator or indicators may be most appropriate for normalization.</li> <li>-Employ more sensitive pathogen signal detection methods, as well as positive controls, for wastewater treatment plant (WWTP) influent sample analyses.</li> </ul> |
| Sampling timing and methods          | <ul style="list-style-type: none"> <li>-Diurnal and day of the week variation in wastewater constituents and flows can markedly impact measurement of pathogen signals in smaller systems, and in systems vulnerable to substantial I&amp;I.</li> <li>-In smaller systems, and in the presence of I&amp;I, analysis of grab samples may, on average, underestimate pathogen signals over time.</li> </ul>                                                                                                                                                  | <ul style="list-style-type: none"> <li>-Compare pathogen signals and trends using grab and composite (time- and/or flow-weighted) samples collected at or over multiple time-points and various days of the week at the same sampling location/s (e.g., WWTP inlet).</li> <li>-Assess and identify potential methodological sources of variation via the use of sequential and split replicates (and field blanks).</li> <li>-Pathogen target/s depending, evaluate the use of larger sample volumes to improve detection.</li> </ul>                                         |
| WWTP staffing and technical capacity | <ul style="list-style-type: none"> <li>-Many smaller WWTPs have relatively few licensed operators and other staff, meaning their ability to collect samples at the WWTP influent and other locations in the sewershed may often be limited.</li> <li>-WWTP staff may not have sufficient experience programming, using, and/or maintaining composite samplers.</li> </ul>                                                                                                                                                                                  | <ul style="list-style-type: none"> <li>-Assess the need for additional funds for staff training and support, and incorporate it into WBS programs.</li> <li>-For WWTPs with sufficient staff and staff availability, training on the use of composite samplers can be provided, initial sampling runs can be conducted in collaboration with relevant state or local-level agencies, and courier services can be used to collect samples at set days and times for transport to state or local laboratories.</li> </ul>                                                       |

## 5. Ethical challenges for rural wastewater-based surveillance implementation

In addition to methodological considerations (as well as associated laboratory and analytic methods beyond the scope of this article), there are ethical dimensions and challenges associated with the implementation of wastewater-based testing for research and public health surveillance.

When wastewater-based testing is used as a tool for public health surveillance systems it can be considered a routine public health practice activity, and thus subject to relevant public health laws, regulations, and existing ethical guidelines for public health surveillance (Heilig and Sweeney, 2010; WHO, 2017). When wastewater-based testing is to be used for research, protocols should first be reviewed and assessed by a research compliance committee, such as a university institutional review board or research ethics board, to help ensure compliance with regulations for the ethical conduct of research. If personally identifiable information or individual-level data or biospecimens will not be collected or used as part of a proposed research project – the norm for most wastewater-based testing research to date – such projects would not typically be considered human subjects research (HHS, 2021), and so would not be subject to associated ethical review and regulatory requirements. This is potentially problematic in a number of respects, and particularly so when sewage samples are collected from small sewersheds where the potential to link indicators of rare disease outcomes with specific cases exists. While researchers in such cases (and indeed in all cases) are still obligated to conduct research in an ethical fashion, given evolving understandings of the ethical issues in this domain, there are increasing calls for the development of standardized ethical guidelines and review procedures for wastewater-based research specifically (Bowes et al., 2023).

Ethical considerations also extend to sufficient forethought as to how wastewater-based research and WBS data are analyzed, disseminated, and used, and the attendant roles and degrees of participation of local government, community groups, and public health agencies. In smaller rural systems, the privacy implications of publicly reporting pathogen concentrations or trends for small rural communities – or especially for distinct housing clusters in a sewershed – are considerable, given the potential for the high-resolution reporting of relatively rare outcomes or conditions to be linked to individuals living in sampling nodes or sewersheds (Jacobs et al., 2021).

As the field evolves and as assays are developed for more targets and markers (e.g., screening wastewater for individual-level DNA) (Boger and Ozer, 2023), privacy related concerns and challenges will continue to grow in both rural and urban settings. Agencies such as the US CDC have delineated population sizes below which WBS results are publicly suppressed (e.g., 3000 people for SARS-CoV-2) (CDC, 2023b), but as wastewater-based testing research and public health surveillance efforts and applications continue to advance, more nuanced and standardized guidelines and ethical guardrails will be needed, particularly for the application of WBS in small rural communities.

## 6. Conclusions

- The public health benefits of increased application of WBS and WBE in rural areas are potentially substantial.
- As the use of WBS continues to evolve and expand to pathogen targets beyond viruses such as SARS-CoV-2, and to various biomarkers and other health-associated indicators, the potential benefits of WBS should be responsibly and ethically extended to small and lower-income rural communities as well as their urban counterparts.
- A number of strategies may be employed to address common challenges for WBS implementation in smaller rural systems, including assessments of factors contributing to inflow and infiltration and associated impacts on pathogen signals, the use of varied sampling

methods, frequencies, and locations, and other context-specific approaches.

- There is arguably a pressing urgency for more rural-focused research to develop and establish best practice guidelines for the reliable, responsible, cost-effective, and ethical use of WBS and WBE for rural communities in low-, middle-, and high-income countries alike.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Aggregated data may be provided upon reasonable request.

## Acknowledgments

We extend our thanks to the Public Service Authority and wastewater treatment plant staff in southwest Virginia for their collaboration and support. Our southwest Virginia research also benefited from partial funding from Virginia Tech, the University of Virginia at Charlottesville, and the National Science Foundation (Award 2125798). The views expressed in this manuscript are those of the authors and do not necessarily reflect those of Virginia Tech, the University of Virginia, or the National Science Foundation. The funders had no role in study design, data collection, data analysis, the decision to publish, or preparation of this manuscript.

## References

- Augusto, M.R., Claro, I.C.M., Siqueira, A.K., Sousa, G.S., Caldereiro, C.R., Duran, A.F.A., de Miranda, T.B., de Moraes Bomediano, C., Cabral, A.D., de Freitas Bueno, R., 2022. Sampling strategies for wastewater surveillance: evaluating the variability of SARS-CoV-2 RNA concentration in composite and grab samples. *J. Environ. Chem. Eng.* 10, 107478 <https://doi.org/10.1016/j.jece.2022.107478>.
- Bertels, X., Demeyer, P., Van den Bogaert, S., Boogaerts, T., van Nuijs, A.L.N., Delpitte, P., Lahousse, L., 2022. Factors influencing SARS-CoV-2 RNA concentrations in wastewater up to the sampling stage: a systematic review. *Sci. Total Environ.* 820, 153290 <https://doi.org/10.1016/j.scitotenv.2022.153290>.
- Boger, N., Ozer, M., 2023. Monitoring sewer systems to detect the eDNA of missing persons and persons of interest. *Forensic Sci. Int.* 349, 111744 <https://doi.org/10.1016/j.forsciint.2023.111744>.
- Bowes, D.A., Darling, A., Driver, E.M., Kaya, D., Maal-Bared, R., Lee, L.M., Goodman, K., Adhikari, S., Aggarwal, S., Bivins, A., Bohrerova, Z., Cohen, A., Duvallet, C., Elimeiry, R.A., Hutchison, J.M., Kapoor, V., Keenum, I., Ling, F., Sills, D., Tiwari, A., Vikesland, P., Ziels, R., Mansfeldt, C., 2023. Structured Ethical Review for Wastewater-Based Testing in Support of Public Health. *Environ. Sci. Technol.* <https://doi.org/10.1021/acs.est.3c04529>.
- Calabria de Araujo, J., Gavazza, S., Leao, T.L., Florencio, L., da Silva, H.P., de Oliveira Albuquerque, J., de Lira Borges, M.A., de Oliveira Alves, R.B., Rodrigues, R.H.A., dos Santos, E.B., 2021. SARS-CoV-2 sewage surveillance in low-income countries: potential and challenges. *J. Water Health* 19, 1–19. <https://doi.org/10.2166/wh.2020.168>.
- CDC, 2023. National wastewater surveillance system [WWW Document]. Centers for Disease Control and Prevention. URL <https://www.cdc.gov/nwss/wastewater-surveillance.html> (accessed 6.8.23).
- CDC, 2023. COVID data tracker [WWW Document]. Centers for Disease Control and Prevention. URL <https://covid.cdc.gov/covid-data-tracker> (accessed 7.6.23).
- Chen, W., Bibby, K., 2023. Making waves: establishing a modeling framework to evaluate novel targets for wastewater-based surveillance. *Water Res.* 245, 120573 <https://doi.org/10.1016/j.watres.2023.120573>.
- Ciesielski, M., Blackwood, D., Clerkin, T., Gonzalez, R., Thompson, H., Larson, A., Noble, R., 2021. Assessing sensitivity and reproducibility of RT-ddPCR and RT-qPCR for the quantification of SARS-CoV-2 in wastewater. *J. Virol. Methods* 297, 114230. <https://doi.org/10.1016/j.jviromet.2021.114230>.
- Cohen, A., Maile-Moskowitz, A., Grubb, C., Gonzalez, R.A., Ceci, A., Darling, A., Hungerford, L., Fricker Jr., R.D., Finkielstein, C.V., Pruden, A., Vikesland, P.J., 2022. Subwashed SARS-CoV-2 wastewater surveillance and COVID-19 epidemiology using building-specific occupancy and case data. *ES&T Water* 2, 2047–2059. <https://doi.org/10.1021/acsestwater.2c00059>.
- Curtin, S.C., Spencer, M.R., 2021. Trends in death rates in urban and rural areas: united States, 1999–2019. Centers for Disease Control and Prevention - National Center for Health Statistics, NCHS Data brief, no. 417. <https://stacks.cdc.gov/view/cdc/109049>.
- Darling, A., Cohen, A., Subwashed & wastewater characterization, enteropathogen quantification and associated implications for wastewater treatment, surface water quality, & environmental health in rural central Appalachia - Pre-specified study protocols. Open Science Framework. <https://osf.io/kbj9r/>.
- Deblonde, T., Cossu-Leguille, C., Hartemann, P., 2011. Emerging pollutants in wastewater: a review of the literature. *Int. J. Hyg. Environ. Health* 214, 442–448. <https://doi.org/10.1016/j.ijheh.2011.08.002>. The second European PhD students workshop: Water and health ? Cannes 2010.
- Douthit, N., Kiv, S., Dwolatzky, T., Biswas, S., 2015. Exposing some important barriers to health care access in the rural USA. *Public Health* 129, 611–620. <https://doi.org/10.1016/j.puhe.2015.04.001>.
- Ellis, J.B., 2001. Sewer Infiltration/Exfiltration and Interactions with Sewer Flows and Groundwater Quality. Urban Pollution Research Centre, Lisbon, Portugal.
- Ellis, J.B., Bertrand-Krajewski, J.L., 2019. Assessing infiltration and exfiltration on the performance of urban sewer systems (APUSS). IWA Publishing. <https://doi.org/10.2166/9781780401652>.
- Fewtrell, L., Kay, D., 2015. Recreational water and infection: a review of recent findings. *Curr. Environ. Health Rep.* 2, 85–94. <https://doi.org/10.1007/s40572-014-0036-6>.
- Garcia, M.C., Rossen, L.M., Bastian, B., Faul, M., Dowling, N.F., Thomas, C.C., Schieb, L., Hong, Y., Yoon, P.W., Iademarco, M.F., 2019. Potentially excess deaths from the five leading causes of death in metropolitan and nonmetropolitan counties—United States, 2010–2017. *Morb. Mortal. Wkly. Rep. Surveill. Summ.* 68, 1–11.
- Gudra, D., Dejus, S., Bartkevics, V., Roga, A., Kalina, I., Strods, M., Rayan, A., Kokina, K., Zajackina, A., Dumpis, U., Ikkere, L.E., Arhipova, I., Berzins, G., Erglis, A., Binde, J., Ansonsa, E., Berzins, A., Juhna, T., Fridmanis, D., 2022. Detection of SARS-CoV-2 RNA in wastewater and importance of population size assessment in smaller cities: an exploratory case study from two municipalities in Latvia. *Sci. Total Environ.* 823, 153775 <https://doi.org/10.1016/j.scitotenv.2022.153775>.
- Guo, Y., Sivakumar, M., Jiang, G., 2022. Decay of four enteric pathogens and implications to wastewater-based epidemiology: effects of temperature and wastewater dilutions. *Sci. Total Environ.* 819, 152000 <https://doi.org/10.1016/j.scitotenv.2021.152000>.
- Hamouda, M., Mustafa, F., Maraqa, M., Rizvi, T., Aly Hassan, A., 2021. Wastewater surveillance for SARS-CoV-2: lessons learnt from recent studies to define future applications. *Sci. Total Environ.* 759 <https://doi.org/10.1016/j.scitotenv.2020.143493>.
- Heilig, C.M., Sweeney, P., 2010. Ethics in public health surveillance. Lee, L.M., Teutsch, S.M., Thacker, S.B., St. Louis, M.E. (Eds.). Principles & Practice of Public Health Surveillance. Oxford University Press, p. 0. <https://doi.org/10.1093/acprof:oso/9780195372922.003.0009>.
- Hey, G., Jonsson, K., Mattsson, A., 2016. The impact of infiltration and inflow on wastewater treatment plants: A case study in Sweden (No. 06). VA-Teknik Sodra, Sweden. <https://va-tekniksodra.se/wp-content/uploads/2016/12/06-2016-Report-on-Infiltration-Inflow-Hey-et-al-2016.pdf>.
- HHS, 2021. 45 CFR 46 [WWW Document]. US Department of Health and Human Services: Office for Human Research Protections. URL <https://www.hhs.gov/ohrp/regulations-and-policy/regulations/45-cfr-46/index.html> (accessed 8.1.23).
- Hrudey, S.E., Conant, B., 2021. The devil is in the details: emerging insights on the relevance of wastewater surveillance for SARS-CoV-2 to public health. *J. Water Health* 20, 246–270. <https://doi.org/10.2166/wh.2021.186>.
- Hughes, J., Whisnant, R., Weller, L., Eskaf, S., Richardson, M., Morrissey, S., Altz-Stamm, B., Drinking water and wastewater infrastructure in Appalachia: An analysis of capital funding and funding gaps. The university of North Carolina at Chapel Hill and the Appalachia Regional Commission. <https://www.arc.gov/wp-content/uploads/2020/06/DrinkingWaterandWastewaterInfrastructure.pdf>.
- IFAD, 2010. Rural Poverty Report 2011: New realities, New challenges, New Opportunities for Tomorrow's Generation. IFAD, Rome.
- Jacobs, D., McDaniel, T., Varsani, A., Halden, R.U., Forrest, S., Lee, H., 2021. Wastewater monitoring raises privacy and ethical considerations. *IEEE Trans. Technol. Soc.* 2, 116–121. <https://doi.org/10.1109/TTs.2021.3073886>.
- Karpf, C., Krebs, P., 2011. Quantification of groundwater infiltration and surface water inflows in urban sewer networks based on a multiple model approach. *Water Res.* 45, 3129–3136. <https://doi.org/10.1016/j.watres.2011.03.022>.
- Kumblathan, T., Liu, Y., Uppal, G.K., Hrudey, S.E., Li, X.F., 2021. Wastewater-based epidemiology for community monitoring of SARS-CoV-2: progress and challenges. *ACS Environ. Au.* <https://doi.org/10.1021/ACSENVIRONAU.1C00015>.
- Lee, L.M., Thacker, S.B., 2011. The cornerstone of public health practice: public health surveillance, 1961–2011. *MMWR* 60, 15–21.
- Lee, W.L., Gu, X., Armas, F., Leifels, M., Wu, F., Chandra, F., Chua, F.J.D., Syenina, A., Chen, H., Cheng, D., Ooi, E.E., Wurtz, S., Alm, E.J., Thompson, J., 2022. Monitoring human arboviral diseases through wastewater surveillance: challenges, progress and future opportunities. *Water Res.* 223, 118904 <https://doi.org/10.1016/j.watres.2022.118904>.
- Leider, J.P., Meit, M., McCullough, J.M., Resnick, B., Dekker, D., Alfonso, Y.N., Bishai, D., 2020. The state of rural public health: enduring needs in a new decade. *Am. J. Public Health* 110, 1283–1290. <https://doi.org/10.2105/AJPH.2020.305728>.
- MacKinnon, N.J., Emery, V., Waller, J., Ange, B., Ambade, P., Gunja, M., Watson, E., 2023. Mapping health disparities in 11 high-income nations. *JAMA Netw. Open* 6, e2322310. <https://doi.org/10.1001/jamanetworkopen.2023.22310>.
- McQuade, E.T.R., Blake, I.M., Brennhof, S.A., Islam, M.O., Sony, S.S.S., Rahman, T., Bhuiyan, M.H., Resha, S.K., Wettstone, E.G., Hughlett, L., Reagan, C., Elwood, S.E., Mirza, Y., Mahmud, A.S., Hosan, K., Hoque, M.R., Alam, M.M., Rahman, M., Shirin, T., Haque, R., Taniuchi, M., 2023. Real-time sewage surveillance for SARS-CoV-2 in Dhaka, Bangladesh versus clinical COVID-19 surveillance: a longitudinal

- environmental surveillance study (December 2019–December 2021). *Lancet Microbe* 4, e442–e451. [https://doi.org/10.1016/S2666-5247\(23\)00010-1](https://doi.org/10.1016/S2666-5247(23)00010-1).
- Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., Brouwer, A., 2020. Presence of SARS-coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in The Netherlands. *Environ. Sci. Technol. Lett.* 7 <https://doi.org/10.1021/acs.estlett.0c00357>.
- Medina, C.Y., Kadonsky, K., Roman, F.A.R., Tariqi, A.Q., Sinclair, R., D'Aoust, P.M., Delatolla, R., Bischel, H., Naughton, C.C., 2022. The need of an environmental justice approach for wastewater based epidemiology for rural and disadvantaged communities: a review in California. *Curr. Opin. Environ. Sci. Health*, 100348. <https://doi.org/10.1016/j.coesh.2022.100348>.
- Metcalf, T.G., Melnick, J.L., Estes, M.K., 1995. Environmental virology from detection of virus in sewage and water by isolation to identification by molecular biology—A trip of Over 50 years. *Annu. Rev. Microbiol.* 49, 461–487. <https://doi.org/10.1146/annurev.mi.49.100195.002333>.
- Parra-Arroyo, L., Martínez-Ruiz, M., Lucero, S., Oyervides-Muñoz, M.A., Wilkinson, M., Melchor-Martínez, E.M., Araújo, R.G., Coronado-Apodaca, K.G., Velasco Bedran, H., Buitrón, G., Noyola, A., Barceló, D., Iqbal, H.M.N., Sosa-Hernández, J.E., Parra-Saldívar, R., 2023. Degradation of viral RNA in wastewater complex matrix models and other standards for wastewater-based epidemiology: a review. *TrAC Trends Anal. Chem.* 158, 116890 <https://doi.org/10.1016/j.trac.2022.116890>.
- Rezaee, M., Tabesh, M., 2022. Effects of inflow, infiltration, and exfiltration on water footprint increase of a sewer system: a case study of Tehran. *Sustain. Cities Soc.* 79, 103707 <https://doi.org/10.1016/j.scs.2022.103707>.
- Scheil-Adlung, X., 2015. Global evidence on inequities in rural health protection. *New Data on Rural Deficits in Health Coverage for 174 Countries (Working Paper)*. International Labour Organization (UN).
- Sims, N., Kasprzyk-Hordern, B., 2020. Future perspectives of wastewater-based epidemiology: monitoring infectious disease spread and resistance to the community level. *Environ. Int.* 139, 10568.
- Singh, K.P., Mohan, D., Sinha, S., Dalwani, R., 2004. Impact assessment of treated/untreated wastewater toxicants discharged by sewage treatment plants on health, agricultural, and environmental quality in the wastewater disposal area. *Chemosphere* 55, 227–255. <https://doi.org/10.1016/j.chemosphere.2003.10.050>.
- UN, 2018. 2018 revision of world urbanization prospects [WWW Document]. United Nations. URL <https://www.un.org/en/desa/2018-revision-world-urbanization-prospects> (accessed 6.6.23).
- USCB, 2019. Rates of uninsured fall in rural counties, remain higher than urban counties [WWW Document]. United States Census Bureau. URL <https://www.census.gov/library/stories/2019/04/health-insurance-rural-america.html> (accessed 6.6.23).
- VDH, 2023. Wastewater surveillance for COVID-19: Wastewater Surveillance Sentinel Monitoring Program [WWW Document]. Virginia Department of Health. URL <https://www.vdh.virginia.gov/environmental-health/wastewater-surveillance-for-covid-19/> (accessed 6.9.23).
- WHO, 2017. WHO Guidelines on Ethical Issues in Public Health Surveillance. World Health Organization.
- Wiesner-Friedman, C., Brinkman, N.E., Wheaton, E., Nagarkar, M., Hart, C., Keely, S.P., Varughese, E., Garland, J., Klaver, P., Turner, C., Barton, J., Serre, M., Jahne, M., 2023. Characterizing spatial information loss for wastewater surveillance using crassphage: effect of decay, temperature, and population mobility. *Environ. Sci. Technol.* <https://doi.org/10.1021/acs.est.3c05587>.
- Wilder, M.L., Middleton, F., Larsen, D.A., Du, Q., Fenty, A., Zeng, T., Insaif, T., Kilaru, P., Collins, M., Kmush, B., Green, H.C., 2021. Co-quantification of crAssphage increases confidence in wastewater-based epidemiology for SARS-CoV-2 in low prevalence areas. *Water Res.* X (11), 100100. <https://doi.org/10.1016/j.wroa.2021.100100>.
- Wilson, W.J., 1928. Isolation of *B. Typhosus* from sewage and shellfish. *Br. Med. J.* 1, 1061–1062. <https://doi.org/10.1136/bmj.1.3520.1061-a>.
- Wu, F., Xiao, A., Zhang, J., Moniz, K., Endo, N., Armas, F., Bushman, M., Chai, P.R., Duvallet, C., Erickson, T.B., Foppe, K., Ghaeli, N., Gu, X., Hanage, W.P., Huang, K.H., Lee, W.L., McElroy, K.A., Rhode, S.F., Matus, M., Wuertz, S., Thompson, J., Alm, E.J., 2021. Wastewater surveillance of SARS-CoV-2 across 40 U.S. states from February to June 2020. *Water Res.* 202, 117400 <https://doi.org/10.1016/j.watres.2021.117400>.
- Zhang, Y., Cen, M., Hu, M., Du, L., Hu, W., Kim, J.J., Dai, N., 2021. Prevalence and persistent shedding of fecal SARS-CoV-2 RNA in patients with COVID-19 infection: a systematic review and meta-analysis. *Clin. Transl. Gastroenterol.* 12, e00343. <https://doi.org/10.14309/CTG.0000000000000343>. –e00343.