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Charting the future of wastewater-based epidemiology for vector-borne diseases: opportunities, challenges, and climate-driven needs

Tahmina Ahmed, ^a Alessandro Zulli, ^b Farah Ishtiaq, ^c Judith Chui Ching Wong, ^d Juliana Calabria de Araujo, ^e Katrin G. Kuhn,^f Alexandria B. Boehm, ^b Rolf U. Halden,^g Kyle Bibby ^a and Jeseth Delgado Vela ^{*h}

Vector-borne diseases (VBDs) pose a growing public health threat globally, driven by climate change, urbanization, and increasing human mobility. Wastewater-based epidemiology (WBE), which has proven valuable for monitoring enteric and respiratory pathogens, is now being explored as a complementary tool for VBD surveillance. This manuscript synthesizes insights from a 2025 National Science Foundation Research Coordination Network (RCN) workshop (Award # 2202361), which convened researchers and public health professionals on March 13, 2025, to assess the feasibility, challenges, and future directions of WBE for VBD surveillance. The application of WBE to VBDs has several technical and biological challenges, including low and inconsistent shedding of arboviruses in feces and urine, RNA degradation in wastewater, availability of sewer networks for identification of hotspots, geography, and the limited performance of clinical qPCR assays in complex environmental matrices. Newer methods such as metagenomic sequencing and digital PCR (dPCR) offer enhanced sensitivity and detection, but are resource intensive and require additional technical specialization. The strategic selection of sentinel sampling locations such as hospitals, airports, and congregate settings can improve early detection, particularly in non-endemic or travel-associated outbreak contexts. The geographical expansion of competent arboviral vectors have been exacerbated by climate change, urging the development of WBE systems that are adaptable, geographically targeted, and integrated with climate and socio-ecological data. We highlight the need for interdisciplinary collaboration, methodological innovation, and public health engagement to translate WBE signals of vector borne pathogens into timely and actionable responses. As global disease landscapes continue to evolve, WBE may serve as an important early warning system for emerging and re-emerging VBD threats.

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Water impact

The expansion of wastewater surveillance has been rapid since the COVID-19 pandemic, especially towards other respiratory pathogens. There is significant opportunity to apply wastewater surveillance towards vector-borne diseases. Here we summarize some considerations and challenges for monitoring endemic and emerging diseases: climate change impacts on the spread and monitoring value of vector-borne diseases, sentinel monitoring, and translating results to public health action.

^a Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame, Notre Dame, Indiana, USA

^b Department of Civil and Environmental Engineering, Stanford University, Stanford, California, USA

^c Tata Institute for Genetics and Society, Bangalore, India

^d Environmental Health Institute, National Environment Agency, Singapore

^e Department of Sanitary and Environmental Engineering, Federal University of

Minas Gerais, Belo Horizonte, Brazil

^f Hudson College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA

^g School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, Arizona, USA

^h Department of Civil and Environmental Engineering, Duke University, Durham, North Carolina, USA. E-mail: jeseth.delgadovela@duke.edu



Introduction

Vector-borne diseases (VBDs) are among the major public health challenges globally, affecting both urban and rural populations and contributing significantly to the global burden of disease.¹ VBDs are transmitted through vectors such as mosquitoes, ticks, and sandflies and are responsible for illnesses including dengue, malaria, Zika virus disease, chikungunya, yellow fever, Lyme disease, and West Nile virus, among others.^{2–5} According to the World Health Organization (WHO), more than four billion people are at risk of contracting VBDs, and these diseases account for over 700 000 deaths annually.^{1,6} The distribution of VBDs is changing rapidly due to factors such as urbanization, increased human mobility, ecological transformation, and most critically, climate change.^{6–8}

Traditional VBD surveillance relies on clinical case reporting and entomological data. While these systems provide critical insights, they often suffer from underreporting, delayed detection, and limited geographic coverage.^{9,10} Wastewater-based epidemiology (WBE) has emerged as a promising complementary tool that enables population-level, non-invasive surveillance of pathogens through analysis of community sewage. Clinical testing capacity can also be overwhelmed during outbreaks, and in many low- and middle-income countries (LMICs), healthcare access and laboratory diagnostic infrastructure remain limited.¹¹ Moreover, VBDs often manifest with nonspecific symptoms that overlap with other febrile illnesses, complicating diagnosis and case confirmation.¹² Entomological data, while useful, may not be available in real time.

With growing interest in extending WBE to vector-borne pathogens, research is now focused on adapting sampling strategies and molecular tools to overcome the biological and infrastructural challenges unique to VBD surveillance. While WBE has been widely used for monitoring enteric pathogens such as norovirus,^{13,14} poliovirus^{15,16} and respiratory viruses such as SARS-CoV-2^{17,18} and influenza virus,¹⁹ its application to vector-borne diseases is still in early stages. The potential of WBE to serve as a cost-effective, early-warning tool for VBDs is promising, but methodological, biological, and infrastructural challenges must be addressed before widespread adoption can occur.

To explore the frontier of this emerging field, the National Science Foundation funded Research Coordination Network (RCN) on Wastewater Surveillance for SARS-CoV-2 and Emerging Public Health Threats convened a workshop on March 13, 2025, titled “Wastewater-based epidemiology (WBE) for vector-borne pathogens” to explore the potential, challenges, and future directions of WBE in monitoring vector-borne diseases. The workshop included expert presentations and facilitated breakout discussions. Key themes emerging from the event include identifying challenges, climate impacts, sentinel surveillance strategies, and the translation of WBE data into public health action. This manuscript synthesizes workshop discussions and highlights research priorities and implementation strategies.

Identifying challenges and barriers

WBE for vector-borne pathogens faces several technical, operational, and biological challenges (Table 1). The primary challenge is the biology of these pathogens: arboviruses and protozoans such as dengue, chikungunya, Zika, and malaria have uncertain and often low concentrations of fecal or urinary shedding.^{7,16,20} In addition to fecal and urinary routes, shedding through other human bodily fluids, such as blood, saliva, sweat, or respiratory secretion may also contribute viral or parasitic nucleic acids to wastewater, though these pathways remain poorly characterized and probably vary by pathogen and infection stage. Although viremia is well documented for many of these infections, evidence of virus excretion into wastewater through non-fecal routes is still limited and requires further study. In contrast, norovirus^{14,21} and rotavirus²² are excreted in large quantities, ranging from 10⁵ to 10⁹ genome copies per gram of stool for norovirus and 10⁶ to 10⁷ for rotavirus, and are consistently detected in wastewater samples across diverse geographic settings.^{14,23,24} Similar detection consistency been observed for SARS-CoV-2.²⁵ However, for most vector-borne pathogens, concentrations in wastewater are typically at or near detection limits,²⁶ quantitative data on their shedding dynamics which include how long, how frequently, and in what quantities pathogens are shed, as well as their incubation periods remain limited.⁸ Both shedding duration and incubation time are critical parameters for assessing whether wastewater signals can

Table 1 Challenges and barriers in WBE for vector-borne pathogens

| Category | Challenge/barrier |
|-----------------------------------|--|
| Molecular detection | Clinical assays underperform in environmental matrices |
| Shedding patterns | Unknown fecal/urine shedding dynamics for many VBDs; limited understanding of shedding duration and incubation period impacts on detection and early warning |
| Sample integrity | RNA degradation in sewage due to temperature and storage condition |
| Infrastructure | Lack of sustained systems and sample transport in low-resource settings |
| Data interpretation | Ambiguity in associating signals with asymptomatic vs. symptomatic cases |
| Regulatory and logistical | Need for integrated frameworks and capacity building |
| Sampling strategy | Labor-intensive sample collection and privacy concerns at hospitals and sensitive sites; need for hotspot identification in urban and rural settings |
| Sampling and pretreatment methods | Choice of sampling fraction (<i>e.g.</i> , solids vs. supernatant), concentration, and extraction approaches affect detection timing and sensitivity |
| Source attribution | Uncertainty in animal vs. human origin of wastewater signals; need for ecological context |



serve as early warning indicators. This makes it difficult to determine whether a single wastewater signal reflects active community transmission, sporadic travel-associated shedding, or background non-human contamination. Without foundational clinical and animal model studies that explore viral kinetics and excretion pathways, interpretation of wastewater signals will remain speculative. Nevertheless, this knowledge gap also presents an opportunity. Expanded WBE studies that correlate arbovirus signals with clinical case data and case ascertainment rates could help estimate detection thresholds, refine pathogen-specific surveillance models, and ultimately assess the potential of wastewater signals to anticipate outbreaks before clinical systems detect them.

Even when shedding occurs, detection can be challenged by RNA degradation under certain environmental conditions. While recent studies suggest that short RNA fragments may remain stable in wastewater for extended periods,²⁷ high ambient temperatures can still accelerate degradation and reduce detection sensitivity.²⁸ A study found that the persistence of Zika virus RNA decreased with increasing temperature, with notably reduced signal at 35 °C compared to lower temperatures.²⁸ This supports findings that in tropical regions, where wastewater temperatures often exceed 30 °C, RNA integrity may be compromised, reducing detection sensitivity for vector-borne pathogens. One panelist from a tropical region noted that despite confirmed clinical cases and the use of targeted assays, dengue RNA was not detected in wastewater samples. This observation highlights the complexity of wastewater surveillance for arboviruses and may reflect a combination of factors, including low fecal shedding, dilution effects in large catchments, and the potential influence of high ambient temperatures on RNA stability.

Molecular tools designed for clinical settings often underperform in wastewater matrices.²⁹ The CDC Triplex RT-qPCR assay, while validated for detecting dengue, Zika, and chikungunya in patient samples,^{5,30} shows reduced sensitivity when used on sewage due to inhibitors and low target concentrations.³¹ Droplet digital PCR (ddPCR) has emerged as a promising alternative with higher tolerance to inhibitors and improved limits of detection,³⁰ offering advantages particularly in challenging wastewater conditions. However, ddPCR platforms remain relatively costly and may not be feasible in low-resource settings. Metagenomic sequencing and semi-targeted enrichment strategies such as hybrid capture method for whole genome sequencing, have also shown greater promise in detecting low-abundance targets across diverse matrices,³¹ but these approaches are technically demanding, and not yet scalable for routine surveillance. While some of these limitations can be addressed through assay optimization and matrix-specific troubleshooting, the need remains for validated, affordable, and context-appropriate tools that balance sensitivity with accessibility for global implementation.

Sample collection itself poses a logistical bottleneck. Collecting, storing, and transporting wastewater samples requires

significant labor, cold chain logistics, and long-term commitment. In low-resource or decentralized settings, where many VBDs are endemic, these constraints are often insurmountable.

Infrastructural challenges, including the lack of sewage connectivity, fragmented sewer networks, and reliance on decentralized or informal waste treatment, further limit the feasibility of WBE in many global regions.³² An example provided by one panelist was new apartment complexes that operate private sewage treatment plants (STPs) and make centralized sampling infeasible. Additionally, public distrust and privacy concerns can hinder sample collection at hospitals, schools, or transportation hubs. In rural areas, where centralized sewage infrastructure is often absent, open drainage systems, canals, or irrigation ditches can potentially serve as alternative sampling points. These sites could be particularly valuable for monitoring zoonotic or vector-borne pathogens among populations with close contact with animals, such as farmers and agricultural workers. However, open systems also present additional challenges, including higher environmental contamination, fluctuating flow rates, and uncertainty in human waste contribution, which complicate quantitative interpretation of pathogen signals.

The ambiguity of wastewater signals is further complicated by the presence of non-human hosts.³³ In urban and peri-urban areas where humans share environments with primates and rodents, distinguishing the source of pathogen signal can be challenging. For example, metagenomic detection of malaria parasites in wastewater can lead to ambiguous interpretations due to the potential presence of avian and simian *Plasmodium* species, especially in regions where native and non-native birds and nonhuman primates co-occur with humans.³⁴ This raises concerns about cross-species attribution and surveillance specificity.⁸ Without supporting entomological, ecological, clinical, and epidemiological data on malaria transmission in the region, WBE findings can be hard to interpret.

The lack of reliable clinical surveillance for many VBDs complicates validation of wastewater signals. For example, clinical testing is often limited, inconsistent, or reactive in many regions including areas with dengue endemicity.^{8,31} Furthermore, not all infected persons are symptomatic and may not seek clinical care. As a result, WBE and clinical case data are not always expected to corroborate, even in settings with relatively strong surveillance. Taken together, this makes it difficult to establish baselines or determine whether wastewater detection precedes, aligns with, or lags clinical case trends. While the expansion of clinical testing was identified as a critical need, the integration of WBE data with case reporting systems and case ascertainment rates was identified as a key opportunity for research.

Despite these challenges, the workshop emphasized that targeted methodological development, collaborative networks, and region-specific strategies could help overcome barriers. Resource-sharing, cross-training, and international standardization of protocols were mentioned as vital steps for advancing WBE for VBDs.



Monitoring value of endemic vs. emerging pathogens

WBE offers distinct insights when applied to endemic *versus* emerging vector-borne diseases, typically provided as an earlier signal of pathogen presence and as a complementary tool to clinical systems.³⁵ To clarify its value within vector-borne disease surveillance, WBE should be understood not as a replacement for entomological monitoring but as a complementary system that captures infections directly at the human source before they are transmitted back to vectors. For arboviruses such as dengue, Zika, and chikungunya, where humans serve as the primary amplifying host, detecting viral RNA in wastewater provides a direct indicator of the human infection reservoir that subsequently drives mosquito infection. Unlike SARS-CoV-2, where comparisons centered on wastewater *versus* clinical testing, the relevant comparison for VBDs is often wastewater *versus* entomological surveillance. Vector trapping and testing typically detect infected mosquitoes only after transmission is already established, whereas WBE can detect human infections earlier, including asymptomatic or unreported cases. The collective experiences of the co-authors have found that the application of WBE varies based on geographic, epidemiologic, and infrastructural contexts. Here, we highlight case studies that underscore the potential and limitations of WBE across a spectrum of use cases.

In regions with endemic transmission of VBDs such as dengue, WBE data may mirror trends in clinical case detection. For instance, in Miami, Florida, repeated detection of dengue virus serotype 3 in municipal wastewater aligned well with clinical data.² Importantly, viral RNA was often observed in sewage prior to the onset of reported clinical cases, suggesting that WBE could offer an earlier indication of community-level transmission.³⁶ This ability to detect and quantify arboviral signals in near real time may offer significant advantages to public health authorities attempting to make time-sensitive decisions about resources deployment, mosquito control interventions, and risk communication. Nevertheless, each wastewater sample necessitated replicate testing using ddPCR, underscoring the need to evaluate resource allocation for such strategies in comparison to clinical testing availability in endemic settings.

Furthermore, WBE applications in tropical megacities such as Belo Horizonte, Brazil, illustrated some of the practical complexities in endemic settings. The study applied both metagenomic sequencing and RT-qPCR methods to monitor wastewater from hospitals and communities.³¹ Despite relatively sparse clinical reports, sequencing revealed viral reads for chikungunya and dengue viruses in 25–31% of wastewater samples, suggesting ongoing low-level transmission.

In non-endemic or emerging VBD contexts, WBE has also demonstrated utility as an early warning system. For example, one workshop panelist reported the unexpected detection of *Plasmodium falciparum* in wastewater samples collected in New Haven, Connecticut, an area with no known locally acquired malaria cases. This raised the possibility of transient shedding

from travel associated or asymptomatic infections. Such findings are particularly relevant given recent concerns about malaria reemergence in the southeastern United States.^{4,37} Similarly, Singapore's integrated wastewater and entomological surveillance approach detected areas of possible Zika transmission in early 2024 and mid 2025, prompting timely public advisories and vector control operations.^{3,38,39} The dual approach: targeted sampling in affected neighborhoods and broader background monitoring, ensured timely outbreak containment with minimal spread.

The interpretive framework for WBE data should be tailored to the pathogen of interest and the local surveillance objective. For endemic diseases, longitudinal data can help define seasonal baselines and trigger alerts based on deviations from historical norms. For emerging threats, broader surveillance using metagenomics may be warranted, especially when outbreaks are suspected or when zoonotic spillover is plausible. One panelist cautions against overinterpreting WBE results in contexts where host specificity is uncertain or pathogen shedding patterns are poorly understood, as is the case for simian malaria or sylvatic dengue transmission cycles.⁷

Climate change impacts on surveillance needs

Climate change is expanding the geographic distribution of vector species.^{6,40} These environmental changes are increasing the risk of VBD outbreaks in regions previously unaffected, and placing new populations at risk of diseases such as dengue, chikungunya, West Nile virus, and Lyme disease.^{41–43} WBE systems must evolve to meet the demands of a rapidly changing climate.⁷ Pathogen-specific assays may be insufficient in the face of changing disease ecology, where shifting disease ecology and the potential for zoonotic or genetically diverse strains complicate detection.^{44,45} Flexible and adaptive surveillance tools are necessary to capture the complexity of transmission cycles as vector populations move, expand, or overlap in new regions. This adaptability includes the use of multiplex and metagenomic assays capable of detecting multiple pathogens in parallel, especially those associated with zoonotic and sylvatic reservoirs, as well as pathogens previously thought to be regionally confined.

Longitudinal surveillance emerged as a central recommendation from the workshop. Understanding the true impact of climate change on VBD incidence requires sustained monitoring over multiple years, across both wet and dry seasons, and through periods of environmental transition. Though costly and slow to yield immediate returns, these long-term datasets are essential for detecting gradual shifts in transmission patterns, tracking changes in pathogen prevalence and establishing baseline concentrations during both low and high incidence periods.

The interplay between climatic and socio-ecological factors was also highlighted as critical to the interpretation of WBE data. Temperature and rainfall may affect vector biology, but transmission intensity is also shaped by human behavior, urban



infrastructure, and water access.⁴⁶ In some tropical regions, dengue outbreaks aligned with monsoon rains⁴⁷ and increased household water storage due to irregular piped supply. In such settings, intermittent water availability compels residents to store water in open containers, inadvertently creating breeding grounds for *Aedes* mosquitoes.⁴⁸ These socio-ecological dynamics are not easily captured through meteorological data alone, and underscore the need for contextual interpretation when evaluating WBE trends. Importantly, environmental change also affects the infrastructure conditions that determine the quality and interpretability of WBE data. High temperatures can influence wastewater chemistry and microbial activity, potentially reducing nucleic acid stability, while intense rainfall or flooding can dilute sewage, overwhelm open drains or combined systems, and disrupt sampling access. In regions relying on open channels, informal drainage, or decentralized sanitation, these climate-driven impacts directly limit the suitability of WBE and complicate temporal and spatial comparisons.

However, the feasibility of applying WBE for dengue surveillance varies significantly across tropical regions. In many low-resource settings, poor sewage infrastructure, high temperatures, and the presence of wastewater inhibitors hinder the detection of stable viral RNA, limiting the utility of WBE as a complementary surveillance tool.^{8,49} In contrast, WBE may be more feasible in countries such as Brazil, particularly in large urban centers with well-developed sewerage infrastructure such as in São Paulo, Rio de Janeiro, and Belo Horizonte, where centralized collection systems, automated sampling, and established laboratory networks help overcome temperature related challenges.^{31,49} Moreover, practical mitigation strategies, such as rapid sampling processing, use of preservatives (e.g., RNAlater or acidification), and maintaining cold-chain transport possibly can minimize RNA degradation and improve detection reliability even in warmer climates. Nevertheless, challenges persist even in well-served areas, including resource-intensive sample collection and processing, limited access to sequencing technology, and the need for sustained funding and trained personnel.

Sentinel monitoring locations

Strategic placement of sampling points is crucial for effective WBE surveillance of VBDs. Because VBD-associated pathogens are often present in low concentrations in human excreta even during periods of high clinical caseload and exhibit variable shedding patterns, selecting high-yield, representative, and logistically feasible locations is essential.⁵⁰ Panelists demonstrated the importance of establishing sentinel sites which is defined as sampling locations that act as early indicators of disease activity within a defined population or geographic region. These sites, if chosen strategically, can serve as crucial anchors in any WBE network, especially for low-prevalence pathogens or pathogens of emerging concern.⁵⁰

Hospitals and clinical facilities emerged as particularly promising sentinel sites. Sewage from hospitals tends to contain higher concentrations of viral or parasitic nucleic acids, reflecting symptomatic individuals with more advanced infections. Panelists highlighted that arboviral sequences, specifically dengue, chikungunya, and Zika have been detected in hospital and municipal wastewater samples through metagenomic sequencing, though such detections have been limited in number and often coincide with periods of known clinical activity. For example, chikungunya RNA was identified in a single hospital sewage sample in Belo Horizonte, Brazil, during a period when over 400 confirmed cases were reported in the municipality, illustrating both the potential and current limitations of wastewater surveillance for arboviruses.³¹ In Bangalore, India, for example, where the municipal government maintains robust dengue surveillance using ELISA, researchers nonetheless struggled to detect dengue or chikungunya RNA in community wastewater. Panelists noted that sampling from hospital sewage or known transmission hotspots may offer a more effective strategy, given the higher probability of concentrated pathogen shedding from hospitalized patients and reduced dilution compared to broader catchment areas. Although published examples of arbovirus detection in hospital wastewater without corresponding known hospitalized patients remain rare, this capability represents a major value add of WBE. In principle, hospital sewage can capture infections among individuals with mild or atypical symptoms, are misdiagnosed, or never undergo confirmatory laboratory testing. This could allow wastewater to flag early or cryptic transmission that clinical systems miss. Even when no known hospitalized VBD cases are present, detection in hospital wastewater could indicate undetected community transmission, spillover from nearby neighborhoods, or shedding from outpatients or visitors. As a result, hospital-based wastewater surveillance has the potential to function as an early-warning or anomaly-detection system, complementing clinical and entomological surveillance.

Airports and major transport hubs were also highlighted as valuable surveillance locations, especially for travel-associated VBDs such as Zika, dengue, and malaria. These settings present opportunities for early detection of imported cases, particularly during viremic phases when travelers may be shedding viral RNA in bodily fluids. Panelists cited examples from countries like Singapore, where coordinated sampling at border crossings and transport terminals has enabled detection of a range of emerging pathogens, highlighting the potential of aircraft surveillance for global public health monitoring.^{51,52} However, these sites pose unique logistical and ethical challenges. Privacy concerns, regulatory restrictions, and infrastructure constraints can complicate sustained monitoring. Installing sampling devices in airports, bus stations, or customs facilities often requires policy considerations, administrative approval, anonymization and community trust. Other congregate settings, such as schools, universities, dormitories, and military barracks were suggested as high-potential sentinel sites. These dense populations share sanitation infrastructure, increasing the



likelihood of signal amplification during outbreaks. Targeted wastewater surveillance in student dormitories and boarding schools has successfully identified clusters of enteric and respiratory illnesses such as during Covid-19 outbreak.⁵³ Similar applications for VBDs are feasible, particularly during seasons of high transmission or in regions where mosquitoes are prevalent. The sampling frequency and timing are crucial to optimizing detection efficiency and resource use. For example, sampling can be intensified during peak transmission seasons or post-monsoon periods, while lower frequency (*e.g.*, monthly) sampling could be maintained during inter-epidemic phases to reduce operational costs. Temporal alignment of wastewater sampling with known seasonal VBD cycles or vector abundance data can enhance the interpretability of WBE signals and improve early warning potential. While VBDs are not transmitted through the fecal-oral route, infected individuals may shed viral RNA in urine, and studies have confirmed that dengue and Zika RNA can be excreted in detectable amounts during acute infection.^{36,54} Understanding the timing and intensity of this shedding is essential for interpreting results from such settings.

In areas lacking full sewer infrastructure where traditional wastewater-based surveillance is limited or infeasible, a hybrid surveillance approach that combines environmental sampling with vector surveillance was recommended. Mosquito ovitraps and larval sampling in peri-urban neighborhoods of India are used to identify breeding hotspots and guide interventions.⁵⁵ These same locations could be prioritized for focused wastewater sampling, even if they are not connected to centralized sewage systems. For example, community toilets, septic tanks, or open drainage systems could provide wastewater access points for localized monitoring. However, these decentralized systems raise additional concerns regarding sample integrity, pathogen nucleic acid degradation, and contamination from non-human hosts.

Despite the promise of sentinel monitoring, challenges remain. First, the representativeness of samples can vary significantly depending on catchment characteristics and human behaviors. A school, hospital, or neighborhood may not capture the full diversity of infection dynamics, especially in areas with mobile populations or mixed sewer sources. Second, assigning detected pathogens to specific areas or infection sources becomes difficult when wastewater from multiple buildings, neighborhoods, or even species converge. To address these complexities, panelists advocated for the use of geospatial and computational tools to support sentinel site planning. Geographic information systems (GIS), combined with sewer network modeling and population density mapping, can help optimize site selection by identifying the areas of highest infection risk, vulnerability, or transmission potential. In addition, combining temporal datasets across multiple sentinel sites allows for triangulation of signals, which can improve outbreak forecasting and distinguish between noise and true emergence events.^{56,57}

Translation to public health action

One of the primary goals of WBE is to inform timely public health responses. However, translating wastewater signals of VBD into action requires trust, data integration, and effective communication. Panelists warned against overinterpreting preliminary signals, emphasizing that missteps can erode public trust. Engaging public health agencies early and communicating uncertainties clearly were cited as essential steps.⁵⁸ Locally tailored guidelines and integration with national public health frameworks were also recommended to support the effective use of WBE data in outbreak management.

Panelists also noted that successful communication requires distinguishing between detection, risk, and actionable outcomes, especially given the complexity of interpreting vector-borne pathogen signals. WBE was seen as potentially complementary to mosquito-based surveillance.^{3,59} For example, in Bangalore, India, where wastewater is reused for agriculture due to water scarcity, there is a higher prevalence of kala-azar and WBE was viewed as a potentially valuable complement to sand fly-based surveillance. This approach may be especially beneficial in rural areas, where sand flies, the vectors of kala-azar, serve as key disease reservoirs. Panelists stressed that integrating WBE into existing public health surveillance systems will require building a strong evidence base, consistent validation, and careful framing of results.

Conclusions

Wastewater-based epidemiology offers transformative potential for monitoring vector-borne diseases, particularly in the context of climate change, urbanization, and global mobility. The insights from this workshop emphasize the importance of developing robust methodologies, validating detection tools for environmental matrices, understanding shedding patterns, and designing WBE systems that are responsive, inclusive, and actionable. Advancing this field will require cross-sectoral collaboration, targeted investment, and ongoing engagement with public health stakeholders.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

No primary research results, software or code have been included and no new data were generated or analyzed as part of this perspective.

References

- 1 WHO, *Vector-borne diseases*, Geneva, Switzerland, 2024.
- 2 M. K. Wolfe, A. H. Paulos, A. Zulli, D. Duong, B. Shelden, B. J. White and A. B. Boehm, Wastewater Detection of Emerging Arbovirus Infections: Case Study of Dengue in the United States, *Environ. Sci. Technol. Lett.*, 2024, **11**, 9–15.



- 3 J. C. C. Wong, M. Tay, H. C. Hapuarachchi, B. Lee, G. Yeo, D. Maliki, W. Lee, N.-A. Mohamed Suhaimi, K. Chio, W. C. H. Tan and L. C. Ng, Case report: Zika surveillance complemented with wastewater and mosquito testing, *EBioMedicine*, 2024, **101**, 105020.
- 4 V. Bansal, J. Munjal, S. Lakhanpal, V. Gupta, A. Garg, R. S. Munjal and R. Jain, Epidemiological shifts: the emergence of malaria in America, *Bayl. Univ. Med. Cent. Proc.*, 2023, **36**, 745–750.
- 5 G. A. Santiago, J. Vázquez, S. Courtney, K. Y. Matías, L. E. Andersen, C. Colón, A. E. Butler, R. Roulo, J. Bowzard, J. M. Villanueva and J. L. Muñoz-Jordan, Performance of the Triplex real-time RT-PCR assay for detection of Zika, dengue, and chikungunya viruses, *Nat. Commun.*, 2018, **9**, 1391.
- 6 D. Campbell-Lendrum, L. Manga, M. Bagayoko and J. Sommerfeld, Climate change and vector-borne diseases: what are the implications for public health research and policy?, *Philos. Trans. R. Soc., B*, 2015, **370**, 20130552.
- 7 M. B. Diamond, E. Yee, M. Bhinge and S. V. Scarpino, Wastewater surveillance facilitates climate change–resilient pathogen monitoring, *Sci. Transl. Med.*, 2023, **15**, eadi7831.
- 8 F. Ishtiaq, Wastewater-based surveillance of vector-borne pathogens: a cautionary note, *Trends Parasitol.*, 2024, **40**, 93–95.
- 9 S. T. Stoddard, A. C. Morrison, G. M. Vazquez-Prokopec, V. P. Soldan, T. J. Kochel, U. Kitron, J. P. Elder and T. W. Scott, The Role of Human Movement in the Transmission of Vector-Borne Pathogens, *PLoS Neglected Trop. Dis.*, 2009, **3**, e481.
- 10 O. J. Brady, A. Osgood-Zimmerman, N. J. Kassebaum, S. E. Ray, V. E. M. de Araújo, A. A. da Nóbrega, L. C. V. Frutuoso, R. C. R. Lecca, A. Stevens, B. Z. de Oliveira, J. M. de L. Jr, I. I. Bogoch, P. Mayaud, T. Jaenisch, A. H. Mokdad, C. J. L. Murray, S. I. Hay, R. C. R. Jr and F. Marinho, The association between Zika virus infection and microcephaly in Brazil 2015–2017: An observational analysis of over 4 million births, *PLoS Med.*, 2019, **16**, e1002755.
- 11 J. N. Nkengasong, K. Yao and P. Onyebujoh, Laboratory medicine in low-income and middle-income countries: progress and challenges, *Lancet*, 2018, **391**, 1873–1875.
- 12 A. Wilder-Smith, D. J. Gubler, S. C. Weaver, T. P. Monath, D. L. Heymann and T. W. Scott, Epidemic arboviral diseases: priorities for research and public health, *Lancet Infect. Dis.*, 2017, **17**, e101–e106.
- 13 A. B. Boehm, B. Shelden, D. Duong, N. Banaei, B. J. White and M. K. Wolfe, A retrospective longitudinal study of adenovirus group F, norovirus GI and GII, rotavirus, and enterovirus nucleic acids in wastewater solids at two wastewater treatment plants: solid-liquid partitioning and relation to clinical testing data, *mSphere*, 2024, **9**, e00736-23.
- 14 M. Hellmér, N. Paxéus, L. Magnusius, L. Enache, B. Arnholm, A. Johansson, T. Bergström and H. Norder, Detection of Pathogenic Viruses in Sewage Provided Early Warnings of Hepatitis A Virus and Norovirus Outbreaks, *Appl. Environ. Microbiol.*, 2014, **80**, 6771–6781.
- 15 A. F. Brouwer, J. N. S. Eisenberg, C. D. Pomeroy, L. M. Shulman, M. Hindiye, Y. Manor, I. Grotto, J. S. Koopman and M. C. Eisenberg, Epidemiology of the silent polio outbreak in Rahat, Israel, based on modeling of environmental surveillance data, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, E10625–E10633.
- 16 J. M. Deshpande, S. J. Shetty and Z. A. Siddiqui, Environmental Surveillance System To Track Wild Poliovirus Transmission, *Appl. Environ. Microbiol.*, 2003, **69**, 2919–2927.
- 17 A. Bivins, D. North, A. Ahmad, W. Ahmed, E. Alm, F. Been, P. Bhattacharya, L. Bijlsma, A. B. Boehm, J. Brown, G. Buttiglieri, V. Calabro, A. Carducci, S. Castiglioni, Z. Cetecioglu Gurol, S. Chakraborty, F. Costa, S. Curcio, F. L. De Los Reyes, J. Delgado Vela, K. Farkas, X. Fernandez-Casi, C. Gerba, D. Gerrity, R. Girones, R. Gonzalez, E. Haramoto, A. Harris, P. A. Holden, M. T. Islam, D. L. Jones, B. Kasprzyk-Hordern, M. Kitajima, N. Kotlarz, M. Kumar, K. Kuroda, G. La Rosa, F. Malpei, M. Mautus, S. L. McLellan, G. Medema, J. S. Meschke, J. Mueller, R. J. Newton, D. Nilsson, R. T. Noble, A. Van Nuijs, J. Peccia, T. A. Perkins, A. J. Pickering, J. Rose, G. Sanchez, A. Smith, L. Stadler, C. Stauber, K. Thomas, T. Van Der Voorn, K. Wigginton, K. Zhu and K. Bibby, Wastewater-Based Epidemiology: Global Collaborative to Maximize Contributions in the Fight against COVID-19, *Environ. Sci. Technol.*, 2020, **54**, 7754–7757.
- 18 G. Medema, L. Heijnen, G. Elsinga, R. Italiaander and A. Brouwer, 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.*, 2020, **7**, 511–516.
- 19 M. K. Wolfe, D. Duong, B. Shelden, E. M. G. Chan, V. Chan-Herur, S. Hilton, A. H. Paulos, X.-R. S. Xu, A. Zulli, B. J. White and A. B. Boehm, Detection of Hemagglutinin H5 Influenza A Virus Sequence in Municipal Wastewater Solids at Wastewater Treatment Plants with Increases in Influenza A in Spring, 2024, *Environ. Sci. Technol. Lett.*, 2024, **11**, 526–532.
- 20 W. Chen and K. Bibby, A Model-Based Framework to Assess the Feasibility of Monitoring Zika Virus with Wastewater-Based Epidemiology, *ACS ES&T Water*, 2023, **3**(4), 1071–1081.
- 21 P. F. M. Teunis, F. H. A. Sukhrie, H. Vennema, J. Bogerman, M. F. C. Beersma and M. P. G. Koopmans, Shedding of norovirus in symptomatic and asymptomatic infections, *Epidemiol. Infect.*, 2015, **143**, 1710–1717.
- 22 A. Bennett, L. Pollock, K. C. Jere, V. E. Pitzer, B. Lopman, N. Bar-Zeev, M. Iturriza-Gomara and N. A. Cunliffe, Duration and Density of Fecal Rotavirus Shedding in Vaccinated Malawian Children With Rotavirus Gastroenteritis, *J. Infect. Dis.*, 2019, **222**, 2035–2040.
- 23 L. Kittigul, K. Rupprom, Y. Thongpanich, T. Neamhom and F. Utrachkij, Quantification and Genotyping of Norovirus in Aerosols from Wastewater Treatment Plants in Thailand, *Food Environ. Virol.*, 2025, **17**, 31.
- 24 C. A. Valdivia-Carrera, A. C. Ho-Palma, A. Munguia-Mercado, K. Gonzalez-Pizarro, C. Ibacache-Quiroga, A. Dinamarca, M.



- Stehlík, M. Rusiñol, R. Girones, M. T. Lopez-Urbina, A. Basaldua Galarza and E. Gonzales-Gustavson, Surveillance of SARS-CoV-2, rotavirus, norovirus genogroup II, and human adenovirus in wastewater as an epidemiological tool to anticipate outbreaks of COVID-19 and acute gastroenteritis in a city without a wastewater treatment plant in the Peruvian Highlands, *Sci. Total Environ.*, 2023, **905**, 167161.
- 25 F. Wu, J. Zhang, A. Xiao, X. Gu, W. L. Lee, F. Armas, K. Kauffman, W. Hanage, M. Matus, N. Ghaeli, N. Endo, C. Duvallet, M. Poyet, K. Moniz, A. D. Washburne, T. B. Erickson, P. R. Chai, J. Thompson and E. J. Alm, SARS-CoV-2 Titers in Wastewater Are Higher than Expected from Clinically Confirmed Cases, *mSystems*, 2020, **5**, e00614-20.
- 26 C. Stauber, L. C. Jacob-Nascimento, C. Grosch, M. da S. Sousa, M. M. Portilho, R. O. Anjos, M. A. Brinton, U. Kitron, M. G. Reis and G. S. Ribeiro, Presence of dengue virus RNA in urine and oral fluid of laboratory-confirmed dengue patients: Implications for wastewater surveillance, *Braz. J. Infect. Dis.*, 2024, **29**, 104484.
- 27 M. Zhang, L. Roldan-Hernandez and A. Boehm, Persistence of human respiratory viral RNA in wastewater-settled solids, *Appl. Environ. Microbiol.*, 2024, **90**, e02272-23.
- 28 K. Zhu, C. Hill, A. Muirhead, M. Basu, J. Brown, M. A. Brinton, M. J. Hayat, C. Venegas-Vargas, M. G. Reis, A. Casanovas-Massana, J. S. Meschke, A. I. Ko, F. Costa and C. E. Stauber, Zika virus RNA persistence and recovery in water and wastewater: An approach for Zika virus surveillance in resource-constrained settings, *Water Res.*, 2023, **241**, 120116.
- 29 K. Farkas, L. S. Hillary, S. K. Malham, J. E. McDonald and D. L. Jones, Wastewater and public health: the potential of wastewater surveillance for monitoring COVID-19, *Curr. Opin. Environ. Sci. Health*, 2020, **17**, 14–20.
- 30 T. E. Colombo, A. F. Versiani, K. R. Dutra, J. G. D. Rubiato, T. M. Galvão, A. F. Negri Reis and M. L. Nogueira, Performance of CDC Triplex qPCR during a dengue outbreak in Brazil, *J. Clin. Virol.*, 2019, **121**, 104208.
- 31 J. Calabria de Araujo, A. P. A. Carvalho, C. D. Leal, M. Natividade, M. Borin, A. Guerra, N. Carobin, A. Sabino, M. Almada, M. C. M. Costa, F. Saia, L. V. Frutuoso, F. C. M. Iani, T. Adelino, V. Fonseca, M. Giovanetti and L. C. J. Alcantara, Detection of Multiple Human Viruses, including Mpox, Using a Wastewater Surveillance Approach in Brazil, *Pathogens*, 2024, **13**, 589.
- 32 S. Adhikari and R. U. Halden, Opportunities and limits of wastewater-based epidemiology for tracking global health and attainment of UN sustainable development goals, *Environ. Int.*, 2022, **163**, 107217.
- 33 R. T. Noble, S. M. Allen, A. D. Blackwood, W. Chu, S. C. Jiang, G. L. Lovelace, M. D. Sobsey, J. R. Stewart and D. A. Wait, Use of viral pathogens and indicators to differentiate between human and non-human fecal contamination in a microbial source tracking comparison study, *J. Water Health*, 2003, **1**, 195–207.
- 34 X. Su and J. Wu, Zoonotic Transmissions and Host Switches of Malaria Parasites, *Zoonoses*, 2021, **1**, 11.
- 35 S. Monteiro, R. Pimenta, F. Nunes, M. V. Cunha and R. Santos, Detection of dengue virus and chikungunya virus in wastewater in Portugal: an exploratory surveillance study, *Lancet Microbe*, 2024, **5**, 100911.
- 36 F. Chandra, W. L. Lee, F. Armas, M. Leifels, X. Gu, H. Chen, S. Wuertz, E. J. Alm and J. Thompson, Persistence of Dengue (Serotypes 2 and 3), Zika, Yellow Fever, and Murine Hepatitis Virus RNA in Untreated Wastewater, *Environ. Sci. Technol. Lett.*, 2021, **8**, 785–791.
- 37 D. Blackburn, M. Drennon, K. Broussard, A. M. Morrison, D. Stanek, E. Sarney, C. Ferracci, S. Huard, W. Brennan, J. Eaton, S. Nealeigh, N. Barber, R. A. Zimler, J. N. Adams, C. Blackmore, M. Gordillo, R. Mercado, H. Vore, K. Scanlan, I. Motie, L. Stanfield, A. Farooq, K. Widell, K. Tomson, N. Kerr, J. Nasir, M. Cone, C. Rice, T. Larkin, E. Hernandez, J. Bencie, C. R. Lesser, M. Dersch, S. Ramirez-Lachmann, M. Clark, S. Rollo, A. Bashadi, R. Tyler, B. Bolling, B. Moore, B. Sullivan, E. Fonken, R. Castillo, Y. Gonzalez, G. Olivares, K. E. Mace, D. Sayre, A. Lenhart, A. Sutcliffe, E. Dotson, C. Corredor, E. Rogers, B. H. Raphael, S. G. H. Sapp, Y. Qvarnstrom, A. D. Ridpath and P. D. McElroy, Outbreak of Locally Acquired Mosquito-Transmitted (Autochthonous) Malaria — Florida and Texas, May–July 2023, *Morb. Mortal. Wkly. Rep.*, 2023, **72**, 973–978.
- 38 Potential Zika transmission at Boon Lay Place after one case reported, persistent virus signals found in area - CNA, <https://www.channelnewsasia.com/singapore/zika-boon-lay-place-mosquito-cases-virus-signals-wastewater-test-moh-nea-4141901>, (accessed 18 August 2025).
- 39 2 Zika cases in Woodlands; NEA says surveillance suggests persistent virus signals in the area, <https://www.asiaone.com/singapore/zika-cases-woodlands-nea-says-surveillance-suggests-persistent-virus-signals-area>, (accessed 18 August 2025).
- 40 J. Rocklöv and R. Dubrow, Climate change: an enduring challenge for vector-borne disease prevention and control, *Nat. Immunol.*, 2020, **21**, 479–483.
- 41 N. Watts, M. Amann, N. Arnell, S. Ayeb-Karlsson, J. Beagley, K. Belesova, M. Boykoff, P. Byass, W. Cai, D. Campbell-Lendrum, S. Capstick, J. Chambers, S. Coleman, C. Dalin, M. Daly, N. Dasandi, S. Dasgupta, M. Davies, C. D. Napoli, P. Dominguez-Salas, P. Drummond, R. Dubrow, K. L. Ebi, M. Eckelman, P. Ekins, L. E. Escobar, L. Georgeson, S. Golder, D. Grace, H. Graham, P. Haggard, I. Hamilton, S. Hartinger, J. Hess, S.-C. Hsu, N. Hughes, S. J. Mikhaylov, M. P. Jimenez, I. Kelman, H. Kennard, G. Kiesewetter, P. L. Kinney, T. Kjellstrom, D. Kniveton, P. Lampard, B. Lemke, Y. Liu, Z. Liu, M. Lott, R. Lowe, J. Martinez-Urtaza, M. Maslin, L. McAllister, A. McGushin, C. McMichael, J. Milner, M. Moradi-Lakeh, K. Morrissey, S. Munzert, K. A. Murray, T. Neville, M. Nilsson, M. O. Sewe, T. Oreszczyn, M. Otto, F. Owfi, O. Pearman, D. Pencheon, R. Quinn, M. Rabbaniha, E. Robinson, J. Rocklöv, M. Romanello, J. C. Semenza, J. Sherman, L. Shi, M. Springmann, M. Tabatabaei, J. Taylor, J. Triñanes, J. Shumake-Guillemot, B. Vu, P. Wilkinson, M. Winning, P. Gong, H. Montgomery and A. Costello, The 2020 report of The Lancet Countdown on health and climate



- change: responding to converging crises, *Lancet*, 2021, **397**, 129–170.
- 42 S. J. Ryan, C. J. Carlson, E. A. Mordecai and L. R. Johnson, Global expansion and redistribution of Aedes-borne virus transmission risk with climate change, *PLoS Neglected Trop. Dis.*, 2019, **13**, e0007213.
- 43 Z. Gentry, L. Zhao, R. A. Faust, R. E. David, J. Norton and I. Xagorarakis, Wastewater surveillance beyond COVID-19: a ranking system for communicable disease testing in the tri-county Detroit area, Michigan, USA, *Front. Public Health*, 2023, **11**, 1178515.
- 44 B. Escudero-Pérez, A. Lalande, C. Mathieu and P. Lawrence, Host-Pathogen Interactions Influencing Zoonotic Spillover Potential and Transmission in Humans, *Viruses*, 2023, **15**, 599.
- 45 S. Seal, G. Dharmarajan and I. Khan, Evolution of pathogen tolerance and emerging infections: A missing experimental paradigm, *eLife*, 2021, **10**, e68874.
- 46 M. N. Hasan, M. Rahman, M. Uddin, S. A. A. Ashrafi, K. M. Rahman, K. K. Paul, M. F. R. Sarker, F. Haque, A. Sharma, D. Papakonstantinou, P. Paudyal, M. Asaduzzaman, A. Zumla and N. Haider, The 2023 fatal dengue outbreak in Bangladesh highlights a paradigm shift of geographical distribution of cases, *Epidemiol. Infect.*, 2025, **153**, e3.
- 47 Y. Sophia, M. K. Roxy, R. Murtugudde, A. Karipot, A. Sapkota, P. Dasgupta, K. Baliwant, S. Saunik, A. Tiwari, R. Chattopadhyay and R. K. Phalkey, Dengue dynamics, predictions, and future increase under changing monsoon climate in India, *Sci. Rep.*, 2025, **15**, 1637.
- 48 N. Vannavong, R. Seidu, T.-A. Stenström, N. Dada and H. J. Overgaard, Effects of socio-demographic characteristics and household water management on Aedes aegypti production in suburban and rural villages in Laos and Thailand, *Parasites Vectors*, 2017, **10**, 170.
- 49 D. S. Barcellos, C. E. R. Barquilha, P. E. Oliveira, M. Prokopiuk and R. G. Etchepare, How has the COVID-19 pandemic impacted wastewater-based epidemiology?, *Sci. Total Environ.*, 2023, **892**, 164561.
- 50 M. B. Diamond, A. Keshaviah, A. I. Bento, O. Conroy-Ben, E. M. Driver, K. B. Ensor, R. U. Halden, L. P. Hopkins, K. G. Kuhn, C. L. Moe, E. C. Rouchka, T. Smith, B. S. Stevenson, Z. Susswein, J. R. Vogel, M. K. Wolfe, L. B. Stadler and S. V. Scarpino, Wastewater surveillance of pathogens can inform public health responses, *Nat. Med.*, 2022, **28**, 1992–1995.
- 51 M. Tay, B. Lee, M. H. Ismail, J. Yam, D. Maliki, K. Y.-H. Gin, S.-R. Chae, Z. J. M. Ho, Y. L. Teoh, L. C. Ng and J. C. C. Wong, Usefulness of aircraft and airport wastewater for monitoring multiple pathogens including SARS-CoV-2 variants, *J. Travel Med.*, 2024, **31**, taae074.
- 52 S. Jin, B. L. Dickens, K. Y. Toh, D. C. B. Lye, V. J. Lee and A. R. Cook, Feasibility of wastewater-based detection of emergent pandemics through a global network of airports, *PLOS Glob. Public Health*, 2024, **4**, e0003010.
- 53 N. H. L. Leung, Transmissibility and transmission of respiratory viruses, *Nat. Rev. Microbiol.*, 2021, **19**, 528–545.
- 54 L. E. Lamb, S. N. Bartolone, M. O. Tree, M. J. Conway, J. Rossignol, C. P. Smith and M. B. Chancellor, Rapid Detection of Zika Virus in Urine Samples and Infected Mosquitos by Reverse Transcription-Loop-Mediated Isothermal Amplification, *Sci. Rep.*, 2018, **8**, 3803.
- 55 D. Dharmamuthuraja, R. P. D., I. Lakshmi M and F. Ishtiaq, Determinants of Aedes mosquito larval ecology in a heterogeneous urban environment- a longitudinal study in Bengaluru, India, *PLoS Neglected Trop. Dis.*, 2023, **17**, e0011702.
- 56 O. E. Hart and R. U. Halden, Computational analysis of SARS-CoV-2/COVID-19 surveillance by wastewater-based epidemiology locally and globally: Feasibility, economy, opportunities and challenges, *Sci. Total Environ.*, 2020, **730**, 138875.
- 57 W. Chen and K. Bibby, Temporal, spatial, and methodological considerations in evaluating the viability of measles wastewater surveillance, *Sci. Total Environ.*, 2025, **959**, 178141.
- 58 M. J. Wade, A. Lo Jacomo, E. Armenise, M. R. Brown, J. T. Bunce, G. J. Cameron, Z. Fang, K. Farkas, D. F. Gilpin, D. W. Graham, J. M. S. Grimsley, A. Hart, T. Hoffmann, K. J. Jackson, D. L. Jones, C. J. Lilley, J. W. McGrath, J. M. McKinley, C. McSparron, B. F. Nejad, M. Morvan, M. Quintela-Baluja, A. M. I. Roberts, A. C. Singer, C. Souque, V. L. Speight, C. Sweetapple, D. Walker, G. Watts, A. Weightman and B. Kasprzyk-Hordern, Understanding and managing uncertainty and variability for wastewater monitoring beyond the pandemic: Lessons learned from the United Kingdom national COVID-19 surveillance programmes, *J. Hazard. Mater.*, 2022, **424**, 127456.
- 59 Wastewater Surveillance, <https://asm.org:443/articles/policy/2024/november/wastewater-surveillance-an-essential-tool-for-publ>, (accessed 31 July 2025).

