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

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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.

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## 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.<sup>1</sup> 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.<sup>2–5</sup> 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.<sup>1,6</sup> The distribution of VBDs is changing rapidly due to factors such as urbanization, increased human mobility, ecological transformation, and most critically, climate change.<sup>6–8</sup>

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.<sup>9,10</sup> 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.<sup>11</sup> Moreover, VBDs often manifest with nonspecific symptoms that overlap with other febrile illnesses, complicating diagnosis and case confirmation.<sup>12</sup> 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,<sup>13,14</sup> poliovirus<sup>15,16</sup> and respiratory viruses such as SARS-CoV-2<sup>17,18</sup> and influenza virus,<sup>19</sup> 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.<sup>7,16,20</sup> 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<sup>14,21</sup> and rotavirus<sup>22</sup> are excreted in large quantities, ranging from 10<sup>5</sup> to 10<sup>9</sup> genome copies per gram of stool for norovirus and 10<sup>6</sup> to 10<sup>7</sup> for rotavirus, and are consistently detected in wastewater samples across diverse geographic settings.<sup>14,23,24</sup> Similar detection consistency been observed for SARS-CoV-2.<sup>25</sup> However, for most vector-borne pathogens, concentrations in wastewater are typically at or near detection limits,<sup>26</sup> 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.<sup>8</sup> 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,<sup>27</sup> high ambient temperatures can still accelerate degradation and reduce detection sensitivity.<sup>28</sup> 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.<sup>28</sup> 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.<sup>29</sup> The CDC Triplex RT-qPCR assay, while validated for detecting dengue, Zika, and chikungunya in patient samples,<sup>5,30</sup> shows reduced sensitivity when used on sewage due to inhibitors and low target concentrations.<sup>31</sup> Droplet digital PCR (ddPCR) has emerged as a promising alternative with higher tolerance to inhibitors and improved limits of detection,<sup>30</sup> 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,<sup>31</sup> 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.<sup>32</sup> 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.<sup>33</sup> 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.<sup>34</sup> This raises concerns about cross-species attribution and surveillance specificity.<sup>8</sup> 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.<sup>8,31</sup> 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.





infrastructure, and water access.<sup>46</sup> In some tropical regions, dengue outbreaks aligned with monsoon rains<sup>47</sup> 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.<sup>48</sup> 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.<sup>8,49</sup> 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.<sup>31,49</sup> 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.<sup>50</sup> 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.<sup>50</sup>

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.<sup>31</sup> 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.<sup>51,52</sup> 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.<sup>53</sup> 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.<sup>36,54</sup> 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.<sup>55</sup> 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.<sup>56,57</sup>

## 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.<sup>58</sup> 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.<sup>3,59</sup> 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.

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