**Defining a research agenda for environmental wastewater surveillance of pathogens**

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**Background**

The World Health Organization has published guidance for polio and SARS-CoV-2 Environmental Surveillance (ES)(1, 2), and established a network of ES sites globally through the Global Polio Eradication Initiative (REF). High-resource settings such as the US and Europe are planning a network of ES sites to enable pandemic preparedness. Whereas healthcare access and hospital-based surveillance are often inequitably distributed globally and within low-resource settings, affordable tools such as ES for surveillance are necessary so that these settings can generate disease burden estimates and are not left behind in reaping the benefits of public health interventions. Most recently, the G7 group of nations have promised support for multipathogen ES. Given the proven benefit of ES demonstrated with polio and SARS-CoV-2, and global funding for and focus on the potential of ES as a platform, the time is ripe to build and sustain an integrated, multipathogen ES infrastructure (that includes polio). From a public health surveillance standpoint, the goal is to leverage resources across countries and disease control programs to integrate information from multiple sources and inform robust public health decisions (Figure 1). These multipathogen ES platforms should address local and global surveillance needs, and as recommended by WHO, should be built in collaboration with local health departments and water sanitation groups.



**Fig 1. Integrated Surveillance as a key component of preparedness in all settings**

**Beyond pathogen-specific surveillance programs**

ES is independent of healthcare access—i.e. ES can be conducted effectively even where people, either by choice or though lack of access, do not avail of care at a clinic or hospital. ES hence provides an equitable view of pathogen circulation and transmission in a population. Indeed, ES can also be used to advocate for better sanitation systems in low-resource geographies.

ES can cater to a variety of use cases (eg: surveillance for disease eradication; surveillance for early warning of increased transmission), which, in turn, influences the design of the surveillance system (Figure 2). For poliovirus, where the goal is eradication, ES is used to monitor spatial and temporal distributions of both wild type and vaccine-derived viruses (3, 4), and any detection leads immediately to vaccination campaigns to eliminate local transmission. In the SARS-COV-2 pandemic, ES has been used to monitor and aid control of ongoing outbreaks by informing use of non-pharmaceutical interventions. Genomics has allowed detection of variants of concern when they are introduced into new geographies, and the identification of novel variants (5, 6). Such genomic data could inform vaccine design and variant-specific vaccine use in the future. Geographically varied and longitudinal ES data on a variety of vaccine-preventable disease pathogens can inform vaccine deployment and allow monitoring of vaccine efficacy (7), particularly when clinical diagnosis of the disease requires infrastructure not widely available, such as for typhoid fever. In all cases, the greatest strength of ES lies in its ability to generate population-level information, whereas case-based monitoring can be costly and universal coverage may be difficult to achieve. With carefully considered sampling strategies, ES could potentially support the detection of multiple pathogens within a single surveillance network at a fraction of the cost of case-based surveillance per capita.

 **Figure 2: Use cases may drive measurement needs and hence, ES system parameters**

**What pathogens should be surveilled using ES?**

The Bill & Melinda Gates Foundation hosted a meeting in May 2022, bringing together academic, manufacturing, and public health decision-making partners to co-develop a vision for multipathogen ES. This section will report on the discussion and outcomes from a roundtable discussion that was held during the *Environmental Surveillance for Public Health Impact* meeting:

ES should be conducted with the explicit aim of impact, employed where interventions can be informed and enacted. Local health department input should be solicited to prioritize pathogens for surveillance; this is essential for creating a well-supported, sustainable ES system. In low-resource settings, there is often a need to prioritize surveillance for diseases that can be severe or difficult to observe through clinical surveillance. During our discussions we focused on seven pathogens of interest (Table 1): poliovirus, *Salmonella* Typhi, *Vibrio cholerae*, SARS-CoV-2, Hepatitis A and E, and Measles virus.

ES for poliovirus has clearly impacted health policies, and detections of the remaining organisms were also deemed to yield actionable data. ES has been used to monitor trends in SARS-CoV-2 infection and to detect new variants of concern. For the remaining pathogenswhere informing vaccine use or vaccine efficacy is the end goal, ES data gathered alongside gold standard surveillance in pilot studies can inform how policy makers should interpret ES to inform disease burden where case data are unavailable.

**Opportunities and challenges for integrated, multipathogen ES**

This section reflects on the capacity and coordination needs of multipathogen ES approaches. These were discussed at a second and third roundtable discussion at the *Environmental Surveillance for Public Health Impact* meeting.

*Sampling sites and frequency, and sampling and concentration methods*

Optimal sampling sites, methods, and collection frequency can vary depending on the goal and pathogen of interest. Whilst well-mapped sewage networks with enumerated populations can inform optimal sampling sites in some regions, sampling site selection in low-resource settings with open drains or riverine networks that receive human waste directly from households is more challenging. Site selection approaches can be informed by hydrological mapping and overlaying different available data sets for elevation, bluelines, and population (e.g. from WorldPop (8)) (7), potentially aided by partners such as Novel-T (9). In these areas, site selection approaches are often iterative, due to uncertainties in the data available and connectivity of the networks. These data must be accurate enough to allow estimation of catchment size and population; whether these data can be gleaned from environmental samples themselves or will always depend on demographic and geographic information is an open question.

Sampling frequency can vary depending on the required data; modelling has shown monthly collections to be sufficient for poliovirus detection (10). Early warning systems, however, as desired for SARS-CoV-2 and *Vibrio cholerae*, could require weekly or potentially even more frequent sampling to inform public health action in a timely manner (11, 12). Sampling frequency and the number of sampling sites may need to be decided in part, based on the cost of sampling, travel to and from sites, cold-chain costs, and the capacity of the laboratory to test samples.

Choice of sampling method can be driven by the need for sensitivity vs. quantitative measurement. Passive or trap sampling (e.g. Moore swabs) can allow greater volumes to be surveyed but can be difficult to translate to quantitative measurements; grab samples provide an absolute sampling volume but survey only at one timepoint. Additionally, lowering the cost of automated samplers for applicability in low-resource settings should be undertaken. Concentration methods such as hydrogel “nanotrap” particles (REF: Ceres) should be tested and optimized for the set of pathogens of interest in a given context. Multipathogen ES would be more cost effective if a single method for sampling and extracting material can be employed for all pathogens. Thorough testing and standardization will be essential to ensure that the method chosen is suitable for pathogens of interest.

*Pathogen detection and genomic sequencing*

As target pathogens will vary geographically, interchangeable detection platforms would be optimal for multipathogen detection. Customizable TaqMan array cards have been used for detection by qPCR of multiple pathogens in ES samples (13). qPCR-based approaches can offer a sensitive method for detection and quantification. However, if genomic data are required, a metagenomic or targeted sequencing approach is necessary. Direct detection, sequencing, and bioinformatics tools for ES need to be developed. Methods employed should be rapid, adaptable to newly emerging pathogens, cost-effective, and easily deployable.

*Data analysis, sharing and communication*

These factors represent some of the largest hurdles for the sustained implementation of ES. Analysis of flow rates, catchment sizes, and population are required for quantitative results, and linkage to clinical or hospital-based case data helps prove the validity of ES findings. Continued engagement with local health departments is required to ensure the data are analyzed and presented in ways that are maximally informative to decision makers. A key concern is the determination of a trigger for action, such as a threshold for quantification, or detection of a specific pathogen serotype. Data and analyses should be accessible to those who may not have a broad scientific background. Dashboards have been demonstrated during the SARS-CoV-2 pandemic to be a useful way to rapidly and visually present data (14).

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Sustainable ES systems require reliable funding. These systems must demonstrate value when integrated into current surveillance methods, ideally providing information about priority metrics (e.g. estimated severe cases, hospitalizations, or deaths). Identifying funding for multipathogen ES could be challenging in low-resource settings if the world focuses on sentinel systems for global health security to the detriment of locally informative surveillance; whilst funding for multipathogen surveillance could also be a challenge where research and public health funds are pathogen-specific, we are encouraged by the promise of support for multipathogen ES from the G7 group of nations (<http://www.g7.utoronto.ca/healthmins/2022-0520-communique.html>). We are also hopeful that networks across low- and high-resource settings where ES is being undertaken could lead to shared tools, approaches, capacity, and ultimately, lower costs to undertake integrated, multipathogen surveillance in low-resource settings.

**Conclusion and call to action**

Tools need to be optimized across the range of methods for multiple pathogens: sampling frequency determination; sampling site selection; sampling and concentration processes; nucleic acid extraction; pathogen detection; genomic sequencing and bioinformatics; modeling and analytics; communication. In addition, multiple pieces of evidence are required in order to build the case for an ES system for integrated surveillance of multiple pathogens.

1. ES for each pathogen must be validated in the field alongside hospital- or clinic-based surveillance using gold standard diagnostics.
2. The cost of population-based ES must be examined, and used to build the case for sustained funding for ES.
3. Best practices for analytics and communication about ES results to policy makers should be developed.

Funders and the WHO will need to coordinate to support the development of standardized approaches and guidelines to ES, acknowledging the varied contexts of sanitation systems between high- and low-resource settings.

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**Author contributions**

AS, CT, and SK assembled minutes and wrote the draft text. DS, DB, SG and NG reviewed the manuscript.

**References**

1. WHO. Environmental surveillance for SARS-COV-2 to complement public health surveillance – Interim Guidance 2022 [Available from: <https://www.who.int/publications/i/item/WHO-HEP-ECH-WSH-2022.1>.

2. WHO. Guidelines for environmental surveillance of poliovirus circulation 2003 [Available from: <https://apps.who.int/iris/handle/10665/67854>.

3. Asghar H, Diop OM, Weldegebriel G, Malik F, Shetty S, El Bassioni L, et al. Environmental surveillance for polioviruses in the Global Polio Eradication Initiative. The Journal of infectious diseases. 2014;210 Suppl 1:S294-303.

4. Manor Y, Handsher R, Halmut T, Neuman M, Bobrov A, Rudich H, et al. Detection of poliovirus circulation by environmental surveillance in the absence of clinical cases in Israel and the Palestinian authority. Journal of clinical microbiology. 1999;37(6):1670-5.

5. Rios G, Lacoux C, Leclercq V, Diamant A, Lebrigand K, Lazuka A, et al. Monitoring SARS-CoV-2 variants alterations in Nice neighborhoods by wastewater nanopore sequencing. Lancet Reg Health Eur. 2021;10:100202.

6. Karthikeyan S, Nguyen A, McDonald D, Zong Y, Ronquillo N, Ren J, et al. Rapid, Large-Scale Wastewater Surveillance and Automated Reporting System Enable Early Detection of Nearly 85% of COVID-19 Cases on a University Campus. mSystems. 2021;6(4):e0079321.

7. Christopher B. Uzzell CMT, Jonathan Rigby, Venkata Raghava Mohan, Jacob John, Dilip Abraham, Rajan Srinivasan, Satheesh Nair, John Scott Meschke, Nicola Elviss, Gagandeep Kang, Nicholas Feasey, Nicholas C. Grassly. Environmental surveillance for Salmonella Typhi as a tool to estimate the incidence of typhoid fever in low-income populations. medRxiv2021.

8. WorldPop. [Available from: [www.worldpop.org](https://bmgf.sharepoint.com/sites/GEM3/Shared%20Documents/Environmental%20Surveillance/ES%20Event%20May%2016%20-%2018/www.worldpop.org).

9. Novel-T. [Available from: <https://www.novel-t.ch/#/home>.

10. Kalkowska DA, Pallansch MA, Cochi SL, Thompson KM. Modeling Poliovirus Surveillance and Immunization Campaign Quality Monitoring Costs for Pakistan and Afghanistan for 2019-2023. Open Forum Infect Dis. 2021;8(7):ofab264.

11. Randazzo W, Truchado P, Cuevas-Ferrando E, Simon P, Allende A, Sanchez G. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. Water Res. 2020;181:115942.

12. Nemudryi A, Nemudraia A, Wiegand T, Surya K, Buyukyoruk M, Cicha C, et al. Temporal Detection and Phylogenetic Assessment of SARS-CoV-2 in Municipal Wastewater. Cell Rep Med. 2020;1(6):100098.

13. Baker KK, Senesac R, Sewell D, Sen Gupta A, Cumming O, Mumma J. Fecal Fingerprints of Enteric Pathogen Contamination in Public Environments of Kisumu, Kenya, Associated with Human Sanitation Conditions and Domestic Animals. Environ Sci Technol. 2018;52(18):10263-74.

14. Wettstone E, Hughlett, L, Reagan, C, Shirin, T, Rahman, M, Haque, R, Blake, I, Taniuchi, M. Dhaka COVID-19 Dashboard [Available from: <https://dhakaesforsars-cov-2.research.virginia.edu/>, Accessed: 24th August 2022.