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Wastewater-Based Epidemiology: A Complementary Approach to Future Pandemic Preparedness

Student:

Niek Stefan Christiaan Henkens

S4916905

Thesis Supervisor:

Dr. Sebastian J. J. Lequime

Virus Ecology & Evolution

Groningen Institute for Evolutionary Life Sciences, University of Groningen

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Abstract

The increasing threat of a viral outbreak calls for adequate preparation of surveillance methods. Viral outbreaks with a similar scale to the SARS-CoV-2 pandemic have large social and economic effects. Although classical surveillance methods have contributed to viral monitoring, limitations like their inability to detect asymptomatic cases, high costs and its limited early-warning capacity highlight the need for complementary or alternative approaches to achieve a more comprehensive monitoring system for viral spread. This paper evaluates Wastewater Based Epidemiology (WBE) as a complement or alternative to classical surveillance and analyses its technical and conceptual limitations, drawing on case studies and molecular techniques such as quantitative PCR (qPCR). Based on this assessment, this paper suggests that WBE could be valuable in future viral monitoring systems due to its scalability, cost-effectiveness, its ability to detect symptomatic and asymptomatic cases and its potential as an early warning system. Despite having some conceptual and technical limitations regarding privacy, infrastructure inequity, sampling challenges and RNA degradation, WBE presents a promising approach for enhancing future pandemic preparedness and public health surveillance.

1. Introduction

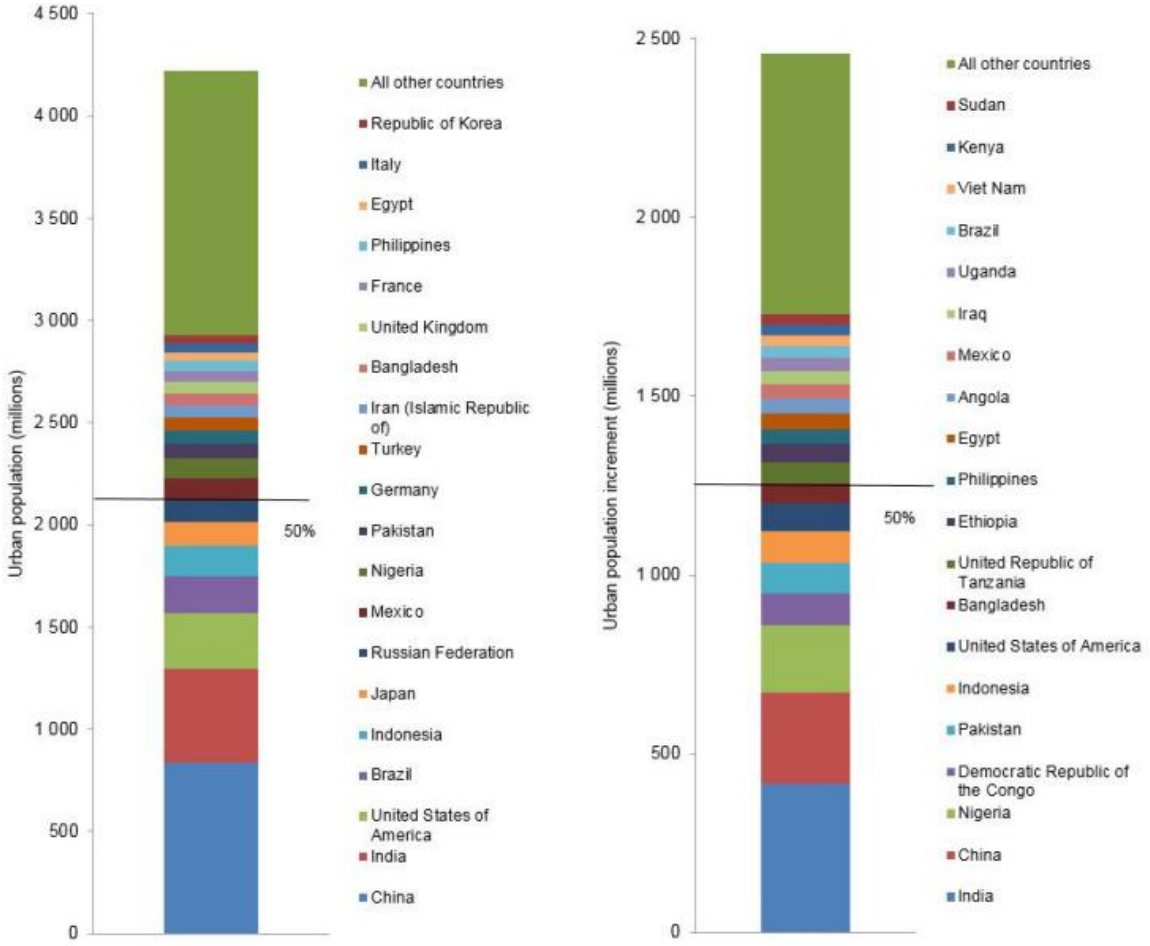
Since the first recorded pandemic, the Antonine plague that hit the Roman Empire in 166AD and the following decade, we have seen many pandemics that affected millions of people and have had various social and economic effects. The risk of diseases spreading and turning into epidemics or pandemics is growing (Haileamlak, 2022). There are several causes of this increasing likelihood, with urbanisation being a major one. In 2018, the United Nations published a revision on the World Urbanization Prospects projecting the increase of the urban population from 2018 to 2050, as can be seen in Figure 1. In 2018, a handful of countries in Asia and Africa already accounted for almost half of the world's urban population, making them key contributors to the projected urbanization trend (United Nations, 2018). On these continents, crowded cities already frequently struggle with infrastructure, sanitation and health care in general, creating conditions that facilitate the emergence and transmission of infectious diseases (Organization, 2025). Furthermore, the increased ease of traveling the globe fuels the spread of infections and diseases. During the Severe Acute Respiratory Syndrome (SARS) outbreak in 2002-2003, the virus spread from Southern China, where it originated, to 29 countries via air travel, causing local epidemics in several countries (Findlater & Bogoch, 2018). Also, the growing contact with animals who have not had human contact before, trade in wildlife (Joi, 2020) and the intensification of agricultural farming lead to higher risk of zoonotic infections (IUCN, 2021). Infections that originate from animals account for about 60% of the emerging human pathogens (Jessica A Farrell, 2021). In the next decades, these factors could lead to a threefold increase in the probability of an extreme epidemic (Marani et al., 2021).

SARS Coronavirus 2 (SARS-CoV-2) belongs to the coronaviruses family and has spread across the earth since 2020. The first death is recorded in China in January 2020, and by July 2021 the total amount of cases stood at about 200 million infections and about 4 million deaths (Baylor College of Medicine, 2021), costing an estimated US$20 trillion (Frieden et al., 2021). The Duke Global Health institute calculated the probability of the occurrence of a pandemic with about the same impact SARS-Cov-2 had using 476 epidemics of different scales. It turns out that a pandemic of this scale will occur once every 59 years. However, it is not the case that because SARS-Cov-2 spread in 2020, the next pandemic will wait another 59 years. The global health system should not wait to prepare for future pandemics. Gabriel Katul, Professor of Hydrology and Micrometeorology at Duke Global Health Institute, made a statement on this in a news article. *"When a 100-year flood occurs today, one may erroneously presume that one can afford to wait another 100 years before experiencing another such event,"* Katul says. *"This impression is false. One can get another 100-year flood the next year."* (Penn, 2023)

Taking Katuls' warning in mind and knowing that the next pandemic could be here any time, epidemiologists and biosecurity experts are immersed in preparedness planning. The global health system should start with data collection, modelling the growth and spread of viruses and raising awareness and detection of pathogens as early as possible (Williams, 2023).

This paper critically evaluates classical surveillance methodologies used during previous pandemics, laying bare some inherent limitations. It approaches Wastewater Based Epidemiology (WBE) as a complementary or potentially alternative approach in future surveillance methods of infectious diseases. While WBE could resolve some of the limitations of classical sampling, it also raises certain conceptual concerns. Additionally, this paper provides a detailed examination of the methods used in WBE, along with the identification of several technical and conceptual limitations and areas requiring further research.

Figure 1; Comparison of urban populations in 2018 and projected increases by 2050 (Shuaib Lwasa & Karen C. Seto, 2021)



2. Classical Surveillance: Overview and Limitations

Classical surveillance as it is approached in this paper, is the process of collecting, analysing and interpreting data focused on viral infections. During the SARS-CoV-2 pandemic, traditional methods were used to track and monitor the disease. For diagnostic testing, which is defined as 'A test used to help figure out what disease or condition a person has based on their signs and symptoms' (Terms, 2025), mostly PCR and antigen tests were used for current infections and past infections, respectively (American Lung Association, 2025). In addition to diagnostic testing, contact tracing was used to control the virus. Contact tracing in the context of the Covid-19 pandemic was defined by the World Health Organization (WHO) as 'the process of identifying, assessing, and managing people who have been exposed to someone who has been infected with the COVID-19 virus'. It is important to stress that these major surveillance methods are focused on identifying symptomatic individuals, which brings us to one of the limitations of classical surveillance.

This widely used approach, focused on symptomatic individuals, reveals a key limitation of classical surveillance as it fails to detect individuals who do not display symptoms. This is illustrated using an example on influenza, an infectious disease caused by the influenza virus and commonly known as the flu (Sara Francis Fujimura, 2003). About two-third of the influenza-infected individuals showed symptoms, meaning that another one-third is asymptomatic. Together with the presymptomatic, those that begin to show symptoms in a later stage, the asymptomatic individuals caused about 33% to 50% of the total influenza transmissions. All these non-symptomatic individuals go undetected while using

regular diagnostic tests like molecular assays and antigen detection tests because those infected people will not have the cue to get themselves tested. A review by Oran and Topol (2020) showed that approximately 40% to 45% of the individuals infected with SARS-CoV-2 will remain asymptomatic, and this group also seems to transmit the virus for an extended period, possibly longer than 14 days (Adhikari & Halden, 2022). Various other viral pathogens , such as adenoviruses and polioviruses are currently also detected through diagnostic testing, although asymptomatic infections are prevalent (Centers For Disearse Control And Prevention, 2024). Up to 70% of the poliovirus infections are asymptomatic, with about 25% experiencing mild symptoms (European Centre for Disease Prevention and Control, 2023). Contact tracing may help reduce the number of undetected asymptomatic infections, as individuals identified as contacts of confirmed cases are more likely to undergo testing, even in the absence of symptoms. In addition to diagnostic testing and contact tracing, obligatory testing, for instance, before going for a night out or using public transport, could partially solve this matter because asymptomatic individuals would then find out about their infection (Patrozou & Mermel, 2009).

Classical surveillance through diagnostic testing also is a costly method. During the first months of the SARS-CoV-2 pandemic in the US, the median price for a COVID-19 diagnostic test was $127, and about half of test charges were priced between $100 and $199. Nearly one in five were priced above $200, researchers reported (LaPointe, 2020). A discrepancy exists between European countries regarding the financial responsibility for COVID-19 diagnostic testing, with some governments covering the full or partial cost of tests, while in others, citizens were financially responsible themselves. Depending on the type of test, whether one has symptoms, and whether the test is conducted at a governmental or private testing location, prices greatly differ (Wintle, 2021).

Especially in the first phase of viral spread, large-scale diagnostic testing is often inefficient. At this stage, the prevalence of infections in the population is relatively low, so most of the tests will have a negative result. Additionally, when in the early-pandemic phase, the first diagnostic tests yield positive results, the pathogen may already have spread more broadly, including symptomatic and asymptomatic infected individuals (Kumar et al., 2021). This argument aligns with the limitations of classical surveillance described above; it explains why diagnostic testing is presumably not the most effective strategy for early detection or as an early warning system during the first stages of a pandemic.

3. Wastewater-Based Epidemiology: A Comprehensive Overview

3.1 Introduction to WBE

WBE makes use of sewage water to assess the quantity of chemical and biological materials in a pooled sample, such as a sewer network or Wastewater Treatment Plant (WWTP). The concentrations of these materials indicate consumption patterns of certain drugs or substances, or pathogen exposure (O'Keeffe, 2021). The first use of this technique was in 2001 as a tool to assess the use of illicit drugs and misused therapeutic drugs in a community. Over the past decades, WBE has become a standardized method in drug detection because of its accuracy. WBE can reveal differences in drug presence and consumption in years, seasons, regions, special events, and weekdays/ weekends (Maria Lorenzo & Yolanda Picó, 2019). WBE became an established method in drug detection, and, for example, tracking down drug labs. The use of WBE in measuring exposure to various chemical substances, such as household or personal care products, or presence and diversity of antimicrobial resistant genes, has broadened over the past two decades. The use of WBE became more widespread during the Covid-19 pandemic, as it assisted in monitoring the spread and evolution of the SARS-Cov-2 virus. Though the use of WBE in viral surveillance is still in its infancy, the possibilities are promising.

Looking back at the example on the influenza virus from the previous chapter (Sara Francis Fujimura, 2003), together with the review of Oran and Topol (2020), the usage of WBE could assist in solving the problem of identifying infections in asymptomatic and presymptomatic individuals that otherwise might have gone undetected. Although the viral RNA of, for example, SARS-CoV-2 is mostly detected in respiratory samples (70-100%) used for diagnostic testing, faecal samples also contain a significant amount of RNA (30-60%) (Jones et al., 2020). By capturing this genetic material of all infected individuals through WBE, including those that may not show symptoms yet, a more comprehensive overview of community transmission can be created.

3.2 Biomarkers

Wastewater contains chemical and biological constituents, such as pharmaceuticals, personal care products, pesticides, bacteria, viruses, parasites, and genetic material from various organisms (Maria Lorenzo & Yolanda Picó, 2019). These compounds, often referred to as biomarkers can provide valuable insights into public health indicators such as community drug use, water quality and prevalence of pathogens.

To ensure reliability of an analysis, biomarkers selected for WBE must meet four essential criteria. At first, they should be readily detectable in the wastewater, which is confirmed through chemical or molecular analysis (Charu Juneja, 2023). Second, the biomarkers should be stable in sewers and during sample storage and processing (Charu Juneja, 2023), a complex criterium because of varying degradation conditions. The third and fourth relate to biological relevance and are generally verified through pharmacokinetic data and literature review. Biomarkers should originate from human metabolism and be excreted consistently across populations, regardless of factors such as age, ethnicity, or lifestyle (Charu Juneja, 2023). The central biomarkers in WBE are environmental deoxyribonucleic acid (eDNA) and environmental ribonucleic acid (eRNA), particles that circulate in water, soil, and air (Davis; Jessica A Farrell, 2021). These environmental nucleic acids (eNAs) originate from materials such as skin, faeces, and mucus, and can exist in either cellular or extracellular form ("Environmental DNA (eDNA)," 2018).

3.3 Metabarcoding and qPCR

One of the primary applications of eDNA is biodiversity assessment in environmental monitoring. In this process, eDNA is extracted from environmental samples and amplified, sequenced and categorized based on its nucleotide sequence. After this categorization, species determination is done, leading to a biodiversity assessment of this environment. (Krista M. Ruppert, 2019) The species identification is achieved through metabarcoding, an extended version of regular barcoding. The basic principle of barcoding determines a sequence of nucleotides and compares it to a reference database to identify which species the sequence belongs to. In metabarcoding, multiple ‘barcodes’ of different species are simultaneously compared to a reference database (Waardenburg). This technique allows researchers to create a list of species present within a given environment.

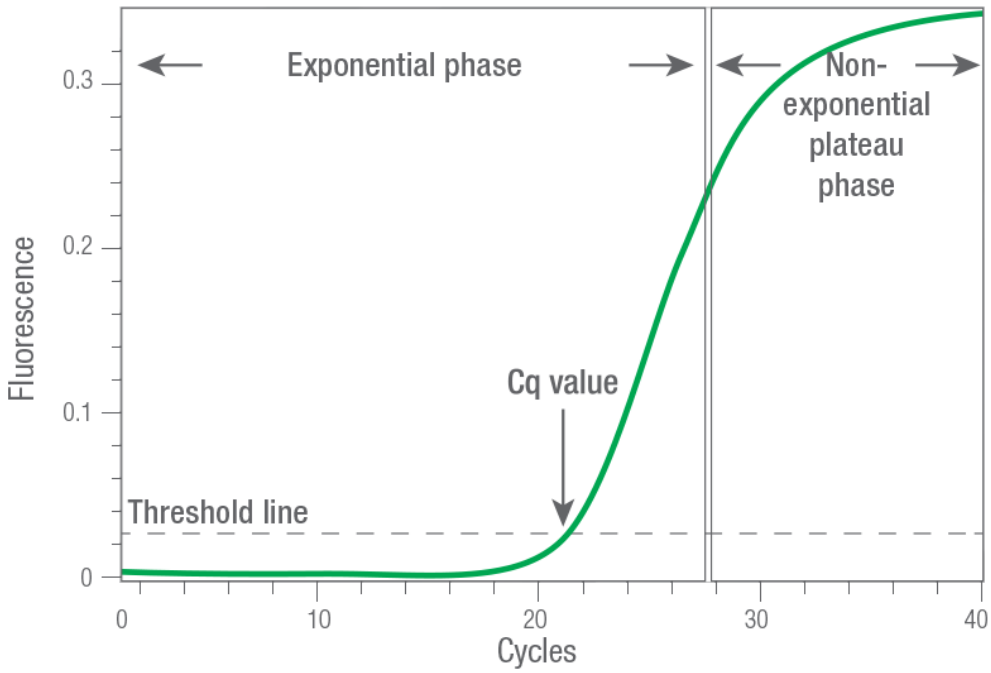
Early adopters of metabarcoding hoped that this method could serve as a quantitative tool, where the number of reads would reliably reflect the abundance or biomass of species in a sample. However, various factors such as species- or tissue-specific differences in secretion and degradation of eDNA, varying DNA extraction efficiency, PCR amplification (Lamb et al., 2019) limit the quantitative accuracy of metabarcoding.

Currently, metabarcoding is predominantly used as a qualitative method in eDNA analysis, allowing for the detection of species presence without providing data on species abundance. In addition, quantitative Polymerase Chain Reaction (qPCR) is used to estimate absolute abundances of the target species, gene,

or viruses by coupling DNA amplification with real-time quantification of target sequences (Pont et al., 2023). qPCR can provide information on the concentration and abundance of biomarkers such as eNAs. The following paragraph will provide a detailed overview of qPCR and narrows down towards its use in viruses.

qPCR is an advanced form of regular PCR that allows both amplification and real-time quantification of specific DNA segments. Regular PCR is a sensitive method that allows for the amplification of target DNA. qPCR builds on this technique by incorporating a fluorescent reporter molecule, enabling real-time detection of amplified DNA as it accumulates (Lilit Garibyan, 2014). Fluorescent labelled sequence-specific primers or probes are added to the reaction mixture, which causes the emitted fluorescence to be directly proportional to the amount of amplified product. Initially, the fluorescence is minimal, but when the amplification process proceeds and more fluorescent product is generated, the signal becomes detectable. The key measurement in qPCR is the quantification cycle (Sq.), which is reached when 'enough amplified product accumulates to yield a detectable fluorescence signal' (**Error! Reference source not found.**). The doubling time of the fluorescence is measured during the exponential period, where the reagents are not yet limited. This doubling time is proportional to the doubling time of the target DNA, and the initial amount of target DNA can be accurately calculated. When the template concentration at the start is high, relatively few amplification cycles are needed to detect a fluorescent signal and make calculations on the initial amount of template. In contrast, when the template concentration is low before amplification, it will take many cycles to reach a detectable fluorescence.

Figure 2; *Exponential and plateau phases of the PCR reaction. (Biorad, 2025)*



Wastewater contains viral RNA from infected individuals, who often shed these eRNA's before symptoms occur. Reverse-transcriptase (RT), a method that converts viral RNA into complementary DNA (cDNA) before amplification and quantification, in combination with qPCR, provides the possibility to perform a quantitative analysis on mRNA and viruses (Kurt & Simsek, 2021). Usually, the first steps of viral analysis methods rely on isolation and purification of the viral RNA (Gregorova et al., 2022). Fiona Rau et al. used qRT-PCR in combination with high-throughput sequencing on the Hepatitis E virus (HEV) to estimate HEV prevalence and identify circulating variants that can impact treatment

outcome (Rau et al., 2024). In short, qRT-PCR is a molecular biology tool that detects and quantifies specific RNA sequences, such as viral RNA, in a sample (Dymond, 2013).

4. The Promise of WBE

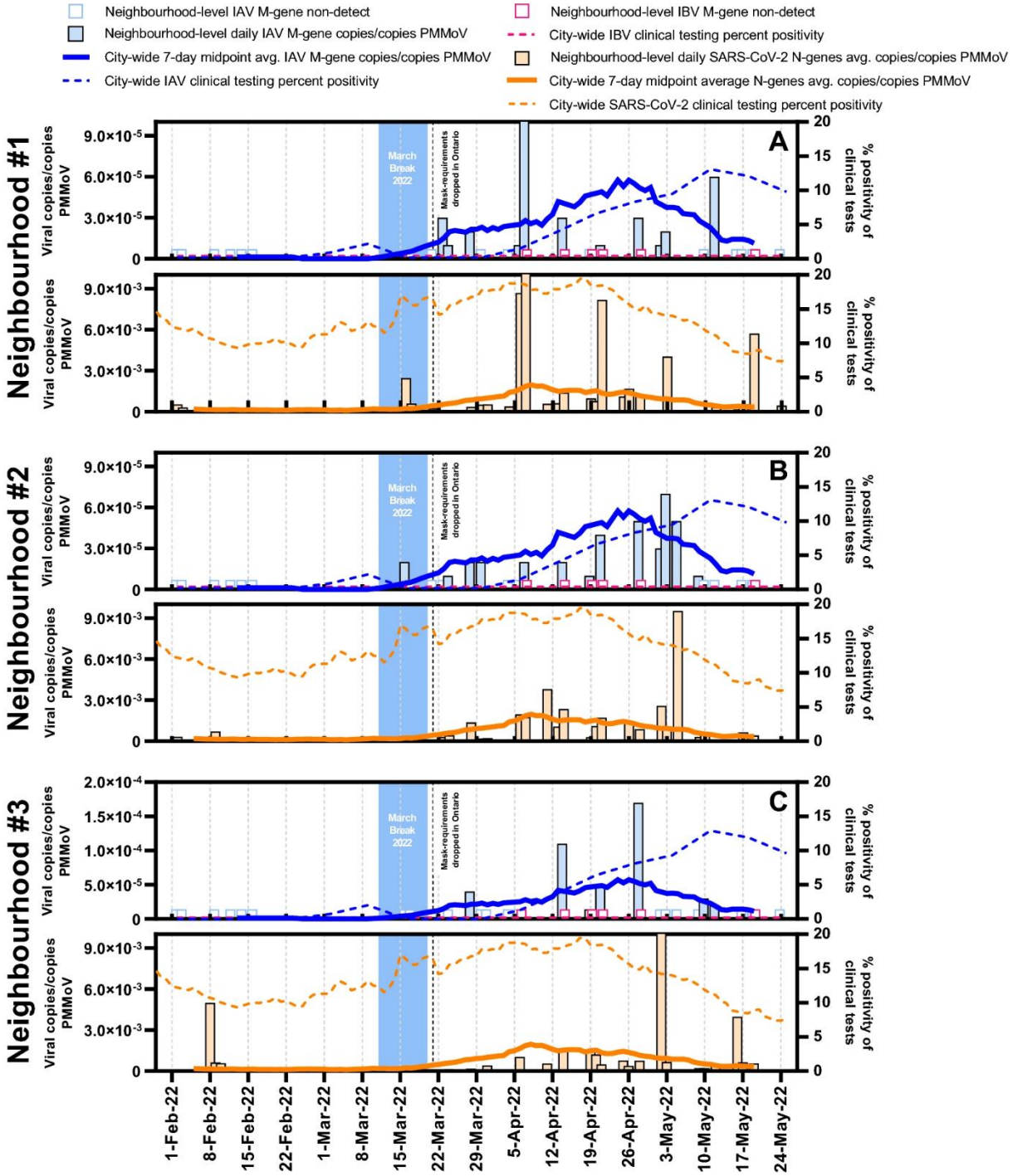
4.1 Scalability

One of the major advantages of WBE is its scalability, allowing it to be effectively implemented at various levels, from individual buildings to entire cities or regions. The use of WBE during the SARS-CoV-2 pandemic in America was mainly focused on large-scale sewer systems like Wastewater Treatment Plants (WWTP). In the United States, 14.748 WWTP's serve 240 million Americans, equating to an average of around 16,275 people per facility (Mattei, 2017). This infrastructure provides the opportunity to monitor viral spread at a relatively large scale. Furthermore, the sewage networks and their connections to specific municipalities, communities, or households enhance the ability to trace the origin of wastewater samples. Knowing where certain wastewater samples originate allows for comparative analysis between geographical regions and demographic groups, adhering to privacy and ethical standards (Gagliano et al., 2023). Appliance of WBE on for example universities, hospitals or nursing homes can be helpful in creating a more targeted public health intervention or to ensure a facility is clear of viruses(Oh et al., 2022). American Universities widely used the viral quantification method qRT-PCR on SARS-Cov-2 to detect outbreaks (White et al., 2024) or ascertain the university was clear of the virus(Bivins & Bibby, 2021).

4.2 Early warning system

Combining wastewater sampling with a quantifying technique could detect the location of the first infections, even before infected individuals show symptoms. During the first weeks of the SARS-Cov-2 pandemic, the KWR Watercycle Research Institute used the sequence of four viral protein-fragments and measured the presence of these viral biomarkers in the sewage systems of 7 big Dutch cities and the airport. On the 6th of February 2020, three weeks before the first SARS-Cov-2 case, measurements showed no indication of these fragments in the wastewater samples. On the 4th and 5th of March, one week after the first positive SARS-CoV-2 test, the samples of 4 cities showed a positive signal for the N1 fragment, while the Dutch National Institute for Public Health and the Environment recorded only 38 official SARS-CoV-2 cases. The number of cases detected through classical surveillance is still quite low, but a significant concentration of viral proteins is already found in the sewage systems of several cities. Analysis on a WWTP in The Netherlands suggests that the N-primer of SARS-CoV-2 already produces a positive signal when around the prevalence was one or even slightly less than one case in 100.000 people This example indicates the possible value of WBE as an early warning system (Medema et al., 2020). Figure 3 shows that the number of detected gene copies of the influenza virus and SARS-CoV-2 start rising earlier than the percentage of positive clinical tests, which is particularly obvious in Neighbourhood #1 and #2 in the graphs on the Influenza virus. Digital Droplet PCR (ddPCR), a novel PCR-based technology, allows for the absolute quantification of DNA by partitioning a sample into thousands of individual droplets, where PCR amplification occurs. Especially for targets at extremely low concentrations, such as SARS-CoV-2 in wastewater, ddPCR outperforms qPCR on sensitivity, precision, and reproducibility. During the SARS-CoV-2 pandemic, ddPCR was still in its infancy and was not yet widely used. However, this technique can become more widely adopted and become a routine test just like qPCR, when the cost for the operating system and chemical reagents are reduced and the total assay throughput is increased (Paruch, 2022).

Figure 3; Comparison of both IAV and SARS-CoV-2 wastewater signals at the neighbourhood level (Mercier et al., 2022)



4.3 Cost-effectiveness

As described previously, classical surveillance through diagnostic testing is costly. Analysis of WWTP's, municipalities, communities or households is much cheaper. Comparing the most used test during the Covid-19 pandemic, a single regular PCR test, to the analysis of a wastewater sample representing an entire population or subpopulation, American laboratories can perform this full analysis for only twice the price of a single PCR test. The costs for wastewater-based analysis is based on operating instrumentation and labour, but not sampling costs and initial equipment investments (Safford et al., 2022). These additional costs are not negligible, however when this process would be scaled up, the costs for sampling and equipment can be divided over many tests and will still be an affordable

alternative to classical surveillance. Cost reduction for qPCR tests on wastewater, but also for the pospective technique ddPCR, is necessary. However, these wastewater-based surveillance methods are undoubtedly more cost-efficient than clinical sampling.

4.4 Advanced molecular techniques

It is important to acknowledge that, beyond the methods extensively explained in previous paragraphs, several advanced molecular techniques hold promise for the future of WBE, although they are not yet widely implemented. Multiplex qPCR (mqPCR) enables the detection and quantification of multiple gene targets within a single reaction, leading to enhanced efficiency and reduced reagent consumption compared to qPCR. High-throughput next-generation sequencing (HT-NGS) offers an untargeted, high-resolution detection of a wide array of pathogens in wastewater(Paruch, 2022). It is especially useful in monitoring genetic diversity and detecting emerging pathogens. Another prospective method is loop-mediated isothermal amplification (LAMP), a technique that amplifies DNA with high specificity and efficiency under isothermal condition. It uses designed primers to target specific sequences and is cost-effective as it eliminates the need for thermal cycling and therefore does not need any specific and expensive instrument. These characteristics make LAMP a deployable technique, in generates amplification that is detectable within 30 minutes, creating possibilities for diagnosis at point-of-care facilities.

In summary, WBE is a cost-effective technique that can complement, or potentially serve as an alternative to, classical surveillance methods. It has the ability to detect viral presence in entire communities, including infections in asymptomatic and presymptomatic individuals. The application of WBE during the SARS-CoV-2 pandemic, particularly in large-scale systems such as WWTPs and institution-level settings like universities, show its practical value and potential in monitoring viral spread.

5. WBE: Conceptual and technical limitations

Using WBE for monitoring viral spread during an outbreak or potentially using it as early detection system comes with some conceptual and technical problems. One of the principal conceptual challenges in the implementation of WBE is the balance between ensuring privacy and sampling scale of WBE, and the analytical reliability of viral detection. To maintain individual privacy, the generally accepted threshold for a sample is at least 10.000 individuals. In the United States, the average WWTP serves approximately 16.275 people. As a result, efforts to maintain privacy standards often limit WBE to large-scale applications, making small-scale sampling strategies ethically challenging ((SCORE), 2016; Sims & Kasprzyk-Hordern, 2020). Strict adherence to ethical guidelines limits WBE to large-scale surveillance, where identification on smaller scales is virtually possible. Although relaxing these guidelines to allow sampling at smaller scales may expand the potential uses of WBE, it introduces ethical concerns regarding individual privacy, potential discrimination, and stigmatization of vulnerable communities (Sims & Kasprzyk-Hordern, 2020).

A study by Adhikari and Halden (2022) posed another conceptual problem concerning populations already at risk of poor hygiene. They start by recalling that half of the sustainable development goals (SDG's) listed by the United Nations (UN) agenda of 2030 may use WBE monitoring at centralized infrastructures. Expansion of WBE to 109,000+ WTTP's in 129 countries would increase the global coverage from the current 300 million individuals to approximately 2.7 billion, a ninefold increase. However, this still leaves an estimated 5 billion without access to centralized sewerage systems. Adhikari and Halden highlight disparities in present-day sanitation infrastructure. They show that certain regions vulnerable to infectious disease outbreaks are also excluded from the protective benefits of

WBE. These doubly disadvantaged populations are at heightened risk due to poor hygiene and a lack of early-warning systems (Adhikari & Halden, 2022).

The stability of viral genetic material in sewage water is influenced by physicochemical factors like temperature, pH, suspended solids, and disinfectants. For example, a study by Xin-Wei Wang et al. showed that SARS-CoV-2 RNA persists for approximately 2 days in hospital wastewater, domestic sewage and dechlorinated tap water at 20 °C. However, it could persist for up to 14 days in wastewater at 4 °C. One study (Bastardo-Mendez et al., 2024) showed that wastewater samples with neutral pH values around 7.1-7.4 yielded more frequent positive SARS-CoV-2 detections than samples with higher pH levels, probably due to the inactivation of the virus in alkaline conditions(Varbanov et al., 2021).

In addition to these variables, travel time of the wastewater in the sewage system greatly impacts viral RNA degradation (Camille McCall, 2022). The impact of variables such as temperature, suspended solids, pH, and disinfectants depends on the in-sewer travel times, where longer travel times contribute to increased decay of RNA. These travel times are influenced by several dynamic factors, including flow rate variations due to rainfall or water consumption, the location of sampling stations and seasonal changes (Xander Bertels, 2022). Consequently, variability in travel time introduces an additional layer of uncertainty in interpreting WBE data.

6. Discussion

This paper explored the fundamental principles and limitations of classical surveillance, particularly in the context of the SARS-CoV-2 pandemic. Classical surveillance, although foundational to public health monitoring, has some drawbacks. Limitations like the focus on primarily symptomatic individuals, high costs, and limited capacity for early detection, call for a complementary or alternative approach. This paper discussed to which extent Wastewater Based Epidemiology (WBE) could resolve these limitations.

WBE offers scalability, cost-efficiency, and the ability to detect viral nucleic acids shed by both symptomatic, asymptomatic and pre-symptomatic individuals, providing a more representative view of viral spread. WBE relies on advanced molecular techniques like RT-qPCR for the detection and quantification of viral biomarkers, which evolved from biodiversity monitoring techniques like eDNA metabarcoding and qPCR.

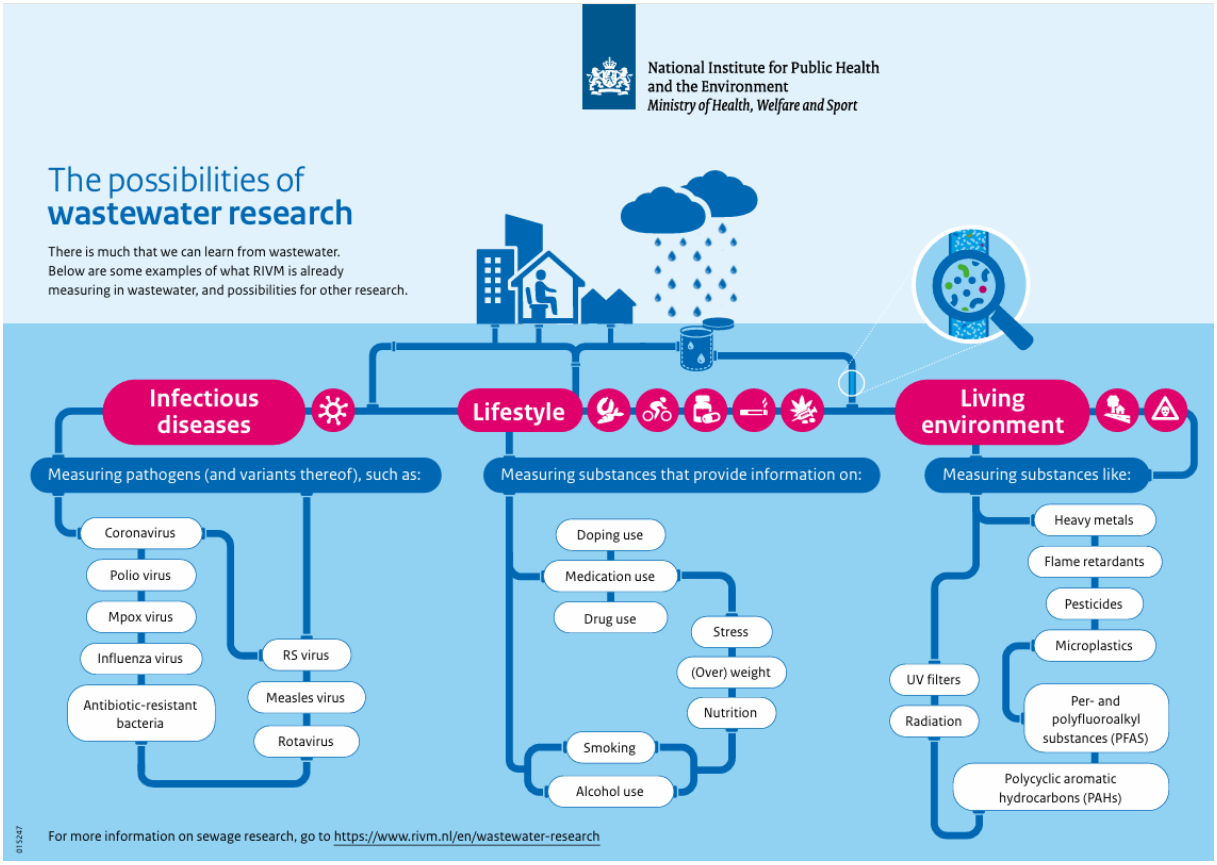
However, WBE has some conceptual and technical limitations. On the conceptual level, some ethical concerns arise in the balance between privacy and sampling scale. Also, disparities in sanitation infrastructure around the globe limits the reach of WBE and brings populations already at risk of infection in a disadvantaged position. On the technical level, factors such as the temperature and pH of sewage water and in-sewer travel time impact the viral RNA stability and presence, increasing the uncertainty of the results.

The application of wastewater in health monitoring is part of the future of viral monitoring. Techniques like metabarcoding, qPCR and RT-PCR, as discussed in this paper, form the foundation for the analysis of eDNA. These methods are not limited to detecting viruses, its relevance extends to broader applications such as monitoring drug consumption trends within populations. Moreover, it could potentially be used to track antimicrobial resistance, hormone levels, or other biochemical markers linked to public health.

Expanding the knowledge and use of molecular analytical techniques will enhance the accuracy and scope of these analyses. Prospective techniques like ddPCR, mqPCR, HT-NGS and LAMP show considerable potential for future wastewater analysis, their application is still in its early stages. Further

research is needed to explore their practical utility and enable broader implementation in WBE. Additionally, strengthening the sampling infrastructure in sewage systems could provide a more consistent and organized approach to health monitoring. This infrastructure not only supports viral detection, Figure 4 shows an infographic provided by the Dutch National Institute for Public Health and the Environment (RIVM, Rijksinstituut voor Volksgezondheid en Milieu) that shows the possibilities in the analysis of infectious diseases, substances that provide information on lifestyle and living environment.

Figure 4; Possibilities of Wastewater Research (RIVM)



It is inevitable that we will face pandemics in the near future, as a consequence of increasing urbanization, global interconnectedness, environmental pressures, and intensified human–animal interactions. Both classical surveillance and WBE will undoubtedly play a significant role in the early warning mechanisms and surveillance of these viral outbreaks. We should not move away from diagnostic testing and contact tracing as they offer high specificity at the individual level, simply confirming infections and guiding clinical interventions. As described in this paper, they overlook asymptomatic and presymptomatic individuals and are cost intensive. WBE captures viral signals in early stages, and with them creates a comprehensive overview of entire populations, regardless of symptom status. Strategic investments in these complementary systems enhances the outbreak detection, allowing for earlier interventions, targeted testing in high-risk areas and more data-driven public health decisions.

This paper proves an in-depth explanation of qPCR, which remains the most established technique for DNA and RNA quantification. In addition to qPCR, several prospective technical processes are discussed, including ddPCR, mqPCR, HT-NGS and LAMP. Although these methods are rapidly gaining attention and show great potential, at this point they are not yet sufficiently developed or standardized

for widespread implementation. Further research is required to optimize qPCR protocols and to advance these emerging techniques. This will be of great value in ensuring the reliability, scalability and cost-effectiveness of WBE as a routine tool.

The routine implementation of WBE can reach from nationwide monitoring through centralized wastewater infrastructure to more targeted surveillance at airports, universities or hospitals. It does not imply universal application at every location; it is the structured integration of WBE into public health systems where it is feasible and valuable. Routine implementation. ensures a stable stream of health data, at various scales, creating long-term datasets which improves the depth and quality of trends and models.

With the rising probability of the occurrence of a pandemic with a similar scale to the SARS-CoV-2 pandemic, strategic investments should be directed towards Wastewater Based Epidemiology, advancement of its underlying technologies and the integrated implementation alongside classical surveillance. Although WBE presents certain technical and conceptual limitations, it offers a scalable, cost-effective and complementary approach to monitoring viral transmission, and will undoubtedly play a role in the future of infectious disease surveillance.

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Appendix; use of AI

Artificial intelligence (AI) tools were used in the process of writing this thesis in several ways. AI provided feedback on the table of content which I created myself and noted the strengths and weaknesses of this table of content. The whole paper was written without using AI. When the first version was finished, AI provided feedback on the structure, leading to some changes in its organization. Additionally, AI helped in improving the spelling, grammar and gave feedback on how to enhance the flow of (paragraphs/chapters of) the paper. In few cases, sentences written by AI were almost literally inserted into the paper, as they were rewritten concisely. In almost all cases, only some (linking) words were added or removed to create a more scientific writing style and enhance the flow.