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Wastewater Surveillance for SARS-CoV-2 RNA in Canada

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Abstract

Wastewater surveillance for SARS-CoV-2 RNA is a relatively recent adaptation of long-standing wastewater surveillance for infectious and other harmful agents. Individuals infected with COVID-19 were found to shed SARS-CoV-2 in their faeces. Researchers around the world confirmed that SARS-CoV-2 RNA fragments could be detected and quantified in community wastewater. Canadian academic researchers, largely as volunteer initiatives, reported proof-of-concept by April 2020. National collaboration was initially facilitated by the Canadian Water Network.

Many public health officials were initially skeptical about actionable information being provided by wastewater surveillance even though experience has shown that public health surveillance for a pandemic has no single, perfect approach. Rather, different approaches provide different insights, each with its own strengths and limitations. Public health science must triangulate among different forms of evidence to maximize understanding of what is happening or may be expected. Well-conceived, resourced, and implemented wastewater-based platforms can provide a cost-effective approach to support other conventional lines of evidence. Sustaining wastewater monitoring platforms for future surveillance of other disease targets and health states is a challenge. Canada can benefit from taking lessons learned from the COVID-19 pandemic to develop forward-looking interpretive frameworks and capacity to implement, adapt, and expand such public health surveillance capabilities.

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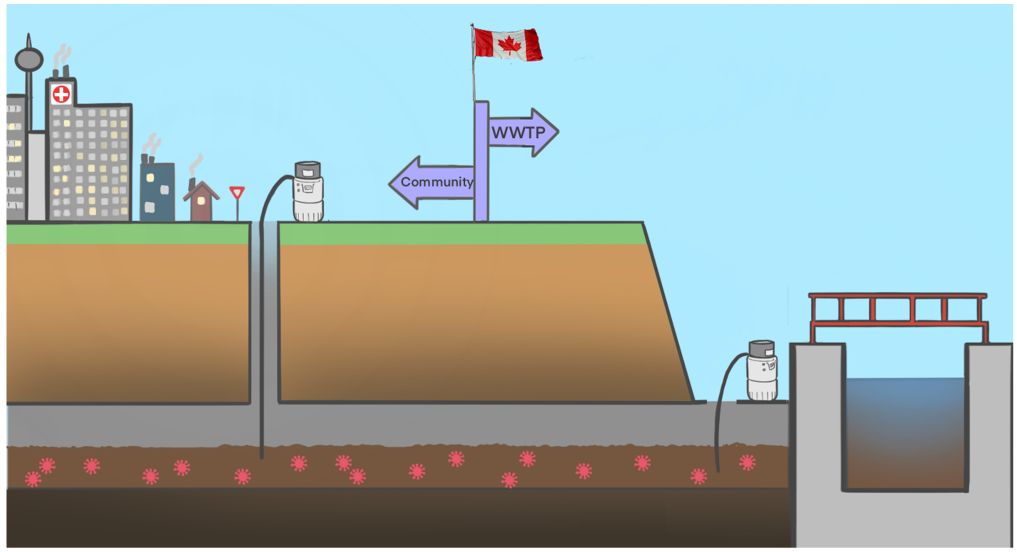

Key words: COVID-19, monitoring, pandemic, public health innovation, bottom-up initiative

1. Background, purpose, and introduction

1.1. Background

Canadian researchers responded rapidly to the World Health Organization's (WHO) declaration of COVID-19 as a pandemic on 11 March 2020. Teams across the country came together to determine how their knowledge and skills could be applied to deal with the enormous public health implications of a global pandemic (Kelly 2011). This pandemic, caused by the highly transmissible and infectious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in more than 601 million cases and an estimated 6.5 million deaths worldwide, as of the end of August 2022 (WHO 2022a). In April 2020, the Royal Society of Canada created the Task Force on COVID-19 which has established, under its oversight as of the time of writing this report, 29 working groups to prepare Policy Briefings on diverse subjects addressing the COVID-19 pandemic.

Early on, the potential for wastewater surveillance for SARS-CoV-2 as part of pandemic management in Canada had been proposed and described in June and December 2020, then August 2021, respectively (Hrudey et al. 2020, Hrudey 2020; MacKenzie et al. 2020; Manuel et al. 2021). Our current report, one of the 28 RSC Policy Briefings to date, seeks to document, explain, and evaluate Canada's experience with implementing wastewater surveillance for SARS-CoV-2 over the past two years of the COVID-19 pandemic to provide recommendations for going forward and to support evidence-informed policy decisions.

1.2. Purpose for this report

Our report highlights what has been achieved with implementation of wastewater surveillance for SARS-CoV-2 and its contribution to date towards managing the COVID-19 pandemic in Canada and beyond. It also underscores the potential for a wastewater surveillance platform to be applied to other biomarker targets, providing decision-makers in Canada with timely and actionable intelligence regarding disease and other public health threats.

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Scientific, logistical, and organizational achievements to date in the field of wastewater-based disease surveillance position it as a catalyst to establishing a long-sought, pan-Canadian public health intelligence network; one that was envisioned twenty years ago by the National Advisory Committee on SARS and Public Health in the aftermath of the SARS-CoV-1 outbreak (HC 2003). The dedicated reader is encouraged to consult the body of literature that outlines the challenges to outbreak and pandemic response that are specific to the Canadian context prior to reading this report addressing wastewater surveillance for SARS-CoV-2 RNA.

This report does not seek to serve as an exhaustive review of all relevant topics or as a technical reference source for those seeking to evaluate or implement a wastewater surveillance program. Rather, it seeks to provide an informative overview of this topic in sufficient detail to inform interested readers and policy makers about key aspects and technical challenges involved with pursuing wastewater surveillance for SARS-CoV-2 RNA.

Early in the pandemic, many Canadian jurisdictions struggled to rapidly perform enough clinical testing of individual patients to accurately track and report the incidence and prevalence of COVID-19 in the population to allow for timely contact tracing. While the national testing rates were comparable to other G7 countries (GCDL 2022), systemic inequalities within and between provincial/territorial health jurisdictions, including shortages of personal protective equipment, contributed to inadequate responses most notably in congregate care settings which ultimately led to high morbidity and death rates (CPHA 2021). Financial and human resource limitations aside, the lack of harmonization and standards in clinical surveillance and reporting across Canada is a reality that is inherent to clinical surveillance systems around the world but is pronounced in the Canadian federation over such a large expanse of land and disparity in health systems. Gaps and inefficiencies in disease surveillance can lead to inaccurate intelligence and subsequent inadequate or even inappropriate responses from key decision makers. Moreover, mitigation of disease incidence using a classic test-trace-isolate strategy, such as the one many jurisdictions in Canada employed up until the advent of Omicron, has poor scalability, requires high rates of public compliance, and is ineffective for mitigating transmission when the etiologic agent is highly contagious (i.e., SARS-CoV-2 Omicron and its sub-variants, Contreras et al. 2021). Thus, emergence of more transmissible variants of the original Wuhan-Hu–1 SARS-CoV-2 virus (often referred to as the wild-type strain) led to increased challenges to clinical test-trace capacity resulting in a variety of changes in policies governing who in the population will be tested (e.g., random, self-selected, contact with cases, high risk, symptomatic only). In addition to regional differences in testing policies, reporting practices also differed across the country and at different times in the pandemic. Such changes in testing policy and lack of standardization between (and even within) public health jurisdictions have undermined the ability of clinical COVID-19 test results (i.e., case counts and their associated metadata) to serve as an effective, high-quality, primary data source that is a requirement for gold standard (Umemneku Chikere et al. 2019) epidemiological modelling and assessment of incidence, prevalence, and other metrics.

With the enormous public health management challenges posed by the COVID-19 pandemic, recognition of wastewater surveillance in Canada as a scientifically valid method to assess population-level disease states and its integration into the public health decision-making framework, has been an uphill struggle for credibility. An understandable skepticism of this apparently new and unfamiliar approach to public health surveillance was expressed by some medical and public health professionals. Likewise, concerns over a lack of clarity about what value wastewater surveillance could realistically provide towards public health decision-making (i.e., how can actionable data be derived from this type of environmental surveillance) have been expressed by health professionals. However, those skeptical perspectives are appropriately viewed in relation to the substantial uncertainty surrounding all knowledge collected in the COVID-19 pandemic, including that derived from classical clinical surveillance methods. Indeed, clinical surveillance may have been treated functionally as a gold standard, but it suffers from inherent biases and limitations that must be recognized in the context of this report (Glennon et al. 2021).

Until effective vaccines and specific medical treatments could be developed, public health management was necessarily limited to non-pharmaceutical interventions (NPIs) which all carry inevitably large uncertainty over their efficacy and underlying evidentiary basis. Under those conditions, any accurate evidence providing insights about the state of the pandemic could prove to be valuable. The evolution of variants of concern (VOC) and (or) waning population immunity that can render individuals susceptible to (re-)infection has made continued vigilance necessary despite widespread and successful vaccination programs in Canada.

The Institute of Medicine IOM (2000) summarized four critical components of public health surveillance as: collection, analysis, dissemination, and response. In a broad sense public health surveillance has derived information from many sources and implementation must adapt to the realities of those different sources. Historically, public health surveillance has been used (IOM 2000): *"to identify cases for investigation, to estimate magnitude of disease, to detect outbreaks, to evaluate response and prevention measures, to monitor changes in infectious agents, to facilitate research, and to measure the impacts of changes in health care practices."* This report explains how various applications of wastewater surveillance have contributed to most of these functions. Reasonable evaluation of wastewater surveillance for SARS-CoV-2 should be based on judging the merits of its contributions to various aspects of public health surveillance, not on fulfillment of the unreasonable expectation that it should be the only means for achieving most or all these objectives. Indeed, data triangulation is a fundamental tenet of the scientific method and of epidemiology specifically. All effort should be made to consider knowledge derived from wastewater surveillance, not in isolation, but in the context of multiple sources of intelligence, including, but not limited to clinical surveillance metrics (e.g., cases, hospitalizations, deaths) and event-based surveillance (e.g., mobility analytics, crowd-sourced syndromic surveillance, etc.).

1.3. Pre-COVID applications of wastewater surveillance for public health purposes

Wastewater surveillance, which has been commonly labeled as "*wastewater-based epidemiology (WBE)*" and can also be referred to as wastewater monitoring, dates back as far as the 1940s (Safford et al. 2022a) and has been used for a wide variety of purposes. This field of study has been commonly described as "*wastewater-based epidemiology*". Despite this common usage, wastewater surveillance is a more accurate label. Definitions of epidemiology include: "*Epidemiology is the method used to find the causes of health outcomes and diseases in populations. In epidemiology, the patient is the community and individuals are viewed collectively. By definition, epidemiology is the study (scientific, systematic, and data-driven) of the distribution (frequency, pattern) and determinants (causes, risk factors) of health-related states and events (not just diseases) in specified populations (neighborhood, school, city, state, country, global).*" (CDC 2011) and "*Epidemiology is the basic science of public health, because it is the science that describes the relationship of health and (or) disease with other health-related factors in human populations, such as human pathogens*." (Detels 2021). Surveillance is an important element of epidemiology and provides a better description of the activity being addressed. "*Public health surveillance is the ongoing systematic collection, analysis, and interpretation of outcome-specific data, closely integrated with the timely dissemination of these data to those responsible for preventing and controlling disease or injury*" (Thacker and Stroup 1994; Groseclose and Bukeridge 2017).

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Choi et al. (2018) reviewed the range of wastewater surveillance applications, including those with public health implications for surveillance of substances consumed by humans (pharmaceuticals, illicit drugs, tobacco, and alcohol), those that assess human exposure to industrial chemicals, and those that monitor spread of antibiotic-resistant microorganisms and infection by microbial pathogens. Choi et al. (2018) documented the rapid growth in overall research publications finding almost no relevant publications in 2007, rising to ~150 publications by 2017. For a more recent perspective on the explosion of the literature relevant to the COVID-19 pandemic, a search of Web of Science that updates the Choi et al. (2018) search with the additional requirement of "*SARS*" reports (as of 21 July 2022) indicates 120 articles published in 2020, 331 in 2021, and 183 so far in 2022. The sheer magnitude of the relevant published literature makes it not feasible to fully review all relevant published papers, therefore, this report prioritizes publications that bear most directly on the applications of wastewater surveillance for SARS-CoV-2 in Canada.

The application of wastewater surveillance for microbial pathogens is the most relevant to our current focus in this report. Prior to COVID-19, the most substantial and impactful application for pathogen surveillance has been in support of the global effort to control and eliminate poliomyelitis through vaccination programs. Duintjer Tebbens et al. (2017) provided a comprehensive overview of information on designs, costs, and effectiveness of these applications for poliovirus. They reviewed 146 studies (published between 1975 and 2016) covering 101 polio-related environmental surveillance activities from 48 countries. These studies ranged tremendously in scope, covering catchment zones as small as 50 people and as large as 7.3 million (median of 500 000). Numerous studies reported detection of polioviruses in wastewater in the absence of evidence of clinical cases. Tapani Hovi has been among the most active researchers in this field with more than 20 published papers on applications. Hovi (2006) confirmed that wastewater often revealed detection of poliovirus before reporting of clinical cases but cautioned that this early-warning strategy could only be cost-effectively deployed in jurisdictions where wastewater is collected from a common sampling point (i.e., a centralized wastewater treatment plant (WWTP)). Within Canada, studies of poliomyelitis, coxsackie and other enteric viruses present in wastewaters from Toronto (Rhodes et al. 1950; Clark et al. 1951), Ottawa (Sattar and Westwood 1977), and Montreal (Payment et al. 1979a, 1979b, 1983) provided pioneering research in this field. Many other enteric pathogens including norovirus, adenovirus, astroviruses, rotaviruses, coxsackievirus, echovirus, hepatitis A virus, and *Cryptosporidium* spp. have been candidates for wastewater surveillance (Clark et al. 1951; Myrmel et al. 2006; Payment et al. 1983; Zhou et al. 2003). Methods of detecting enteric pathogens in early wastewater surveillance studies were both rudimentary and laborious, requiring injection of animals with raw sewage (following bactericidal treatments) and observing diagnostic signs of infection, or later, by incubating cells with processed sewage and then determining viral titers. The advent of PCR technology in the late 1980s and later, quantitative PCR (qPCR), allowed rapid, sensitive, and specific detection of enteric pathogens in sewage. Heijnen and Medema (2011), working in the Netherlands, were the first to apply qPCR technology successfully to detect a respiratory virus, pandemic influenza A (H1N1 2009), in wastewater.

A related application of wastewater surveillance has been monitoring of antimicrobial resistance markers. This topic became one of active international research (Hendricksen et al. 2019) prior to the COVID-19 pandemic. Bouki et al. (2013) reviewed this application and reported that wastewater contained high proportions of antimicrobial (including antibiotic) resistant bacterial populations and provided evidence that conditions in WWTPs are conducive to transfer of antimicrobial resistance genes among bacterial flora.

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Fig. 1. University of California – Merced website summary of locations using wastewater surveillance of SARS-CoV-2 (arcg.is/1aummW).

1.4. International pursuit of wastewater surveillance for SARS-CoV-2 RNA

Early in the COVID-19 pandemic, several water researchers recognized the opportunity to apply knowledge of the published genetic sequence for SARS-CoV-2 to develop molecular analytical (PCR) methods to detect fragments of its genome in wastewater. By April to July of 2020, several teams submitted proof-of-concept findings for refereed publication (e.g., Ahmed et al. 2020a; Gonzalez et al. 2020; La Rosa et al. 2020; Lodder and deRoda Huisman 2020; Medema et al. 2020a; Nemudryi et al. 2020; Peccia et al. 2020; Randazzo et al. 2020a, 2020b; Sherchan et al. 2020; Wu et al. 2020; Wurtzer et al. 2020). This remarkably rapid dissemination of methods and results led to rapid uptake of applications of wastewater surveillance for tracking the pandemic in different municipal settings around the world. The phenomenal growth in adoption of wastewater surveillance for SARS-CoV-2 has been followed by a group led by Professor Colleen Naughton at the University of California – Merced which has maintained a website (summarized in **Fig. 1**, Naughton et al. 2021) that seeks to document the number and location of sites that are using this approach. As of 28 July 2022, this site lists 3 536 sites in 68 countries believed to be performing some aspect of wastewater surveillance for SARS-CoV-2.

Outside of Canada, major national and international research initiatives to evaluate and implement wastewater surveillance for SARS–CoV-2 include:

- Water Research Foundation, Denver, USA (waterrf.org/event/virtual-international-water-research-summit-covid-19; waterrf.org/resource/covid-19-wastewater-surveillance-symposium-global-update)
- European Commission, Brussels, Belgium (as well as several member States) (ec.europa.eu/environment/pdf/water/recommendation_covid19_monitoring_wastewaters.pdf)

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- Collaboration on Sewage Surveillance of SARS-CoV-2, ColoSSoS, Water Research Australia, Adelaide, Australia (waterra.com.au/research/communities-of-interest/covid-19/)
- South African Medical Research Council, Durban, South Africa (samrc.ac.za/wbe/index.html)
- UK Wastewater testing coverage data for the Environmental Monitoring for Health Protection (EMHP) programme (gov.uk/government/publications/wastewater-testing-coverage-data-for-19-may-2021-emhp-programme/wastewater-testing-coverage-data-for-the-environmental-monitoring-for-health-protection-emhp-programme)
- National Wastewater Surveillance System, US Centres for Disease Control & Prevention (cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-surveillance.html), partnering with Biobot Analytics (biobot.io/press-release/u-s-centers-for-disease-control-and-prevention-selects-biobot-analytics-to-expand-national-wastewater-monitoring/), the Federal Drug Administration (fda.gov/food/whole-genome-sequencing-wgs-program/wastewater-surveillance-sars-cov-2-variants), and state-level initiatives and programs

In addition, many other nations have adopted national wastewater surveillance programs (e.g., Turkey, Israel, Singapore). The foregoing list is limited to those with currently active websites describing their programs.

The Water Research Foundation hosted an international online summit (WRF 2020) of early adopters of wastewater surveillance for SARS-CoV-2 in May 2020. This early consultation event predicted potential uses including trends and changes in occurrence, assessment of community prevalence, and viral evolution.

1.5. Initiation of wastewater surveillance for SARS-CoV-2 RNA in Canada

This section deals with the initiation of wastewater surveillance for SARS-CoV-2 RNA as it began and evolved in Canada. A high-level overview of this evolution is presented but does not provide details of those activities, some of which are covered in subsequent sections and in more detail for a number of Canadian locations with the case studies that are listed in Part 1 of the **Supplementary Information**.

There has been a huge investment of time and energy by numerous researchers and institutions, the scope and detail of which is not possible to do full justice to in this report. Rather, this introduction focuses on over-arching initiatives that have shaped Canadian activities.

Prior to the COVID-19 pandemic, wastewater surveillance as a technique in support of public health had received limited attention and only specialized research study in Canada. Public Health Agency of Canada (PHAC), a few provincial public health agencies, and academic laboratories across Canada were investigating public health indicators such as pharmaceuticals, pathogens, and antimicrobial resistance in wastewaters. For example, Statistics Canada led Canadian Wastewater Survey (CWS) program to monitor cannabis and illicit drug use in five cities (Metro Vancouver, Edmonton, Toronto, Montreal, and Halifax) and 14 wastewater treatment plants across Canada (Werschler and Brennan 2019). With the onset of the COVID-19 pandemic, about seven or eight academic laboratories that were already analyzing environmental water samples for genetic markers of microorganisms pivoted their efforts in the period of March to May 2020 to include or to completely focus on the detection and quantification of SARS-CoV-2 and its variants in Canadian wastewaters. The federal CWS program was leveraged in May 2020 to collect and store samples for the monitoring of SARS-CoV-2 by PHAC when approvals and their method development had proceeded sufficiently to do so.

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With the intent of creating an effective and enabling space to enhance and catalyze more effective national collaboration among the disconnected individual efforts to apply wastewater sampling and analysis to COVID-19, the Canadian Water Network (CWN) established the COVID-19 Wastewater Coalition (cwn-rce.ca/covid-19-wastewater-coalition/) in April 2020. The Wastewater Coalition was created with the goal of informing a better understanding of *if*, *how* and *where* wastewater surveillance for SARS-CoV-2 might provide value for public health decisions. This national collaboration brought together municipalities, utilities, researchers, public health agencies, and governments to advance Canada's ability to support public health protection and surveillance in the face of COVID-19 by endorsing the shared goal of better connecting research to public health decisions. Efforts also benefitted from CWN participation with the Global Water Research Coalition (globalwaterresearchcoalition.net/about-us/gwrc-members/), comprising representatives of water research agencies or water utility organizations from nine countries, and by active participation in international fora.

The Wastewater Coalition established a National Research Advisory Group and a Public Health Advisory Group, built on members from the provincial agencies and academic laboratories engaging in SARS-CoV-2 testing of wastewaters and set out four guiding principles for Coalition participants to adopt in support of its shared goal.

- ***"Principle #1 – Adopting the Coalition Framework***

Activities conducted within the COVID-19 Wastewater Coalition will be organized in a way that is consistent with the Draft Wastewater Coalition Framework that better connects research areas to key public health decision-makers. Those participating in the COVID-19 Wastewater Coalition will attempt to best articulate the position of their work or research within the framework.

- ***Principle #2 – Research and Activities Framed by End-User Needs and Decisions***

Rather than emphasizing research interests and expertise areas, all research and activities conducted within the COVID-19 Wastewater Coalition will be framed and implemented in direct response to how the research outcomes will address end-user needs and support public health decision-making.

- ***Principle #3 – Open Sharing of Ideas***

Rapid sharing of ideas is imperative to group learning and achieving the collective goal. Participants in the COVID-19 Wastewater Coalition will actively share their knowledge with each other, prioritizing collective progress over individual or institutional recognition or advancements.

- ***Principle #4 – Open Sharing of Results***

Within the bounds of existing privacy protection agreements with partner municipalities or governments, all results of COVID-19 Wastewater Coalition-related work will be openly shared. Work conducted within the COVID-19 Wastewater Coalition will not be used for commercial gain, nor unduly held up due to publication or peer review requirements. The COVID-19 Wastewater Coalition will regularly share results with its international partners."

An early recognition that emerged through the Coalition's work was that wastewater testing for SARS-CoV-2 is fundamentally an application of public health surveillance that must be governed by appropriate ethical guidance. During the spring of 2020, the Coalition developed ethics and communications guidelines (CWN 2020a) for conducting research on SARS-CoV-2 in wastewater and engaging effectively with public health agencies and communities about wastewater surveillance data. Internationally, the Coalition's guidelines have been adopted by the European Commission for

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implementation in the EU Sewage Sentinel System for SARS-CoV-2 and have been recognized by the WHO.

The infrastructure and scientific knowledge and methodology developed during the pandemic for the use of SARS-CoV-2 wastewater surveillance in Canada, particularly over the first year, was largely built from grassroot efforts led by groups in academic laboratories in partnership with wastewater utilities and public health agencies across the country. These efforts were achieved largely by leveraging existing collaborations between laboratories with the necessary expertise and equipment working with the cooperation of local municipalities and in many cases with local public health agencies. That cooperation and collaboration has resulted in substantial activity to respond to the COVID-19 pandemic in different jurisdictions across the country. These local initiatives were in large part supported by the allocation of discretionary funds held by research groups at universities and in a few cases from short-term funding from research agencies. Although these initial groups were funded by various means independently, numerous weekly informal meetings and information exchange sessions were conducted and with active participation within the country, these exchange sessions furthered the advancement of wastewater surveillance in Canada.

Beginning in May 2020, CWN collaborated with the PHAC's National Microbiology Laboratory (NML) to design and implement an inaugural national inter-laboratory study to evaluate the capability of eight participating Canadian laboratories (CWN 2020b; Chik et al. 2021) to analyze a wastewater sampled in Winnipeg on 31 August 2020, at a time when there were only 85 clinically reported active COVID-19 cases in this city of ~750 000. This wastewater sample was treated as a "*blank*" that was spiked by NML with surrogates and both a low and high level of gamma inactivated SARS-CoV-2. Aliquots were then shipped to participating laboratories for analysis and reporting to the Consortium for interpretation (CWN 2020b; Chik et al. 2021). Overall, all methods included in this study yielded comparable results at the conditions tested. Use of consistent methods to explore wastewater SARS-CoV-2 temporal trends for a given wastewater system, given appropriate quality control protocols, would be expected to succeed. An additional seven interlaboratory evaluations have been organized by the Ontario WSI provincial surveillance program. The subsequent seven interlaboratory evaluations included the 13 academic laboratories in the province along with PHAC and other Canadian and American academic and commercial laboratories. This sample sharing initiative has led to consensus use of gene target regions, positive controls normalization targets, and QA/QC strategies across the province.

The earliest work by laboratories pivoting to wastewater applications for COVID-19 surveillance occurred at laboratories located in seven different provinces (BC, Alberta, Manitoba - NML, Saskatchewan, Ontario, Québec, and Nova Scotia). Some of the earliest successes and a focus on a more coordinated program occurred in Ontario.

The City of Ottawa with the University of Ottawa, the Children's Hospital of Eastern Ontario (CHEO) Research Institute, and Ottawa Public Health was a leading group in Canada that initiated wastewater sampling in April 2020. The Ottawa group achieved the first early detection of a COVID-19 wave in Canada in July 2020. They initiated Canada's most frequent sampling and reporting times of 7 d a week with 24 h laboratory turn-around by September 2020 and in collaboration with the Ottawa Hospital Research Institute, developed Canada's first extensive public reporting dashboard (613covid.ca/wastewater/) in September 2020. Ottawa Public Health used these data to triangulate COVID-19 incidence and to inform application of interventions for the city during the December 2020 wave.

In Ontario, early in the pandemic, wastewater analyses for SARS-CoV-2 were also developed in other regions, with the universities of Waterloo and Windsor joining University of Ottawa in providing leadership in development and application of the technology. These institutions provided a core leadership group supporting development of capacity in other Ontario universities. Drawing on that expertise and particularly the Ottawa experience as a template, the province of Ontario provided preliminary support for provincial wastewater surveillance in November 2020 ($750 000), and subsequently built upon that to create the Ontario Wastewater Surveillance Initiative (WSI) program in January 2021 (see Part 1 **Supplementary Information**). The WSI is the largest program in Canada and includes 13 academic laboratories in addition to PHAC. The program provides surveillance data and VOC tracking data to all public health units in the province of Ontario, for systems corresponding to locations that collectively represent over 70% of the provincial population. The Ontario WSI includes over 170 sites across the province that range from wastewater treatment plants, neighborhoods, long-term care facilities, correctional facilities, shelters, universities, and First Nations. The Ontario COVID-19 Science Advisory Table advising the Province of Ontario has used the wastewater surveillance data as a primary indicator of disease in the province since December 2021 (Draaisma 2022).

The Alberta Provincial Laboratory of Public Health jointly with the University of Alberta leveraged previous research studies on pathogens in municipal wastewater to secure a competitive 1-year proof-of-concept grant from the Canadian Institutes of Health Research (CIHR) that supported sampling from May 2020 and was extended across 12 Alberta municipal WWTPs (initially covering 79% of Alberta's population) from July 2020. Likewise, an interdisciplinary group at the University of Calgary obtained the only other 2020 CIHR grant for wastewater surveillance in June 2020 and initiated WWTP sampling in and around Calgary, as well as more localized neighbourhood and in-building sampling, including tertiary care hospitals. In October 2021 this work resulted in the provincial Pan-Alberta surveillance program that monitors 22 wastewater treatment plants across the province, with the data being public-facing, and captures over 80% of the provincial population (see Parts 1 and 2 **Supplementary Information**).

Researchers at Ecole Polytechnique and McGill in Québec were among the initial research groups developing wastewater techniques for SARS-Co-2 detection in 2020. The Québec government Institut National de Santé Publique (INSPQ) has recently initiated a surveillance program in March 2022 in collaboration with CentrEau of Université Laval, McGill University and Polytechnique Montreal. The program started with four cities and will be expanded to approximately 20 municipalities aiming to cover 70% of the provincial population.

The British Columbia Centres for Disease Control (BCCDC) Public Health Laboratory (PHL) leveraged an existing collaboration with Metro Vancouver focusing on enteric viruses in wastewater so that methods for the quantification of SARS-CoV-2 in wastewater were developed in May 2020. By October 2020, these methods were applied to the surveillance of 5 WWTPs in Metro Vancouver, covering nearly 50% of the B.C. population with a 24 h turn-around time for reporting to provincial epidemiologists and modellers.

These and other surveillance programs across the country, including Nova Scotia and Saskatchewan, have evolved with follow-up investments from local, provincial, and federal funding sources.

At the federal level in Canada, the PHAC pilot program for SARS-COV-2 monitoring began at 15 Canadian WWTPs in November 2020 and has expanded to over 65 locations across the country. In February 2021, PHAC supported the University of Saskatchewan to implement wastewater sampling in Saskatoon and 5 First Nations Communities and in March 2021, established a molecular testing laboratory in the Northwest Territories.

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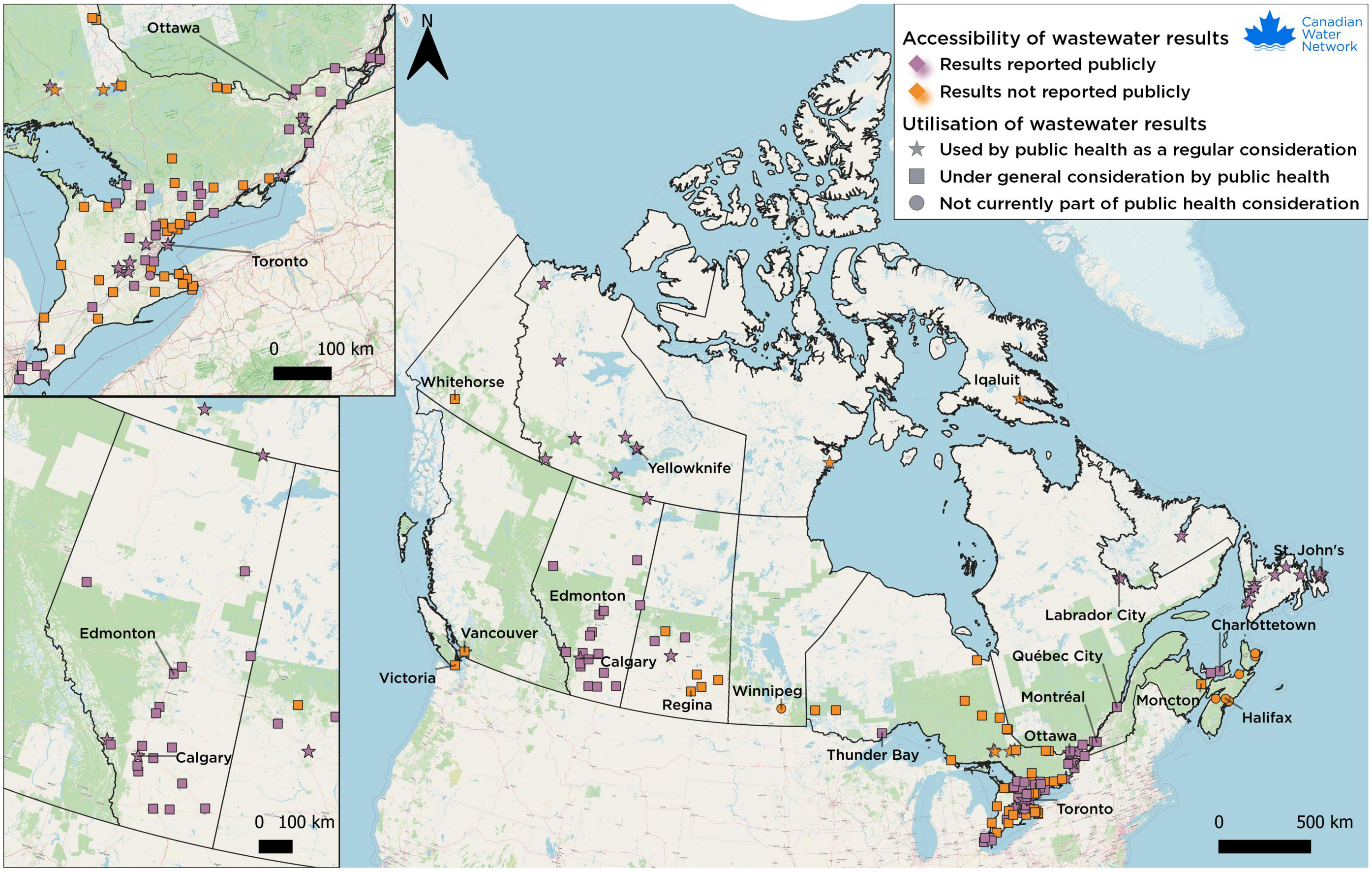

Fig. 2. Wastewater sampling and analysis of SARS-CoV-2 in Canada (as of July 12, 2022) as known to the Canadian Water Network (CWN).

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As of 12 July 2022, the CWN Coalition is aware of 152 municipalities or locations in Canada that have been performing wastewater surveillance (often at multiple sampling sites) for SARS-CoV-2 RNA shown in Fig. 2. Case studies of a variety of programs are presented in Part 1 of the Supplementary Information. The ability to have rapidly implemented this level of activity in Canada is a tribute to an unprecedented level of cooperation and collaboration among academic researchers, wastewater utilities, public health officials and in some cases, private sector contributors.

In most cases, implementation of wastewater surveillance occurred when individuals with relevant expertise identified an opportunity to contribute that expertise to the emerging efforts mounted in different jurisdictions to respond to the COVID-19 pandemic. These local initiatives were in large part supported by allocation of discretionary funds held by individuals at universities and a few cases of short-term funding from research agencies.

2. Public health measures for a pandemic

2.1. Introduction

Although this report is focused on wastewater surveillance for SARS-CoV-2, the need for such surveillance and its merits or limitations should be judged within the context of what public health interventions are possible in a pandemic and how confident anyone can be about the effectiveness

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of interventions. A pandemic has been defined as an epidemic occurring worldwide, or over a very wide area, crossing international boundaries, and usually affecting a large number of people (Porta 2008). Furthermore, as will be discussed further in Section 5 with regard to ethical guidelines, an overriding ethical requirement for judging any public health-motivated activity is that the activity must have a clear public health purpose based on a well-developed plan for data collection, analysis, use, and dissemination. The CWN, in launching the Canadian Coalition on Wastewater-Related COVID-19 Research made clear that there is a need for programs pursuing wastewater surveillance for SARS-CoV-2 RNA to keep public health at their core. These observations are made with full recognition of the important role of discovery research in supporting and advancing wastewater surveillance for SARS-CoV-2, but as is required for discovery research in the health sciences, the ethics of such research must be addressed and cannot be short-circuited in the name of achieving research discoveries.

2.2. Non-pharmaceutical interventions (NPI) for a pandemic

Inherently, public health surveillance is a fundamental form of an NPI. Wastewater surveillance for SARS-CoV-2 seeks to inform public health interventions in response to a pandemic by providing relevant evidence about COVID-19 occurrence in the monitored population. The potential information value of wastewater surveillance should be viewed in relation to the inevitable uncertainty about modes of transmission of a new pathogen and corresponding interventions aimed at reducing transmission.

A variety of non-pharmaceutical interventions (NPI) exist to reduce infectious disease transmission (CDC 2007). NPI refer to measures such as quarantine, physical distancing, mask-wearing, avoidance of crowds, etc. that do not involve reliance of administration of medicines, as elaborated in **Table 1**.

Table 1. Potential non-pharmaceutical public health interventions that could mitigate an influenza pandemic [after Low (2008) adapted from Aledort et al. (2007)].

Human surveillance & individual preventive measures	Case reporting
	Early rapid viral diagnosis
	Disinfection
	Hand hygiene
	Respiratory etiquette
	Surgical and N95 masks
	Other personal protective equipment
Patient management	Isolation of sick individuals
	Provision of social support services to the isolated
Contact management	Quarantine
	Voluntary sheltering
	Contact tracing
Community restrictions	School closures
	Workplace closures
	Cancellation of group events
	International and domestic travel restrictions

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Such measures are especially important in early pandemic stages, before targeted vaccines and therapeutics have been developed. In a study of the 1918–1920 flu pandemic, Markel et al. (2006) concluded that available data fail to show that any NPI, aside from "*protective sequestration*" (i.e., quarantine) was or was not effective in preventing viral spread.

Markel et al. (2006) reported that "*protective sequestration … if enacted early enough in the pandemic, crafted so as to encourage the compliance of the population involved, and continued for the lengthy time period in which the area is at risk, stands the best chance of guarding against infection*." The practicality of this degree of quarantine for controlling COVID-19 in modern societies is challenging, although something approaching this extreme has been attempted by China, Taiwan, and Tonga. Several other jurisdictions have pursued a so-called "*zero COVID*" model that seeks to reduce COVID-19 transmissions to minimal levels without necessarily compelling total lockdown and enforced quarantine of all cases and contacts. Markel et al. (2006) go on to conclude: "*available data from the second wave of the 1918–1920 influenza pandemic fail to show that any other NPI (apart from protective sequestration) was, or was not, effective in preventing the spread of the virus*." Given the tools available to public health authorities in 1918 and the inevitable challenges of researching the success of such interventions almost 80 years afterwards, a determination that evidence about outcomes for various interventions is inconclusive should not be surprising.

The Asian avian flu (H5N1) epidemic and the first Severe Acute Respiratory Syndrome (SARS) epidemic, both of which emerged in 2003, sparked renewed interest and concerns about preparedness for a human influenza pandemic and motivated several expert reviews of available non-pharmaceutical public health interventions. Bell et al. (2006) provided a high-level summary of WHO guidance about national and community measures available for this purpose. Low (2008) took a more detailed look at potential NPIs for mitigating an influenza pandemic. Low noted that the U.S. Centers for Disease Control (Qualls et al. 2017) had released guidance for the use of NPIs for an influenza pandemic including a pandemic severity index based on the case fatality ratio and projected number of U.S. deaths. This pandemic severity index would have classified the COVID-19 pandemic as a Category 4 (out of 5) pandemic. Waterer (2011) reviewed the public health measures taken for the 2003 SARS-CoV-1 and H1N1/09 epidemics (CDC 2019), concluding that measures aimed at preventing international spread of a viral pandemic showed minimal efficacy. Waterer concluded that effective pandemic prevention strategies must incorporate improved surveillance, more flexible planning and response, and improved diagnostic testing while retaining a focus on basic hygiene measures.

To explore NPIs in more detail, Aledort et al. (2007) performed a systematic review (considering 2552 articles and ultimately selecting 168 as relevant, including 9 systematic reviews) and elicited expert opinion from a meeting of interdisciplinary experts in January 2006 to review and evaluate evidence for effectiveness of NPIs for a hypothetical influenza pandemic. Participants included: experts in biomedical research, virology, clinical practice, infection control, epidemiology, public health, ethics, law, history, and health policy, all North American except for one. The interventions considered were consistent with those summarized by Bell et al. (2006) and Low (2008) and are summarized here in **Table 1**.

Aledort's expert consultation considered 56 specific interventions reviewed across four stages of pandemic (i.e., only overseas cases, no cases yet locally, early localized - some local cases and wide-spread transmission nationally). Only nine interventions were recommended for use by a majority of the experts in the consultation for the first two stages and 14 in the last two stages. A majority recommended against using between six (first stage) and 12 specific interventions (last stage).

The recommendations from these experts are summarized in **Tables 2** and **3**, respectively. These findings must be viewed in the context from which they arose – consultation of a diverse, interdisciplinary

Table 2. Agreed-upon measures according to pandemic stage (adapted from Aledort et al. 2007).

Non-Pharmaceutical Interventions	Stage of the Pandemic: No cases in Country	Stage of the Pandemic: Cases in country, none local	Stage of the Pandemic: Early localized cases	Stage of the Pandemic: Advanced – wide-spread transmission
Hand hygiene - hospital	Majority recommendation for use	Majority recommendation for use	Majority recommendation for use	Majority recommendation for use
Hand hygiene - ambulatory	Majority recommendation for use	Majority recommendation for use	Majority recommendation for use	Majority recommendation for use
Hand hygiene - community/home	Majority recommendation for use	Majority recommendation for use	Majority recommendation for use	Majority recommendation for use
Respiratory etiquette - hospital	Majority recommendation for use	Majority recommendation for use	Majority recommendation for use	Majority recommendation for use
Respiratory etiquette - ambulatory	Majority recommendation for use	Majority recommendation for use	Majority recommendation for use	Majority recommendation for use
Human surveillance	Majority recommendation for use	Majority recommendation for use	Majority recommendation for use	Majority recommendation for use
Case reporting	Majority recommendation for use	Majority recommendation for use	Majority recommendation for use	Disagreement - no majority
Rapid viral diagnosis and triage	Majority recommendation for use	Majority recommendation for use	Majority recommendation for use	Disagreement - no majority
Voluntary advisories on departures from international affected regions	Majority recommendation for use	Disagreement - no majority	Disagreement - no majority	Disagreement - no majority
Voluntary self-isolation of the sick in home	–	Majority recommendation for use	Majority recommendation for use	Majority recommendation for use
Provision of social support services (to isolated or quarantined persons) - hospital	–	Disagreement - no majority	Majority recommendation for use	Majority recommendation for use
Provision of social support services (to isolated or quarantined persons) - ambulatory	–	Disagreement - no majority	Majority recommendation for use	Majority recommendation for use
Other PPE - hospital	Disagreement - no majority	Disagreement - no majority	Majority recommendation for use	Majority recommendation for use
N95 Respirators - hospital	Disagreement - no majority	Disagreement - no majority	Majority recommendation for use	Majority recommendation for use
Respiratory etiquette - community/home	Disagreement - no majority	Disagreement - no majority	Majority recommendation for use	Disagreement - no majority
Surgical masks - hospital	Disagreement - no majority	Disagreement - no majority	Disagreement - no majority	Majority recommendation for use
Surgical masks - ambulatory	Disagreement - no majority	Disagreement - no majority	Disagreement - no majority	Majority recommendation for use
Provision of social support services (to isolated or quarantined persons) - home	–	Disagreement - no majority	Disagreement - no majority	Majority recommendation for use
N95 Respirators - ambulatory	Majority recommendation against use	Disagreement - no majority	Disagreement - no majority	Majority recommendation for use

Note: Legend: Majority recommendation for use (green); Majority recommendation against use (red); Disagreement - no majority (white); – not relevant.

panel about actions to be taken in a non-specific, hypothetical pandemic. There is no universal flowchart for a pandemic because important particulars such as the nature of the contagion and its infection dynamics will determine the efficacy of specific intervention measures. More detailed scenarios that consider different classes of pathogens with pandemic potential are beyond the scope of this brief.

An early focus in the COVID-19 pandemic, based on prior experience with SARS and other respiratory viruses (Jefferson et al. 2020), was on cleaning surfaces and on individual handwashing. While a commitment to handwashing is always good advice for reducing infectious disease transmission, surface-borne transmission is no longer believed to be a major factor in transmission of SARS-CoV-2 (Kampf et al. 2020).

Person-to-person transmission of SARS-CoV-2 was shown likely to occur primarily by fine airborne particulates arising from normal speech (Stadnytski et al. 2020), a reality that was inconsistent with

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Table 3. Measures disagreed-upon according to pandemic stage (adapted from Aledort et al. 2007).

Non-Pharmaceutical Interventions	Stage of the Pandemic: No cases in Country	Cases in country, none local	Early localized cases	Advanced – wide-spread transmission
N95 Respirators - ambulatory	Against			For
Limited case-by-case home-based mandatory quarantine (of exposed - home)	–			Against
Contact tracing				Against
Mandatory restrictions on arrivals from affected international regions				Against
Exit screening of travelers from affected international regions to unaffected U.S. regions				Against
Entry screening of travelers from affected international regions to unaffected U.S. regions				Against
Exit screening of travelers from affected U.S. regions to unaffected U.S. regions	–			Against
School closures	–	Against		
Work closures	–	Against		
Case-by-case cancellation of public events	–	Against		
Mandatory restrictions on arrivals from affected U.S. regions	–	Against		Against
Entry screening of travelers from affected U.S. regions to unaffected U.S. regions	–		Against	Against
Mandatory restrictions on departures from affected international regions			Against	Against
Surgical masks - community	Against	Against		
Surgical masks - home	Against	Against		
N95 Respirators - community	Against	Against	Against	
N95 Respirators - home	Against	Against	Against	Against
Mandatory restrictions on departures from affected U.S. regions	–	Against	Against	Against
Other PPE - community	Against	Against	Against	Against
Other PPE - home	Against	Against	Against	Against

Note: Legend: Majority recommendation for use (green); Majority recommendation against use (red); Disagreement - no majority (white); – not relevant.

early WHO guidance that did not adequately reflect that airborne risk (Morawska and Cao 2020). The May 2022 Québec coroner's report (Kamel 2020) into the deaths of 53 senior residents of long term care facilities included expert testimony revealing how failure to recognize the possibility of inhalation transmission contributed to COVID-19 illness and deaths. Knowledge of airborne transmission is vital to valuing the efficacy of wearing N95 masks (Howard et al. 2021) as confirmed by the recent findings of Andrejko et al. (2022). They found with a case control study (652 cases, 1176 controls) that various types of face coverings substantially reduced the risk of SARS-CoV-2 infection, with N95 masks reducing the odds of COVID-19 infection to 0.17 (0.05–0.64; $p < 0.01$) relative to wearing no face covering (odds = 1.00). Cloth masks reduced the odds of COVID-19 infection to 0.44 (0.17–1.17; $p = 0.10$) and surgical masks reduced the odds to 0.34 (0.13–0.90;

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$p = 0.03$). Although airborne transmission of SARS-CoV-2 is evident, modelling of a COVID-19 outbreak by Peng et al. (2022) in comparison with other airborne communicable diseases found that the ancestral Wuhan Hu-1 virus may not have been as readily transmitted as measles. It is now clear that transmissibility of subsequent variants has increased substantially, with Omicron variants, potentially rivaling measles in their ability to spread with unprecedented speed in a naïve population (Liu and Rocklöv 2022). Differences in degree of transmissibility can exist for airborne transmission, adding to uncertainty about interventions. Wang et al. (2021) provided an excellent overview of the early misunderstanding and miscommunication about the benefits of mask-wearing, early in the COVID-19 pandemic, for different types of masks. They distinguished the benefits of protecting others vs. protecting the mask-wearer while stressing that no mask can be effective unless it is worn properly.

In any case, uncertainty and conflicting views portend what has occurred in the pervasive and increasingly divisive debates over public health measures for the COVID-19 pandemic over the past two years. The reasons that individual experts held for supporting or opposing any particular measure are not available from the Aledort study. However, some aspects of the uncertainty about efficacy reflected in Aledort et al. (2007) are particularly applicable to the topic of this report.

The lack of any mention of ventilation as an NPI by these experts, even for the hypothetical influenza pandemic, is striking. Improved ventilation in schools has likely been an important feature of minimizing COVID-19 transmission in schools (Gettings et al. 2021; Ding et al. 2022)

For Canada, most of the past two years of COVID-19 have been spent in the fourth pandemic phase (advanced, wide-spread transmission). **Tables 2** and **3** show that even the experts consulted by Aledort et al. (2007) have lacked unanimity about measures that should be implemented, even allowing for the generic nature of the consultation: foreshadowing the lack of unanimity or even consensus among experts that has manifested as COVID-19 has persisted. What is generally lacking at the moment is credible evidence about the effectiveness of most individual public health measures in mitigating COVID-19. Over the next few years, there will likely have been enough natural experiments across different jurisdictions that developing an improved evidence-base to judge the efficacy of various measures should be possible.

Public health measures taken to prevent our healthcare systems from being overwhelmed have affected virtually every Canadian to some extent, causing everyone to have opinions about these public health measures that has a bearing on the importance of ethical guidance discussed in Section 5. What is clearly evident from the predictions about what measures should be taken in any pandemic and the controversies that have subsequently unfolded during the COVID-19 pandemic is that there is substantial uncertainty about many of the possible non-pharmaceutical public health interventions. Uncertainty creates conditions for misunderstanding. None of these NPI measures are free from some negative aspects for the affected population except for ventilation which was not mentioned.

Compelling evidence for what measures work best, sufficient to justify such inevitable negative impacts, is very limited to non-existent. The one intervention of any kind that has both compelling evidence of success combined with negligible negative consequences for most individuals is vaccination. Yet, vaccination remains controversial among substantial fractions of the population. This anomaly of human behaviour demonstrates that conventional scientific evidence alone cannot motivate all of our population. Regardless, seeking more evidence and better understanding about the evolution of the pandemic, including issues like vaccine hesitancy, are clearly rational goals for public health authorities to pursue.

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The consequence from the evidence cited above is that there is substantial uncertainty about the efficacy of many NPIs. Our RSC report strives to address this uncertainty for one particular NPI—public health surveillance.

2.3. Surveillance measures for a pandemic

The non-pharmaceutical interventions outlined in the previous section reflect considerable uncertainty about their efficacy making surveillance to provide evidence about outcomes particularly important.

Epidemiological evaluation of infectious disease is founded on a classical concept of a triad among a (1) causal agent, (2) a host (the infected individual) and (3) the environment through which the causal agent is transmitted to the host (**Fig. 3**). This triad is also sometimes depicted as involving a balance between agent and host that is governed by the infectivity of the agent, the susceptibility of the host, and nature of the environment in facilitating disease transmission. Determining for any disease how this triad is functioning relies on surveillance that determines the nature and extent of the exposure to the infectious agent and the state of host infection.

This triad suggests two main foci for public health surveillance to understand the infectious disease transmission. The first focus is on determining the infectious agent in the host (clinical surveillance); the second is on tracing the infectious agent in the environment through which the agent reaches the host. Experience with COVID-19 has demonstrated that SARS-CoV-2 spreads primarily through airborne transmission (Lewis 2022; Moriawska and Cao 2020; Zhang et al. 2020) and not as likely by contact with contaminated surfaces.

Wastewater surveillance for SARS-CoV-2 may be misunderstood in this triad because wastewater is known to be an environmental factor and a potential vector for disease transmission in certain contexts, i.e., enteric pathogens contaminating drinking water. Viable SARS-CoV-2 has rarely been isolated from faeces of infected patients despite high levels of RNA detected (Kim et al. 2020; Wölfel et al. 2020) and SARS-CoV-2 transmission via the water cycle is not a major concern (Sobsey 2022). Monteiro et al. (2022) found that SARS-CoV-2 is not viable across secondary wastewater treatment, meaning that treated wastewater does not pose a significant transmission risk for COVID-19 when discharged to the aquatic environment. The reality is that wastewater surveillance of SARS-COV-2 RNA levels are used as a biomarker indicator for infected individuals shedding the virus in the community. Wastewater surveillance is a passive method of pooled observation of infected hosts in a community served by a sewer system.

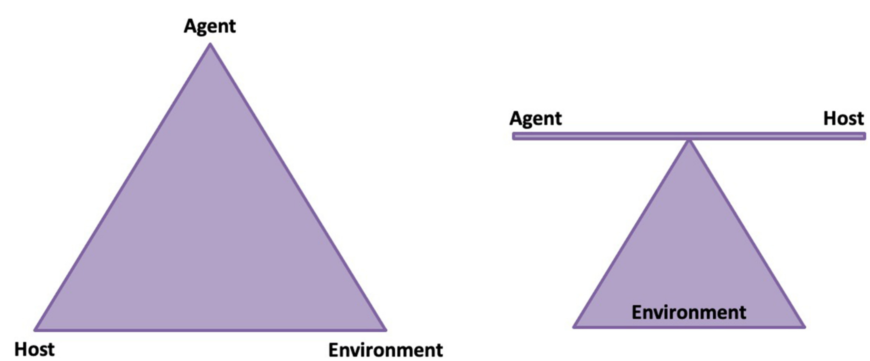

Fig. 3. The epidemiologic triad and balance in disease transmission.

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PCPHN (2015) reviewed preparedness for Canadian pandemic influenza, noting that Canada's public health infrastructure resides primarily within the provincial and territorial ministries or departments of health that partner with the PHAC under Canada's federal governance structure. Notably, effective public health surveillance is necessary to ensure timely application of non-pharmaceutical interventions and public health policy decisions. Effective pandemic management requires public health surveillance mechanisms that have the ability and capacity to identify and (or) trace cases, as well as following how the disease manifests in populations (i.e., epidemiology). Some of the challenges to achieving this goal are associated with the nature of the disease. The expected disease burden that was cited in planning for an influenza pandemic (PCPHN 2015) progressed upward from a base of patients who were asymptomatic infected, up to symptomatic (self-care), medically attended (in the community) to hospitalized and ultimately deceased.

Experience with the COVID-19 pandemic and concerns with overloading healthcare systems suggested a more detailed view of the disease burden (Medema et al. 2020b) progressing upward from a base of all infections: (pre) symptomatic and asymptomatic, all symptomatic infections, symptomatic individuals tested, cases reported, hospitalizations, ICU occupancy, and ultimately deaths.

With conventional communicable disease surveillance, only the top four categories of patients are normally captured by conventional surveillance programs. Of these, the top three may be considered the most tangible and serious indicators of COVID-19 prevalence in the community. Unfortunately, these are also lagging indicators that arise more than a week after initial infection. They represent the burden on the healthcare system that public health interventions seek to flatten the curve of incident cases to avoid a crisis in healthcare system capacity (**Fig. 4**). With growing concern for "*long COVID*" representing an important impact on public health (morbidity as well as mortality), the upper part of the influenza disease burden pyramid may need to be adjusted to include persistent, chronic conditions that have not been a concern with influenza.

PCPHN (2015) planning for an influenza pandemic referred to WHO interim global surveillance standards as providing concepts that recognize:

- "The importance of monitoring both mild and severe influenza;
- The efficiency of sentinel surveillance in collecting high-quality data in a timely way;
- The need for a standardized approach to data collection;
- Recognition that surveillance case definitions are not intended to be used for diagnostic purposes or for treating influenza or influenza-like illness (ILI);

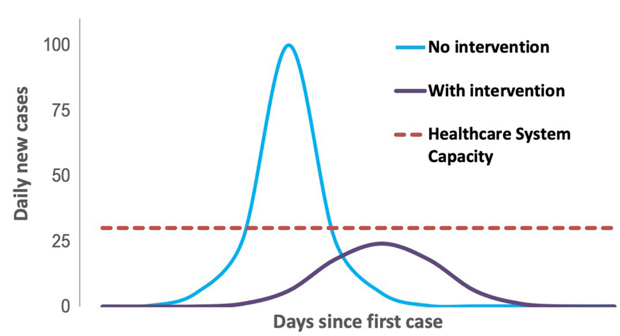

Fig. 4. Flattening the COVID-19 epidemic curve to protect healthcare capacity.

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- The value of having historical (baseline) seasonal surveillance data against which to assess the impact and epidemiological features of the evolving pandemic;
- The integration of influenza surveillance programs into existing public health systems;
- The adaptation of surveillance activities as the pandemic proceeds; and
- Sharing of surveillance data with policy-makers and feedback to those who provided data."

Elements of an effective communicable disease surveillance system can include (HC 2003; Nsubuga et al. 2006; Thacker et al. 1996):

- Early warning: identifying the infectious agent and understanding modes of transmission.
- Trendsetting: monitoring incidence and prevalence of pandemic disease.
- Data gathering and reporting: balancing accuracy with timeliness.
- Contact tracing and data granularity: mitigates transmission and enhances understanding of the disease and effects of interventions.

The ability of wastewater surveillance for SARS-CoV-2 to deliver these capabilities differs from conventional surveillance for infectious diseases. Those capabilities, particularly as experienced in Canada, are elaborated in the next section The niche for wastewater surveillance in the overall surveillance framework for COVID-19 is illustrated in **Fig. 5** (WHO 2022c).

3. Applications of wastewater surveillance for SARS-CoV-2 RNA

3.1. Introduction

In this section, we document the evolution of applications of wastewater surveillance for SARS-CoV-2 in Canada and abroad at various geographic scales. In the subsequent section, the essential components of these surveillance systems are critically examined and explained.

Infectious disease surveillance in most circumstances does not typically reach to the lowest echelons of the disease burden pyramid; only symptomatic patients seeking medical attention are identified. Such a disease prevalence indicator is subject to underreporting relative to the true prevalence of a disease within a community because identified cases will be dependent upon the characteristics of testing policies – something that differed substantially from one provincial jurisdiction to another and from one wave of the COVID-19 pandemic to another. For example, early in the pandemic, in May 2020, Alberta was performing among the highest number of COVID-19 tests per capita in Canada. After more than a year, by 29 July 2021, testing policy was changed such that testing of asymptomatic individuals was no longer recommended. Disease burden indicators higher in the pyramid are typically lagging indicators because they normally only follow initial symptoms.

Wastewater surveillance could potentially provide meaningful early signals of disease in a community, provided that the candidate biomarker(s) for the specific disease under surveillance fulfill the following criteria:

1. is present in human excreta discharged to wastewater,
2. can be reliably detected and quantified in wastewater, and
3. is related in some manner to the number of individuals infected (ideally one that increases and decreases contemporaneously with infections).

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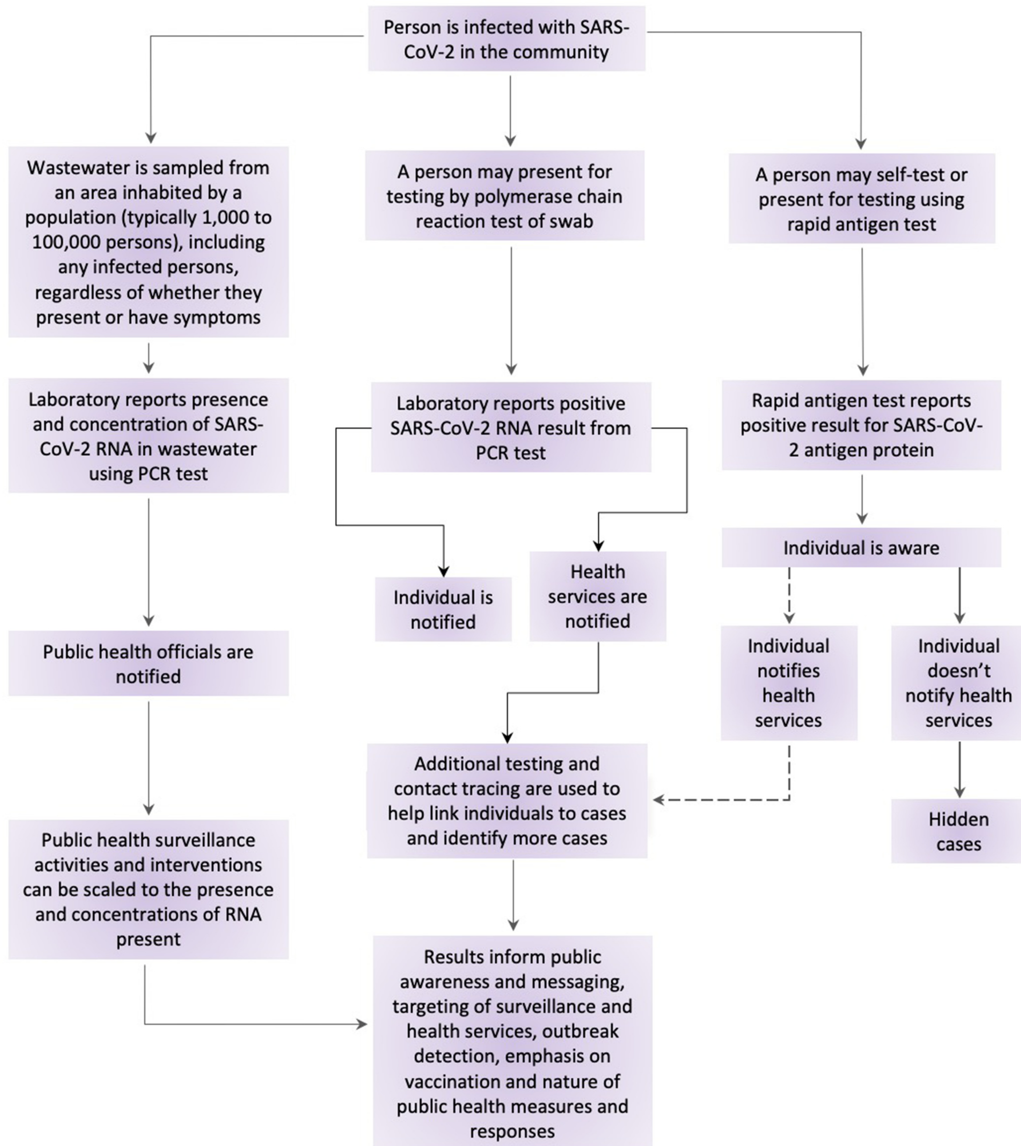

NB:

- The emphasis on different test methods may vary during different phases of the pandemic
- The timeframe from sampling to visualizing test results is of the order 15 min for rapid antigen diagnostic tests and approximately 0.5 to 2 days for both diagnostic and ES PCR tests (sometimes more depending on backlogs and turnaround times).
- The early warning offered by ES comes from its ability to detect the virus in pre-symptomatic and asymptomatic persons in the community that shed the virus but that might not have presented for diagnostic testing.
- In some contexts, results are shared directly with the community at the same time as the public health agency

Fig. 5. Overall COVID-19 surveillance that includes wastewater surveillance (WHO 2022c).

An obvious candidate biomarker for wastewater-based COVID-19 surveillance is the causative agent, SARS-CoV-2. Detection of its genetic material from nasopharyngeal as well as rectal swabs by PCR has formed the basis of clinical diagnosis of COVID-19 since the beginning of the pandemic. Regarding the first requirement that SARS-CoV-2 be known to be excreted to wastewater,

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Zheng et al. (2020) established by means of a survey in China from January to March 2020 that SARS-CoV-2 RNA was detected in the faeces of 59% of a cohort of confirmed COVID-19 patients and that those signals were detected for a median duration of 22 days, a few days longer than in respiratory samples tested in parallel. Notably, Xiao et al. 2020 reported positive detection of SARS-CoV-2 RNA from faeces in 23% of patients even after it had disappeared from the respiratory tract. Kitajama et al. (2020) provided an early extensive review of wastewater surveillance that cited 13 additional references about excretion of SARS-CoV-2. Studies (Lescure et al. 2020; Pan et al. 2020; Wölfel et al. 2020) reported up to 10^8 RNA copies per gram of faeces from COVID-19 patients.

Li et al. (2022) reviewed published evidence on excretion of SARS-CoV-2 from COVID-19-infected patients and reported a similar result with greater than 50% probability of faecal excretion of SARS-CoV-2 along with over 80% probability of saliva excretion. The published evidence base is far from complete in quantitative terms. What matters is that excretion of SARS-CoV-2 to wastewater from a COVID-19-infected population is expected. Of interest is that among 1/3 of patients showing sputum production, 98% of sputum samples were positive for SARS–CoV-2 RNA. Based on this information Li et al. (2022) included sputum excretion of SARS–CoV–2 RNA in a model predicting for its detection in wastewater based on estimated excretion levels and found that including estimates of sputum in wastewater over including faecal excretion alone dramatically improved their model predictions. Although their analysis is not definitive, it does suggest a possible role for the presence of sputum in determining the quantities of SARS-CoV-2 RNA detected in wastewater and in possibly explaining some of the variability in wastewater detection.

Regarding the second requirement of reliable detection and quantification of the biomarker(s), an effective surveillance program requires a well-designed monitoring system supported by an analytical procedure that is able to reliably capture and quantify changes in disease biomarker levels over time. Demonstration that wastewater surveillance was useful for following COVID-19 trends in targeted populations required proof-of-concept studies showing that in addition to appreciable quantities of SARS-CoV-2 RNA being shed by infected individuals, the virus signal persists in wastewater and, owing to the complex nature of the matrix, the detection method can detect relevant quantities of SARS-CoV-2 RNA with false positives only likely if sample or laboratory contamination occurs (Ahmed et al. 2022c).

For the third element to be demonstrated, the wastewater-based SARS-CoV-2 signal needs to correlate with clinical case counts, hospitalizations, and (or) deaths from COVID-19 within the corresponding geographic region served by the sewer system upstream of sampling location.

The earliest longitudinal, near real time, proof-of-concept study for wastewater surveillance was performed in the Netherlands (Medema et al. 2020a). In this context, "*near real time*" refers to intentional collection and analysis for SARS-CoV-2 of fresh wastewater samples over a period of time with contemporaneous reporting using RT-qPCR, versus older, more time-consuming methods or by retroactive analyses of a limited number of archived wastewater samples. As noted in Section 1 of this report, there has been a rapid proliferation in publications related to wastewater surveillance for SARS-CoV-2 since the first proof-of-concept (see Section 1.3), far too many to individually review within the scope of a policy brief about applications for Canada.

Medema et al. (2020a) collected composite samples of influent wastewater at each of six wastewater treatment plants (WWTPs) between February 5 and 7, 2020, about three weeks before the first clinical cases of COVID-19 were reported on 27 February 2020. In their second round of sampling between March 4 and 5, SARS-CoV-2 gene fragments (targeting 3 locations on the N gene, and one location

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on the E gene) were detected in three of the six WWTPs, including Amsterdam's Schipol International Airport. Subsequent rounds of sampling (March 15-16 and March 25) showed positive detection in all six locations. One wastewater sampling location (Amersfoort) was positive for SARS-CoV-2 RNA on March 5 despite the first two clinical cases not being reported for this location until March 11. The last two rounds of sampling (March 15-16 and 25) in this proof-of-concept study showed detectable SARS–CoV–2 RNA at all WWTPs and when results were pooled for all WWTPs and the signals obtained from all 4 targeted regions of the SARS-CoV-2 genome result showed a strong positive correlation with cumulative clinically confirmed cases.

The first applications for sampling influent wastewater at WWTPs provided evidence about COVID-19 for entire served communities. Several other applications of the surveillance concept have emerged since, including sampling in sewer networks to focus on neighbourhoods, sampling of specific institutions (universities/colleges, long term care facilities, hospitals, prisons, industrial worksites), sampling of transportation hubs (cruise ships, airplanes, airports), and tracking of the emergence of SARS–CoV–2 variants. For those interested in a high-level perspective after two years of practice Lok-Wah-Hoon et al. (2022) have produced a set of questions and answers about wastewater surveillance for the WHO Regional Office for Europe.

3.2. Surveillance at wastewater treatment plants (WWTPs)

Applications of SARS-CoV-2 RNA surveillance at WWTPs have generally focused on sampling influent wastewater or primary sludge. Terminology for conventional municipal wastewater (sewage) treatment refers to primary treatment as a sedimentation stage to remove settleable solids, with secondary treatment being a biological stage (e.g., activated sludge, fixed film processes) to remove dissolved and colloidal organic matter, including reduction of biochemical oxygen demand of wastewater and tertiary treatment being additional treatment for specific constituents such as inorganic nutrients like phosphorus and nitrogen. An early review of surveillance practice, mostly at WWTPs, was published by Medema et al. (2020b), and was followed by more recent reviews of what can and cannot be achieved by wastewater surveillance for SARS-CoV-2 RNA (Buonerba et al. 2021; Hrudey and Conant 2022 and Shah et al. 2022) which are elaborated below.

Medema et al. (2020b) summarized early proof of concept monitoring results obtained at WWTPs from nine refereed publications and nine non-refereed preprints (four subsequently refereed and published). These limited results (from 2 to 126 samples, median of 14) demonstrated the feasibility of SARS-CoV-2 RNA detection in primary wastewater or sludge samples. Medema et al. (2020b) noted the challenge of obtaining and accurately reporting clinical cases for the same population as was served by any WWTP. Hrudey and Conant (2022) summarized 16 publications (all refereed) claiming to provide early detection of SARS-CoV-2 RNA at WWTPs before clinical cases were reported but only a publication from Ottawa (D'Aoust et al. 2021a) reported sufficient frequency of sampling to demonstrate an authentic, near real time early warning signal from wastewater (primary sludge) in advance of confirmed clinical cases. Other claims were either based on retroactive analyses (not real time) from sample dates preceding clinical case confirmation or had insufficient detailed evidence to validate an early warning claim. The subject of early warning is reviewed further with regard to the value of wastewater surveillance for SARS-CoV-2 in Section 5.

Shah et al. (2022) provided a formal, systematic review for publications on wastewater surveillance for SARS-CoV-2 from 1 January 2020 to 31 July 2021. From an initial sample of 451 publications, following removal of duplicates, 152 full text studies were reviewed for inclusion, leading to a final set of 92 different studies that were reported in their review. Of the latter, 87 were judged to be research papers and five were reports of government surveillance programs. The research papers came from 34 countries, including three from Canada (Acosta et al. 2021; D'Aoust et al. 2021a; D'Aoust et al.

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2021b). Sampling from WWTPs was reported by Shah et al. (2022) in 69 of the research papers, reflecting the dominance of this approach to wastewater surveillance for SARS-CoV-2 because of a number of advantages for sampling at WWTPs. This is to be discussed subsequently in Section 6.

The summary by Shah et al. (2022) reported that eight studies at WWTPs reported detection of the virus in wastewater days before clinical cases were reported, in some cases because of slow reporting of the latter, but only half of these studies appear to have been done in real time, with the others being retrospective analyses of archived samples. Once COVID-19 had become widely prevalent, the utility of SARS-CoV-2 RNA detection in wastewater to serve as early warning potential had becomes more challenging, moving beyond a binary positive/negative criterion and requiring higher signal resolution to follow increases and decreases over time at sufficient sampling frequency to demonstrate trends. Overall, 23 studies reported an association between positive detection and the number of cases in a community. This supports the idea that wastewater surveillance can be used in many circumstances as an additional or alternate independent tool to monitor the prevalence of COVID-19 in communities.

D'Aoust et al. (2021c) tackled the challenge of sampling small wastewater systems, a wastewater lagoon for a community of less than 5 000 population. Specifically, they compared 24 h composite samples from an upstream pumping station with those taken from an access port to the lagoon, finding that the latter were undetectable for SARS-CoV-2 while the former were consistently detectable over a 5-week sampling period. These findings suggest the need to sample upstream of a lagoon to avoid apparent instability of the virus in lagoon samples.

3.3. Surveillance in sewer networks to identify COVID-19 cases in neighbourhoods

Publications with detailed documentation about sampling in sewer networks were much fewer than those sampling at WWTPs. Albastaki et al. (2021) monitored nine pump stations weekly and 49 sewer regions biweekly between late April and early July 2020 in the United Arab Emirates. Results did provide information on geographic distribution in sewers, but the data were not reported in relation to known cases. Chavarria-Miró et al. (2021) performed sewer sampling that found positive detections after WWTP monitoring had declined to non-detectable levels of SARS-CoV-2 in May 2020.

Wong et al. (2021) reported experience with monitoring a sewer from a high-rise apartment building after a cluster of COVID-19 cases was detected in two unrelated apartment units in July 2020. SARS-CoV-2 RNA was detected in the building sewage despite removal of the clinically detected cases. Phone interviews of quarantined contacts found none with fever or respiratory symptoms, but clinical swab samples confirmed one case who had experienced diarrhea. Xu et al. (2021) implemented sewer sampling to detect locations of cases from buildings in a district hotspot during Hong Kong's third wave, early June to end of September 2020. Wastewater detections were reported on July 27, followed by clinical testing for the affected apartments that found two positive clinical cases on July 29 and a third on 7 August 2020.

Prado et al. (2021) collected sewer samples from 17 sewer locations to monitor neighbourhoods and favelas from 15 April to 25 August 2020 in metropolitan Rio de Janeiro, Brazil. The sewer monitoring results were plotted on heat maps to provide the public with knowledge of apparent COVID-19 hot spots in the community. Similarly, Kumar et al. (2021) performed sewer surveillance at eight sites in urban Ahmedabad, India also producing heat maps to inform the population about apparent COVID-19 hot spots in the community.

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3.4. Wastewater surveillance of COVID-19 in institutions

Among the institutions that have been subject to surveillance of sewers are educational institutions, hospitals and long-term care facilities, prisons, and industrial sites. In these studies, a variety of sampling approaches have been taken (including grabs, autosamplers, and various passive samplers) to overcome some of the logistical issues associated with upstream sewer sites.

3.4.1. Educational institutions

A particularly popular application of wastewater-based COVID-19 surveillance has been on university and college campuses, primarily in U.S States, where academic laboratories engaged in this research had the opportunity to test their monitoring methods in well-controlled environments where clinical testing was often regular and comprehensive. These circumstances facilitated proficient comparisons between the two types of surveillance, demonstrated the potential value of wastewater's early warning capability, and provided estimates of analytical sensitivity and biomarker load on a per case (capita) basis. Harris-Lovett et al. (2021) describe a consortium of 25 U.S. educational institutions that performed on-campus wastewater-based COVID-19 surveillance. An early publication by Betancourt et al. (2021) reported successful detection of COVID-19 cases in student residences at the University of Arizona (Tucson, 47 000 students) by means of monitoring sewers serving residences during late August (start of the fall semester) of 2020. Wastewater detection of SARS-CoV-2 RNA led to three students being confirmed positive by clinical testing who were relocated and quarantined. For this application the testing provided an 79.8% positive predictive value and an 88.6% negative predictive value. The latter value is important, often going unreported, and indicates that false positives (wastewater detection without accompanying recorded case detection), at least in this particular context, were found to be fairly rare.

Gibas et al. (2021) reported details about experience with practical realities and logistics for operating an on-campus student residence sampling network at the University of North Carolina (Charlotte, 24 000 undergraduate students). They used 19 on-campus sewer sampling sites to monitor 17 student residences, with sampling three times per week from late September to late November 2020, with rapid laboratory turn around within 26 to 30 hours of sample receipt allowing for residence lockdown within 36 hours of sample collection. Gibas et al. (2021) noted that this program tested 332 sewer samples (out of 475 samples attempted) over eight weeks vs. testing 3 000 students, three times per week for a total of 72 000 clinical samples. They concluded this program could detect one asymptomatic case in a resident student population of 150 to 200.

Karthikeyan et al. (2021) undertook a major campus monitoring program at the University of California (San Diego, 9 700 undergraduate and graduate students living on campus) during the fall 2020 term. They used a large-scale GIS (geographic information systems)-enabled building-level wastewater monitoring system associated with the on-campus residences of 7 614 individuals using 68 automated samplers to monitor 239 campus buildings. They developed an extremely rapid turnaround on wastewater analyses using an automated, high-throughput wastewater processing pipeline with capacity of processing 96 wastewater samples in 4.5 h. This program benefited from a requirement for all students to undergo clinical testing every other week. Over the period from 23 November to 31 December 2020, 59 cases were diagnosed among on-campus students residing in buildings monitored by the wastewater program and 84.5% (n=50) of these individual case diagnoses were preceded by positive wastewater samples (either in the days prior or the day of diagnostic testing). The monitoring program was judged to be able to detect a single asymptomatic case in a building of 415 residents.

Brooks et al. (2021) performed sewer sampling at a residential college in Maine based on weekly grab samples of sewers serving residences housing 605 students and twice weekly 24 h composite sampling

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from sewage lift stations spanning a 13-week period from late August to late November 2020. A sewage lift station is a facility that pumps wastewater from a lower elevation to a higher elevation. They identified two COVID-19 outbreaks among resident students with 76% of cases identified in the weekly grab samples (< 7 d) before clinical confirmation of cases.

Scott et al. (2021) performed sewer sampling at Tulane University (New Orleans, with over 14 000 students) from the middle of August to the end of November 2020 with weekly grab samples from nine sewer locations yielding 117 samples over the duration of surveillance. Wastewater surveillance provided complementary data to document an outbreak in student residences in early November, but weekly sampling was likely too infrequent to provide a clear early warning.

Reeves et al. (2021) performed sewer sampling at 20 locations on the University of Colorado (Boulder, 30 000 undergraduate students) campus from late August to late November 2020 collecting a total of 1 512 samples. They considered six possible scenarios for their sampling regime in which sewer samples found SARS-CoV-2 RNA to be: (1) absent, (2) low and stable, (3) low and increasing, (4) high and increasing/stable, (5) high and decreasing, and (6) decreasing to absent. Reeves et al. (2021) concluded that sewer sampling could provide an early warning in scenarios (1), (2) and (3). Specifically, detection of increasing concentrations during the first two weeks of sampling led to public health officials contacting residents and employees of identified buildings within 12 h of result reporting directing them to submit saliva samples for testing. This action revealed individual cases not identified by a routine clinical testing program.

Wang et al. (2022) performed wastewater COVID-19 surveillance on the campus of Emory University (total student population over 15 000) from the middle of July 2020 to the middle of March 2021 using weekly Moore swab (Liu et al. 2022) samples from 25 sewer sites serving student residences. They found that weekly sampling using Moore swab sampling was not sensitive enough (only 6 of 63 times) to reliably detect one or two sporadic cases in a residence building, but during the spring 2021 semester SARS-CoV-2 RNA was detected in wastewater from most student residences from one to two weeks before COVID-19 cases grew rapidly on campus. Liu et al. (2022) provided a detailed assessment of the advantages, limitations and costs of the Moore swab sampling approach that was used.

In Canada, Corchis-Scott et al. (2022) performed a sewer surveillance program for a student residence at the University of Windsor (normal student population of over 16 000) initially for seven weeks from early February to late March 2021 based on three samples per week using a grab sample that yielded negative results. This was modified to using passive sampling with a modified Moore swab that provided a positive detection of SARS-CoV-2 RNA only two days after starting this sampling approach. A feminine hygiene tampon was used as an absorbent swab to collect an integrated sample. The swab was suspended in the sewer for about 20 h at a time. The detection was confirmed to be the Alpha (B1.1.7; an emerging and not widespread VOC at the time) variant using a variant-specific assay (Section 4.5) leading to a case finding program the following day that confirmed two cases among 200 students tested. The confirmed cases were quarantined to a separate residence and an on-campus outbreak was likely averted. Wastewater surveillance has been performed on other Canadian universities (e.g., University of Guelph) with results posted on public-facing websites and in other cases with reporting to administration and public health agencies (e.g., University of Waterloo, University of Toronto, University of British Columbia)

3.4.2. Long-term care facilities and hospitals

Wastewater surveillance of healthcare facilities has been widely practiced during the pandemic, but generally with a different purpose for active treatment hospital facilities than for long-term care

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facilities (LTCF)/nursing homes, because COVID-19 cases being treated are expected in the former while COVID-19 cases must be avoided in the latter with its highly vulnerable aged population. Canada's COVID-19 death toll is strongly influenced by early deaths that occurred in LTCF. As of December 2021, LTCF residents accounted for 3% of all COVID-19 cases and 43% of COVID-19 deaths in Canada (CIHI 2021).

Colosi et al. (2021) undertook a proof-of-concept study for wastewater SARS-CoV-2 surveillance at a LTCF with residential complexes housing 105 and 66 occupants. Wastewater surveillance results were validated first using hospital wastewater known to contain SARS-CoV-2 RNA then against clinical testing of LTCF residents for an 8-week period. Across all hospital and LTCF samples collected after methods validation, they obtained 25 true positives, 0 false positives, 9 true negatives, and 1 apparent false negative, yielding an apparent sensitivity of 96.2% and an apparent specificity of 100%. Given an intent of detecting possible COVID-19 cases entering a LTCF, Colosi et al. (2021) noted a concern that their surveillance could not distinguish new cases from convalescent patients previously infected who were still shedding the virus. From this perspective, if convalescent virus shedding was considered to be a false positive, sensitivity was 100%, but specificity was only 45%. This highlights the importance and the need of establishing baselines and adequate sampling frequency to be able to distinguish new cases (high shedding) from convalescent shedding. Work towards this goal is continually evolving and a clearer path has been recently elucidated by Welling et al. (2022) who found that two consecutive day detections in wastewater is most predictive of case detection in the context of building-level surveillance.

Xu et al. (2021) validated their methodology for detecting SARS-CoV-2 RNA in wastewater from a Hong Kong hospital treating COVID-19 patients. Gonçalves et al. (2021) studied wastewater from a small hospital in Ljubljana, Slovenia, sampling one composite wastewater sample per day from June 1 to 15, 2020, starting before any patients with COVID-19 were being treated (it went from 1 up to 4 COVID-19 patients during the 2-week sampling period). They found that they could detect SARS-CoV-2 RNA when only one COVID-19 patient was being treated. The Jørgensen et al. (2020) study at a Danish hospital estimated being able to detect a COVID-19 prevalence rate as low as a 0.02%–0.1% (i.e., between two virus-shedders per 10 000 persons and one virus shedder per 1000).

In Canada, Acosta et al. (2021) studied wastewater directly from three tertiary-care Calgary hospitals with a combined total of more than 2 100 in-patient beds between August and December 2020. Tertiary-care refers to a hospital providing specialized medical treatment referred from primary and secondary health care providers. They noted that many hospitalized COVID-19 patients and certainly those with severe enough symptoms to require intensive care would not be using toilet facilities feeding the sewer. Rather, they would be diapered and faecal wastes would be handled as medical biohazard waste. Accordingly, this approach was sensitive to the detection of new hospital-acquired infections, revealed by wastewater data. This is despite studies suggesting that 52%-56% of hospital employees avoid defecating while at work in a hospital, undermining the ability of sewer sampling to monitor staff for COVID-19 infection. Toileting patterns are thus a fundamental factor in determining the ability of sewer sampling to monitor staff at any facility for COVID-19 infection making behavioural factors an important consideration in study design.

3.4.3. Industrial plants and correctional facilities

Other outbreak risk locations that might offer promise for future wastewater surveillance included industrial plants, most notably meat processing facilities, and prisons. Despite that potential, published reports of implementing wastewater surveillance at such sites are scarce, owing to facility operators choosing to keep such information confidential. Dyal et al. (2020) reported that by the end of April 2020, the U.S. had 115 meat or poultry processing plants in 19 states report COVID-19

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outbreaks accounting for 4 913 cases and 20 confirmed deaths. Alberta Health Services (AHS 2021) reported that Alberta had experienced COVID-19 outbreaks at five meat and poultry facilities between April and November 2020. Pokora et al. (2021) performed a cross-sectional epidemiology study to determine risk factors for COVID-19 infection at 22 German plants totalling over 19 000 employees of which seven plants with more than 10 COVID-19 cases had a disease prevalence of 12.1%.

Piché et al. (2022) summarized clinical cases in Canadian correctional facilities between 1 December 2021 and 28 February 2022 when there were over 12 000 newly reported COVID-19 cases (8375 prisoners and 3961 staff). These cases during this time frame correspond to 55% of the total cases in these provincial/territorial and federal correctional institutions since the onset of the pandemic. Arora et al. (2020) reported on wastewater surveillance in Jaipur, India that included a WWTP serving the city centre that showed a hotspot in May 2020 that was attributed to a jail being located in the sewershed serviced, but no details were provided to focus on wastewater from the jail.

3.4.4. Surveillance of wastewater to identify COVID-19 cases associated with transportation

Recognition that international transmission of COVID-19 was enhanced by travel led to a number of international travel bans early in the pandemic. This reality prompted a few investigations into the potential for wastewater surveillance as a complementary data source to evaluate risk for COVID-19 transmission by disembarking passengers from international travel modes. Ahmed et al. (2020b) evaluated two wastewater samples (one influent, one treated) from a cruise ship, with only crew on-board about a month after passengers had disembarked (estimated that 24 passengers may have been infected with COVID-19) and three wastewater samples from separate international flights: Los Angeles to Brisbane (117 passengers, 26 April), Hong Kong to Brisbane (19 passengers, 7 May) and New Delhi to Sydney (185 passengers, 10 May). The results of this pilot study that evaluated a number of sample preparation and analytical approaches documented many of the challenges (e.g., not every passenger on a flight defecates during the flight, a high proportion of paper in airplane wastewater) facing wastewater surveillance of these sources. Only the cruise ship influent wastewater sample and wastewater from the first flight provided consistently positive results. At the time of this study, it was estimated that over 60% of COVID-19 cases in Australia were infected overseas and it had restricted international air travel for non-Australians since late March 2020. Albastaki et al. (2021) studied wastewater from 198 incoming aircraft from 59 airports on all six continents at Dubai International Airport, United Arab Emirates before September 2020, finding that 13.6% had positive signals for SARS-CoV-2.

Ahmed et al. (2020b) performed a follow-up study on wastewater from 37 long distance charter flights to Darwin, Australia, arranged for re-patriating Australians from overseas between the middle of December 2020 and the end of March 2021. All passengers were quarantined for 14-day post arrival during which clinical testing identified 112 cases of COVID-19. Wastewater from 24 (64.9%) of the flights tested positive for SARS-CoV-2 RNA and results demonstrated a positive predictive value (PPV) of 87.5% and a negative predictive value (NPV) of 76.9%, suggesting flight-wide surveillance results complementary to clinical testing.

Ahmed et al. (2022a) collected an aircraft wastewater sample from a November 25 flight from Johannesburg, South Africa to Darwin, Australia and retrospectively, detected RNA fragments of the Omicron variant. Omicron was declared a VOC by the WHO the day after, on 26 November 2021 and caused massive waves of COVID-19 infection around the world over the following months. Although all passengers on this flight were tested prior to boarding and after disembarking, a single passenger was confirmed on 29 November to be infected by the Omicron VOC by genetic sequencing

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of a clinical swab sample collected after arrival. Agarwal et al. (2022) reported detection of the Omicron VOC in wastewater samples collected from wastewater on 2 and 23 of November from the Frankfurt International Airport as well as at the Frankfurt city WWTP.

3.4.5. Summary

There is a wide range of different applications of wastewater surveillance that can be applied to institutions. We have provided only a relevant sampling of what has been described in accessible publications that should provide some perspective on what is possible and what challenges need to be overcome.

4. Elements of a wastewater monitoring / detection system for SARS-CoV-2

4.1. Introduction

All current wastewater-based COVID-19 surveillance platforms are designed to detect the ribonucleic acid (RNA) associated with the SARS-CoV-2 virus. Most are also capable of quantification of the RNA target, and this section will focus on the methodology used to achieve that goal. The infectious SARS-CoV-2 virus particle (the virion) that causes COVID-19 in humans contains a single, long molecule of genomic RNA (gRNA) encapsulated within a protein structure (the nucleocapsid). Infection of animal cells with virions produces a population of other, smaller RNAs (termed sub-genomic RNAs; sgRNAs) that serve as templates to produce viral proteins that lead to more copies of gRNA and viral particles being produced. In wastewater, researchers continue to study the relative contributions of gRNA and sgRNA, and the form that these molecules take while transiting wastewater collection systems, but there is a consensus in the field that the RNA targets exhibit degradation and fragmentation, making it challenging to reconstruct genomes from this matrix and leading to variability over time and between sampling locations.

Tiny amounts of these RNA fragments (as little as a few copies) present in wastewater must first be extracted, detected, and quantified using polymerase chain reaction (PCR)-based approaches which rely on conversion of the RNA target to DNA prior to amplification of this signal billions of times over from which the final result is derived. This technology exists in many different iterations, however, reverse transcription quantitative PCR (RT-qPCR) is the most common in both, clinical diagnostics of COVID-19 using RNA extracted from e.g., nasopharyngeal swab samples and the wastewater surveillance fields. A major difference in applying this technology to wastewater samples vs. clinical samples includes frequent under-sampling in the former due to very low concentrations and its complex colloidal properties; thus, requiring different sample processing steps for concentrating and extracting the RNA. Wastewater comprises not only human excreta, but also contributions from other domestic, commercial, and institutional water uses. Analytical sensitivity (the limit of detection or the smallest amount of target the test can detect) is thus routinely compromised because of compounds present in the wastewater matrix that can inhibit critical enzymes used in the process like reverse transcriptases (enzymes that converts RNA to DNA) and DNA polymerases (enzymes central to PCRs for exponential amplification of the signal). Nucleic acid complexity (i.e., the number of different combinations of nucleotide polymers in solution) is much greater in wastewater due to many contributing organisms. This can potentially lead to reduced assay specificity when using PCR detection technologies. The probability of detecting SARS-CoV-2 RNA can also be reduced because of dilution effects from additional inputs such as groundwater infiltration and stormwater inflow. Provided that the complexities of these samples are recognized and mitigated through clean-up and concentration steps, RNA in wastewater can be assessed using similar RT-qPCR technology to that used in clinical detection of SARS-CoV-2 RNA.

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4.2. Wastewater sample collection

Sampling locations for wastewater collection can present a challenge in some circumstances because of weather. Freezing conditions can cause blockages in autosamplers, while excessive heat in summer months could compromise sample integrity. Depth of flows for sampling in sewers can also present a challenge, as can security of the sampler if the sample sites are not located on controlled property; the latter is especially relevant when sampling from sewer man (maintenance) holes in public spaces. In some instances, an optimal sampling point from a public health perspective (i.e., covering a defined segment of residents) poses logistical difficulties (i.e., needing to stop busy traffic to access an autosampler via a manhole). Although safety is a concern at all field sites, accessing manholes has many additional concerns that must be considered, including traffic, personal protection, moving heavy covers, open holes, confined spaces, toxic gas and asphyxiation risk, etc. Sampling manholes presents major safety issues and requires specific training and supervision of personnel to be able to fully access them. There is also a challenge in accurately knowing what human population is represented by any particular sample location if wastewater results are intended to be compared with COVID-19 cases diagnosed clinically. These challenges need to be overcome through detailed study of engineering diagrams of how sewersheds in communities or buildings have been developed and are organized. Those doing these assessments have been quick to point out how obvious it is that the sewersheds were not designed with this kind of public health surveillance sampling in mind. The importance of being able to better normalize/correct the SARS-CoV-2 signal in upstream sites to address changes in flow or organic contributions, is apparent and is a clear research need.

4.2.1. Wastewater treatment plants (WWTPs)

Community-scale monitoring programs at municipal wastewater treatment facilities are often designed to capture trends, so these applications necessarily need to be able to estimate SARS-CoV-2 RNA concentrations in the wastewater over time. To achieve a representative sample, community wastewater is typically collected through deployment of industrial autosamplers that collect a time- or flow-weighted composite sample over 24 h (**Fig. 6**). Depending on available resources and the intended purpose of the surveillance program, these composite samples can be collected at a wide range of frequencies. Initial programs aimed at proof of concept sampled from random daily to bi-weekly, but it has become clear at least three times per week (CDC 2022a) is required to track COVID-19 trends and offer any realistic chance of providing early warning for public health decision-makers and the public.

For the most common surveillance purpose of tracking trends of SARS-CoV-2 in wastewater or detecting its emergence in communities with low COVID-19 prevalence, sampling at WWTPs or centrally located pumping stations is the most widely deployed approach. Because SARS-CoV-2 RNA and (or) viral particles can be excreted in faeces by infected individuals, and has been shown by several groups to preferentially partition to the solids/colloidal phase of wastewater (D'Aoust et al. 2021a; Graham et al. 2021; Peccia et al. 2020), sample preparation often focuses on concentrating solids from pre-treated "*raw*" wastewater. Some researchers have advocated sampling primary sludge (collected after the primary treatment process of sedimentation) to leverage the concentration of solids and maximize detection of SARS-CoV-2 (D'Aoust et al. 2021a, 2021b; Graham et al. 2021; Peccia et al. 2020). However, not all municipal wastewater treatment facilities necessarily have accessible sampling locations for primary sludge (e.g., lagoons). Moreover, an understanding of system-specific WWTP hydraulics and operational conditions is especially important when primary sludge is collected, because the age of the sludge (residence time distributions) and return flows in the system are important considerations for interpretation. The wastewater matrix of choice will have further implications for sample analysis strategies (e.g., PCR inhibition – see QA/QC section).

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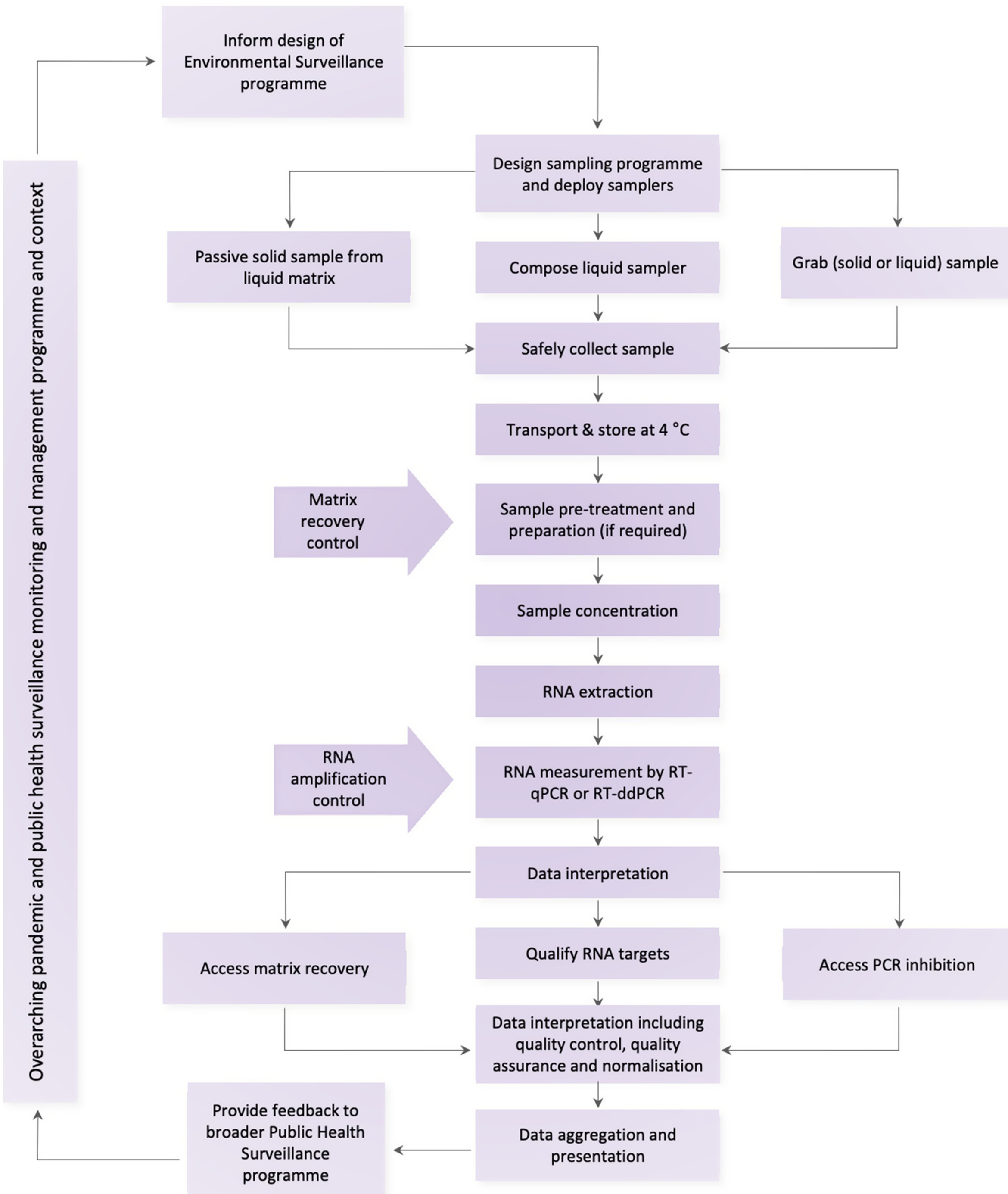

Fig. 6. Typical workflow for wastewater surveillance for SARS-CoV-2 RNA, after WHO (2022c).

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4.2.2. Sewer network sampling

Sampling from a sewer network monitoring upstream of a WWTP has been pursued where there is an objective source to monitor wastewater for SARS-CoV-2 provided wastewater flows of sufficient depth can be sustained. Autosamplers have been deployed, with some consideration for winterization needs to avoid frozen sampling ports. Sewer sampling also encounters problems with the sampling device becoming fouled with extraneous items that are flushed down toilets despite warnings against such actions (paper, tampons, condoms). In some circumstances, where deploying a large autosampler is not feasible (due to depth of manhole), correlations from grab samples with composite samples can be established to provide estimates. Using a consistent time of day and time of week for grab sampling is an important consideration.

Further upstream at the facility level, some sewer access locations may require discretion where industrial autosamplers cannot be used (e.g., access in an office setting). In such cases, portable units have been developed and deployed (lightweight briefcase-sized autosampler from CEC Analytics or collecting in-building time-weighted composite samples). In many upstream sampling locations (and most facility level surveillance) where wastewater flows are more intermittent and cannot be sustained, passive samplers (Bivins et al. 2022; Habtewold et al. 2022; Hayes et al. 2021, 2022; Liu et al. 2022; Rafiee et al. 2021, Schang et al. 2021) have been deployed to provide qualitative (presence/absence) results. Consequently, the goal of passive surveillance in this context is typically for qualitative "*first detection*" rather than tracking trends quantitatively. Habtewold et al. (2022) and Hayes et al. (2021) evaluated different sorbent materials for use in passive samplers.

Access to upstream sewer locations generally requires additional determination of responsibilities and coordination with the responsible utility to ensure strategic sampling locations with respect to the corresponding population contributing to the sewershed, as well as the essential, rigorous implementation of safety precautions (e.g., confined space hazards, access through manhole in a roadway), and training of facility personnel to collect wastewater samples.

4.3. Wastewater sample preparation and analysis

As discussed above, dilution effects, degradation, and inhibitors contribute to reduced analytical sensitivity of RT-qPCR in RNA extracted from a wastewater matrix. A major challenge in the field is in collecting and processing a representative volume of wastewater such that it will yield sufficient RNA extract for analysis. PCR platforms are limited to the analysis of microliters of extracted and concentrated nucleic acid per sample. Insufficient sample volume processed in the context of low COVID-19 prevalence (i.e., sub-sampling) can lead to imprecision in the SARS-CoV-2 RNA concentration estimate.

4.3.1. RNA extraction

Regardless of the choice of PCR platform (discussed below), the sample processing step (i.e., extracting the RNA to be later probed by PCR, **Fig. 7**) contributes to most of the variability and uncertainty in SARS-CoV-2 concentration estimates from wastewater (Chik et al. 2021; Griffiths et al. 2021; Pecson et al. 2021).

To achieve optimal precision and sensitivity to track trends in the wastewater, laboratories undertaking wastewater surveillance activities across Canada have deployed a wide range of processing methods to extract sufficient quantities of RNA. Various physical-chemical approaches to obtain the RNA extract include chemical precipitation, affinity binding columns, filtration, sedimentation, and centrifugation. Accordingly, the degree of concentration achieved, the targeted fraction(s) of wastewater captured by the approach, and interaction effects depending on the specific wastewater

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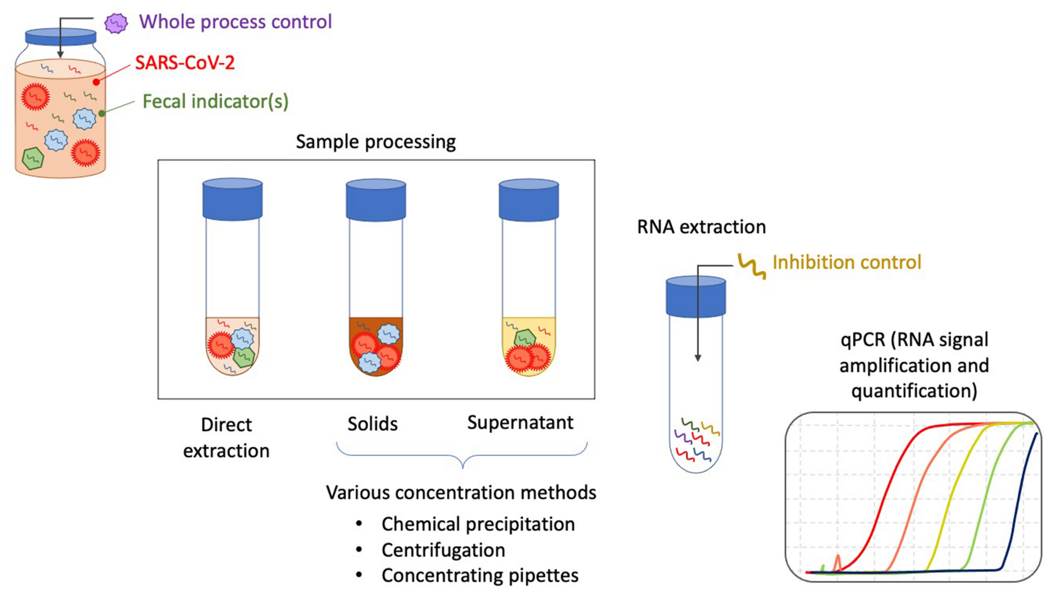

Fig. 7. Generic sample preparation and RT-qPCR Analysis (Credit: A. Chik).

matrix examined, contribute to substantial variability between different methods compared to the precision that may be achievable using a single method.

In the Ontario inter-laboratory program, split sample testing was conducted amongst participating laboratories every two months. Despite obvious method-specific biases, the level of intra-laboratory variability (i.e., precision of concentration estimates) achieved allowed participating laboratories to discern similar trends reflecting the quantitative differences between samples over multiple rounds (Chik personal communication 2022).

4.3.2. PCR-based detection and quantification

Following RNA extraction, the principles applied for detection and quantification of SARS-CoV-2 RNA in wastewater is consistent with PCR-based approaches used for clinical samples. PCR was invented almost 40 years ago, and the inventor shared the Nobel Prize for chemistry in 1993. This method has revolutionized clinical diagnostics and biotechnology. Application of PCR to water and wastewater and various environmental matrices has seen some commercial applications and has been central to many lines of research across multiple disciplines. As described above, RT-qPCR is in common use amongst laboratories engaged in wastewater surveillance. However, a major advance in the last ten years has been the advent of RT-digital droplet PCR (RT-ddPCR) technology and its more recent iteration RT-digital PCR (RT-dPCR) which have the potential to provide greater analytical sensitivity and precision. While all of these platforms typically rely on measurement of fluorescence being generated as the polymerase chain reaction proceeds, RT-qPCR converts its signal by comparing outputs to fluorescence signals from a series of PCR reactions with reference standards of known quantities of target nucleic acids, i.e., a calibration curve or "*standard curve*". Inherently RT-qPCR facilitates relative quantification of the target of interest. On the other hand, d/dd-PCR relies on partitioning a sample into thousands of individual reactions. The occurrence or absence of a fluorescence signal in each partition after PCR cycling is used to facilitate absolute quantification of the analyte and therefore precludes the need for a calibration curve. While d-/dd-PCR may afford better precision of concentration estimates at low-levels of SARS-CoV-2 in wastewater by eliminating

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systematic biases attributable to RT-qPCR and the need for standard calibration, RT-qPCR is the more established technology, and these platforms are widely deployed in laboratories across Canada (specifically probe-based methods which can provide superior analytical sensitivity and specificity). RT-qPCR platforms generally also allow for greater sample throughput.

4.3.3. Learning to live with variability

Multiple processing and analysis pipelines exist across different laboratories and even within laboratories. Until the variability between methods can be reduced or compensated appreciably, site-specific surveillance activity for monitoring trends at a given location should be conducted by a single laboratory using a single method (Chik et al. 2021; Griffiths et al. 2021; Pecson et al. 2021). For this reason, it is imperative that sample analyses not be randomly transferred between laboratories, nor should quantitative results between different laboratories (or different sample processing methods applied within the same laboratory) be assumed to be equivalent or aligned. Sample storage conditions can also affect quantitative results, such that storage should be consistent and any storage effects ascertained. Site-specific methodological consistency still enables longitudinal data sets for a given location to be established, which is arguably one of the most important uses of wastewater surveillance because it enables trend analysis for a given community to provide evidence that can inform public health actions (see Section 5).

Imposing a standardized, common sample processing approach would certainly help reduce variability between different laboratories processing wastewater samples for SARS-CoV-2 surveillance. In retrospect this would have been difficult to achieve in Canada over the past two years. As investigators faced multiple challenges responding to rapidly changing conditions there was a disincentive for adopting new methods. In fact, given the bottom-up, grassroots approach that has made this venture successful across the country, the lack of standardization may have been a factor that contributed to the rapid emergence of testing programs in Canada. Progress towards standardization is desirable although the path for this is not yet clear. Reliance on existing laboratory infrastructure and limitations of supply chain issues encountered earlier on during the pandemic present obstacles. In Québec, a locally standardized protocol was shared by key laboratories, facilitating troubleshooting efforts when an unusual result was encountered. In Alberta and British Columbia, provincial public health laboratories and the National Microbiology Laboratory (Alberta Precision Laboratories albertaprecisionlabs.ca, B.C. Centre for Disease Control bccdc.ca, National Microbiology Laboratory of the Public Health Agency of Canada, canada.ca/en/public-health/programs/national-microbiology-laboratory.html) provide potential platforms for development and dissemination of standardized procedures as they have done historically for clinical microbiology procedures. However, in the broader Canadian context, practical geographical and jurisdictional constraints effectively preclude timely coordination of wastewater surveillance activities through a central, federal laboratory in Canada, such as is currently done in the Netherlands. RIVM, the National Institute for Public Health and the Environment laboratory monitors wastewater from 300 WWTPs, four times per week, covering 17 million Dutch residents (rivm.nl/en). These constraints have largely dictated the patchwork of methods that are currently deployed for wastewater surveillance applications in Canada. Analogous issues are faced in the United States and reportedly presented a challenge with the Centers for Disease Control and Prevention (CDC) delaying the roll out of clinical testing protocols for COVID-19 in the early stages of the pandemic (Evans and Clayton 2020).

Despite there being no single standard method for quantification of SARS-CoV-2 biomarkers in wastewater in the short-term, longitudinal application of a consistent method by a qualified laboratory will yield useful quantitative estimates to facilitate tracking of temporal trends, provided that stringent QA/QC procedures are followed (e.g., Ahmed et al. 2022c, Chik et al 2021, MECP 2022). Given the predominance of RT-qPCR platforms deployed for wastewater surveillance across

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Canada and other parts of the world, a key focus has been on ensuring the quality of the standard calibration curve and streamlining best practices for the choice of standard materials.

Another area of focus is ascertaining whether PCR (an enzyme-dependent method) is inhibited by physical and chemical characteristics of the wastewater matrix, leading to underestimated levels or false negatives. However, QA/QC procedures for these methods were not widely standardized before the COVID-19 pandemic and few operational definitions have been adopted among laboratories. These realities have complicated efforts to bring the datasets together in a comparable manner. In Ontario, to support the range of process pipelines used (which generally consist of sampling, concentration, extraction, detection, and normalization) that are deployed amongst 13 academic institutions conducting surveillance across the province (see case studies in Part 1 of the **Supplementary Information**), Ontario Clean Water Agency led the development of technical guidance (MECP 2022) in collaboration with international experts and stakeholders to establish minimum performance expectations based on streamlined operational definitions and the current state-of-the-knowledge. This process included recommendations for streamlining the choice of a certified quantified RNA standard material among laboratories in Ontario, and verification of assay sensitivity to identify SARS-CoV-2 variants of concern.

4.3.4. QA/QC verification and validation

Given the importance of QA/QC, the implementation of quality management frameworks in the laboratories responsible for generating these data is underscored. Quality management frameworks provide checks and balances at various levels of laboratory operations to ensure the generation of data that are reliable and fit-for-purpose. However, a key challenge to implementing quality management frameworks broadly is the need to tailor them for the sectors relied upon for generating these data.

In industry, accreditation frameworks are used to verify that commercial laboratories have appropriate quality management systems and can perform the tests according to their scope of accreditation. This includes stringent requirements for data quality, documentation, personnel, infrastructure, and participation in inter-laboratory comparisons to demonstrate proficiency. However, early conversations with accreditation bodies during the pandemic suggested that they lack tailored accreditation checklists for PCR methods in environmental matrices, ostensibly due to lack of client demand (Alex Chik, personal communication, 2021). Coupled with the fact that results from these types of tests are not used in clinical diagnosis and do not report substances that might be directly harmful to public health (in contrast to other substances, e.g., heavy metals), there is no strong incentive from either government or industry to develop accreditation programs tailored to wastewater surveillance. That said, recent advances have started to address this gap: the Ontario technical guidance (MECP 2022) as developed and first published in August 2021, and the American Council of Independent Laboratories (ACIL) released a draft accreditation checklist (acil.org/news/597076/Wastewater-Surveillance-for-SARS-CoV-2.htm) in February 2022. The American Public Health Association (APHA) has also released additional guidance in March 2022, and an International Standards Organization (ISO) ongoing working group (ISO/TC 147/SC 4/WG 26, SARS-CoV-2 in wastewater - aphl.org/aboutAPHL/publications/Documents/EH-2022-SARSCoV2-Wastewater-Surveillance-Testing-Guide.pdf) has been formed.

The desire and opportunity for standardization must be balanced with a framework that allows flexibility, and alignment with client goals. The innovation cycle in the wastewater surveillance field is very short, thanks to significant interest (and accompanying investment) from both government and industry. Arguably, this has been aided by the lack of regulatory bodies and accreditation that can disincentivize rapid knowledge dissemination and translation. Academic laboratories are typically engaged in the ongoing development and optimization of new methods and technologies, rather than

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strict adherence to prescribed QA umbrellas regulating existing methods and technologies. University research groups generally employ personnel who lack professional designations (in contrast to certified medical laboratories). For these reasons, academic laboratories typically do not participate in existing accreditation frameworks for quality management. Consequently, surveillance programs dependent on predominantly academic laboratories may require greater oversight and emphasis on internal QA/QC policies and processes (e.g., coordination of inter-laboratory studies, split sampling, establishing minimum requirements for documentation, and reporting) by the entity administering the surveillance program. There are cases in Canada where laboratory staff and leads are cross appointed between academic institutions and provincial public health laboratories (e.g., Alberta, British Columbia). The current pandemic has emphasized the need for coordinated networks to ensure methodological advancements (often undertaken by academic laboratories) are consolidated in a manner that facilitates rapid adoption of best available practices for these tools in industry and public health laboratories that can scale-up to meet the needs of large-scale surveillance networks. Protocol changes should be undertaken cautiously to mitigate disruption to longitudinal data generation so that the ability to compare trends within a given surveillance program over time is maintained. In any case, this reality presents one of the challenges to achieving standardized methods across jurisdictions.

4.4. Interpretation of analytical results

4.4.1. Following trends of SARS-CoV-2 RNA levels in community wastewater

The use of wastewater surveillance data to establish trends was deemed "*very feasible*" by experts early on (WRF 2020). There is general confidence that significant changes in the SARS-CoV-2 RNA signal in a wastewater source can be tracked over time. These trends can provide a useful indication of the impacts of interventions implemented in the community served by the sewershed that is sampled. However, like other types of environmental data, wastewater surveillance data are inherently "*noisy*". This means that establishing trends requires careful interpretation that considers variability in the data attributable to extraneous and (or) confounding factors.

To overcome the noise in the data and help elucidate trends, a range of approaches have been taken. Simple data smoothing techniques such as calculating moving averages to more complex smoothing algorithms have been applied on SARS-CoV-2 wastewater surveillance data (Arabzadeh et al. 2021; Ai et al. 2021), with and without normalization to adjust for variability attributable to the faecal content captured within a sample (e.g., D'Aoust et al. 2021a ; see section 4.4.3 below). Pileggi et al. (2022) presented a quantitative statistical linear trend analysis approach, based on recommendations by US CDC, to systematically use points of inflection to segment wastewater surveillance time series data with associated linear regression to establish whether or not an observed trend in a given segment was statistically significant. While there is not a universally accepted approach for trend analysis, it requires an important trade-off to be considered: approaches that are more intuitive ("*manual*" expert interpretation) are more time-consuming, whereas more systematic algorithms that are automated might not integrate other factors that can influence trend interpretation.

Since the Research Summit in April 2020, various studies globally have shown that SARS-CoV-2 wastewater signal fluctuations often trended with clinical case fluctuations in many systems. Although work is ongoing to examine whether wastewater surveillance data can be used to yield credible estimates of COVID-19 cases, there is a growing body of evidence that suggests wastewater surveillance trends can provide an unbiased estimate of changes to disease prevalence and spread in a community. This is important, as changes and trends at the community level have great value for informing public health officials and the public. Wastewater surveillance data is often interpreted alongside other conventional epidemiological metrics corresponding to population served by the

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sampled sewershed. A coordinated effort is required from municipalities (provision of sewershed boundaries) and public health units (e.g., clinical case testing, vaccination statistics) to facilitate exploration of these trends. Many public dashboards across Canada have been established during the COVID-19 pandemic (see case studies in Part 1 of the **Supplementary Information**), featuring wastewater signal data along with corresponding clinical testing data.

4.4.2. Interpreting and understanding SARS-CoV-2 RNA results from wastewater

Just as with clinical samples, a PCR signal specific to SARS-CoV-2 RNA in wastewater does not report how much, if any, infectious virus is present; rather it *only* indicates the presence of small fragments of its genetic footprint. The detected "*raw*" RT-qPCR signal, regardless of type of specimen tested is a continuous variable generally expressed as a cycle threshold value (Ct; ranging from 1–40, where higher Ct indicates *lower* amounts of the target present in the sample). It is generally accepted that SARS-CoV-2 viral load in nasopharyngeal specimens is linearly correlated with infectious viral load (Puhac et al. 2022), and this is likely true of faecal shedding as well. Thus, individuals with high viral loads, on average will be expected to shed more SARS-CoV-2 RNA during the course of their infection. This analogue information (amount of RNA) is used by clinical laboratories in a standardized analysis process to render a binary outcome, namely, "*positive*" or "*negative*" calls. Throughout the COVID-19 pandemic, a RT-qPCR specimen that is "*positive*" has been designated an active SARS-CoV-2 case. In the clinical context, these cases are treated as discrete variables (i.e., counts) in public health reporting.

In contrast, a wastewater sample can be considered a pool of multiple clinical specimens with contributions of viral RNA shed from multiple infected individuals who are contributing to the sewershed and the composite wastewater samples being collected. At present, this pooled sample is viewed as extremely convoluted and cannot provide any insight into which individual (i.e., age, vaccination status, etc.) or how many individuals might be infected. The wastewater-based RT-qPCR signal is a continuous variable and at its core represents an estimate of the number of specific RNA fragments per volume of wastewater (i.e., a quantifiable concentration). This eponymous feature of RT-qPCR is achieved via detection of increasing fluorescence that varies directly with each PCR amplification cycle, resulting in exponentially increasing fluorescence that is expressed as Ct. Knowledge of the Ct value allows the original number of RNA fragments present in the sample to be estimated based on standard curves of reference. Wastewater surveillance via RT-qPCR thus provides quantitative information with a reasonable expectation that higher concentrations are indicative of a greater number of infected individuals excreting the virus into wastewater in that sewershed catchment area. However, in the absence of reasonable and relevant estimates of the amount of viral RNA excreted per person, wastewater-derived units expressed as equivalent COVID-19 cases could be grossly over- or underestimating this parameter. There may be other tractable strategies to minimize this error (e.g., by adjusting the model using wastewater signal at a time period when clinical testing was high and case estimates were more accurate). Presently, however, the authors are of the opinion that there is no rationale or strong empirical evidence available that serves as a basis for translating the concentration of specific genetic fragments in wastewater into a number of infected individuals in a given sewershed. However, even if equivalent case numbers cannot be estimated with reasonable certainty, it is reasonable and valuable to interpret upward/downward trends in the wastewater signal as increase/decreases in the number of active cases in that community.

4.4.3. Normalization of quantitative data to deal with dilution

Any source of wastewater dilution will reduce the concentration of RNA being measured. Efforts to account for this dilution include characterization of different physical, chemical and (or) biological parameters to estimate the amount of excreta captured in a sample which varies with the size of the contributing population in a given sewershed. Municipal wastewater can be diluted by a variety of

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sources free of SARS-CoV-2, including: stormwater in municipalities where a sanitary sewer is not isolated from any stormwater inputs; groundwater infiltration into sewers (i.e., from rain and snow events), other sources of non-toilet wastewater (household greywater - as a component of municipal sewage greywater refers to all household water discharges through the sanitary sewer including that from laundry, bath, shower and kitchen. Li et al. (2022) have argued that laundry, bath, and shower wastewater that will contain sputum is as important as wastewater containing faecal sources because of the high concentration of SARS-CoV-2 in sputum) and other non-sanitary industrial and institutional discharges to a sewer network (all of which have both liquid and solid components that contribute to dilution or changes in organic load). These sources of dilution can vary over time from sample to sample, affecting the magnitude of the wastewater SARS-CoV-2 concentration estimates. Earlier studies on impacts of stormwater on pathogens in sewers have demonstrated how complex relationships can be (Tolouei et al 2019a, 2019b). Where that quantitative magnitude is being relied upon to demonstrate trends that are correlated with the number of COVID-19 cases in the population being sampled, variation in dilution over the period analyzed will weaken that correlation. For example, different waves of COVID-19 in Canada have coincided with the 2021 and 2022 spring seasons (when snowmelt enters the wastewater system in some municipalities). Concern around diluted signal (whether in liquid- or solids-based processing pipelines) has led to the evaluation and adoption of monitoring for a variety of substances in wastewater that can be interpreted as indicators of faecal contribution to wastewater composition. The rationale being that expressing SARS-CoV-2 signal as a proportion of the amount of a human biomarker (or multiple biomarkers) in a sample will correct for any non-human contributions to the amount of material used in the assay. This has the effect of increased precision of the signal estimates over time.

While there is no doubt that random dilution of wastewater with water that is free of SARS-CoV-2 will interfere with being able to track quantitative trends in excretion of SARS-CoV-2 by means of concentration measurements in wastewater, the solution to this issue is not as clear as is sometimes assumed. Normalizing RT-qPCR results from wastewater with a parameter that is known to represent the faecal content of wastewater has been investigated extensively by different teams. Candidate analytes have been drawn from a list of parameters that have been useful for tracking sanitary sewage discharges within receiving waters (Bivins et al. 2020; Jmaiff Blackstock et al. 2019) such as: *Escherichia coli* (faecal bacteria in all humans), *Bacteroides*HF183 (faecal bacteria in all humans), crAssphage (viral phage infecting *Bacteroides* and found in 50% of a large set of human faecal samples), pepper mild mottle virus (PMMoV, one of the most abundant RNA viruses present in human faeces), faecal sterols, bile acids (present in all human faeces), caffeine, common over-the-counter pharmaceuticals, common artificial sweeteners, common personal care products, and optical brighteners/fluorescent whiteners (indicative of laundry wastewater).

CrAssphage and PMMoV have been the most widely used faecal content markers by wastewater surveillance groups around the world. In Canada, some laboratories are using PMMoV normalization while others are not, with no consensus approach for how to account for dilution in either the liquid or solid fractions. When normalized to PMMoV concentrations, D'Aoust et al. (2021a) reported improved correlation between wastewater-based SARS-CoV-2 RNA signal and reported clinical cases using a processing method based on solids. Graham et al. (2021) reported no change which is unsurprising given that PMMoV levels did not change significantly within the study time period, while Feng et al. (2021) using a processing method based on influent liquid where PMMoV partitions, rather than solids, found a decreased correlation. Although PMMoV has been widely used and is recognized as a viral indicator that is readily recovered from wastewater, the fact that its presence is determined by diet (PMMoV is endemic to many pepper cultivars around the world and is present in many processed foods that are part of the human diet, but levels of PMMoV differ according to source) means it may not be the best indicator of faecal content in wastewater. This is a conspicuous

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concern when attempting to use PMMoV to normalize for faecal content in near-source applications where fewer individuals contribute to the signal. Small changes in diet over time could lead to wild variation in PMMoV levels and subsequent inaccuracies in reported SARS-CoV-2 signal. A faecal or human excreta biomarker that is more closely linked to metabolic activity (e.g., faecal sterols, bile acids) may prove superior. This topic needs to remain an active area of research.

Xie et al. (2022) reported success in normalizing the SARS-CoV-2 signal using acesulfame, a widely used dietary sweetener. This parameter has been widely used as a tracer for wastewater impacts on receiving waters. While possibly more consistent than PMMoV, acesulfame is still governed by dietary consumption. Cluzel et al. (2022) have reported success in normalizing for sanitary sewage content with a mathematical approach incorporating the following markers: ammonia, conductivity and chemical oxygen demand.

There is clearly value in being able to compensate for random dilution of human excreta by water known or expected not to contain SARS-CoV-2 RNA (e.g., stormwater, groundwater infiltration, industrial and commercial wastewaters, and their associated solid components) to arrive at more accurate and precise estimates of SARS-CoV-2 RNA as a fraction of assay input. The ideal indicator would be equally persistent as SARS-CoV-2 RNA in wastewater, would not be confounded with other non-human faecal water sources from communities, and its recovery should be comparable to that of SARS-CoV-2 RNA. Different sewersheds will have different requirements that could influence the choice of the "*ideal*" indicator and may require a period of baseline monitoring to establish a case for choosing one vs. another. There's a distinct possibility that multiple normalizers could be employed to arrive at better estimates of signal. There is a real risk that as clinical testing for COVID-19 is further reduced, fewer opportunities will be available to benchmark different normalization methods and researchers will have to depend on retrospective analyses of potentially compromised archived samples. Another important question to resolve from this research is whether similar normalization schemes can be applied to targets other than SARS-CoV-2, which would be desirable for a wastewater-based platform with expanded applicability.

4.4.4. Communication of results

Wrangling disparate data into a common format facilitates collaborative research, large-scale analytics, and dissemination of results to stakeholders. Early in the pandemic, researchers quickly understood that a data model (i.e., defining data elements and their relationships) would be needed so that, for example, wastewater surveillance data generated in Edmonton can be accessed, understood, analyzed, and interpreted by a research group in Montreal, and beyond. In the first year of the pandemic, Dr. Doug Manuel created the "*Ottawa Data Model*", an important component of the open science approach used by Ottawa's wastewater surveillance project since summer 2020. This work defined minimum data elements and metadata associated with wastewater-based SARS-CoV-2 surveillance. Today, this renamed Public Health Environmental Surveillance Open Data Model (PHES-ODM) has broadened its data dictionary to encompass several environmental sample types beyond wastewater. It is entirely open source and its development is supported by the research community and CoVaRRNet (covarrnet.ca/wastewater-surveillance-group/). Several entities managing wastewater surveillance across 23 countries have so far adopted this data model or are planning to implement it and include: Ontario's WSI, the Canadian National Microbiology Laboratory, and the European Union. The PHES-ODM will also incorporate data dictionaries of other international repositories including the US CDC's National Wastewater Surveillance System. A data repository will be built on top of PHES-ODM and will be accessible to the public. In Ontario, the WSI manages a closed database that includes combined wastewater and clinical analytics such as trend reports. The province's 34 public health units can access this information through a centralized web portal. In the near future, this information will be accessible to the public. Currently in Ontario, the Ontario

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Science Table serves aggregated wastewater trends over time for over 100 sampling sites on their public online dashboard (covid19-sciencetable.ca/ontario-dashboard/). Many public health units across the province are also providing local wastewater data and analytics through their own dashboards and (or) through those set up by the academic laboratories doing the servicing (e.g., 613covid.ca/wastewater/). Similar dashboards are being used in other jurisdictions across Canada (see **Supplementary Information** Part 2: a curated list of Canadian sites is also accessible and around the world, Naughton et al. 2021). Many researchers around the world are also directly reporting and interpreting wastewater results via social media.

The importance of direct and two-way communication between the data collection teams and the public cannot be underestimated. Although anecdotal, researchers have reported that some members of the public might put more trust into wastewater surveillance data and may even alter their own behaviour based on local trends in wastewater surveillance metrics. This communication can benefit from good relationships and mutual understanding between scientists and media outlets that play a large role in disseminating information and updates to the public. Not only must the relationship between researchers and the public be cultivated and maintained, so must relationships between researchers and local public health units. This is due to the fact that wastewater surveillance data reported in isolation can be difficult to interpret, but can be made easier through regular, two-way communication and mutual accountability between these parties.

4.5. Identification and tracking of variants of concern (VOC) in wastewater

4.5.1. How can VOC tracking reduce societal risk and impact of a pandemic?

Infection with SARS-CoV-2 consists of viral replication within host cells, a process that is prone to the introduction of mutations in the viral genome. Mutations allow for natural selection resulting in the emergence of novel genetic variants of the virus being shed by people and (or) animal hosts. Currently, several organizations (i.e., WHO, USCDC, ECDC, UKHSA, PHAC) classify variants based on their potential or known risk to public health. VOC can exhibit any combination of enhanced transmission (spread) in communities, increased pathogenicity, or ability to escape immunity (failure of protection from infection or disease) relative to the ancestral (wild-type) virus and thus pose an increased risk to both regional and global public health. Early discovery and tracking of emerging variants have the potential to enable public health risk reduction in both pandemic and pre/post-pandemic contexts by:

1. Providing advance notice to public health authorities and governments affording them time to plan and implement mitigation measures, such as public messaging, restrictions, and standing-up health care teams.
2. Minimizing the lead time from design-to-testing of new treatments and interventions specific to the emergent VOC (e.g., updating vaccines or anti-viral treatment plans).
3. Determining whether new mutations might reduce sensitivity of current detection methods (e.g., increased false negative rate in wastewater or clinical PCR, or in rapid antigen tests).
4. Identifying new mutations that might increase the chance of immune escape.
5. Improving interpretation of results from clinical and wastewater surveillance by means of better understanding of the occurrence and distribution of VOCs within defined populations.

4.5.2. Which tools are employed to track VOC in Canada?

A summary of VOCs and variants of interest (predicted to behave as a VOC but for which epidemiological evidence is very preliminary or unclear) that have been classified by WHO is provided in

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Table 4. SARS-CoV-2 Variants of Concern and Variants of Interest – World Health Organization as of July 28, 2022. WHO (2022b).

WHO Label	Pango Lineage Rambaut et al. (2020a)	Earliest Documentation	Currently circulating in Canada
Variants of Concern			
Alpha	B.1.1.7	United Kingdom, September 2020	no
Beta	B.1351	South Africa, May 2020	no
Gamma	P.1	Brazil, November 2020	no
Delta	B.1.617.2	India, October 2020	yes
Omicron Currently includes BA.1, BA.2, BA.3, BA.4, BA.5 BA2.12.1, BA.2.9, BA.2.11, BA.2.13, BA.2.75 sub-lineages and descendent lineages	B.1.1.529	Multiple countries, November 2021	yes
Variants of Interest			
Epsilon	B.1.427	USA, March 2020	no
Zeta	P.2	Brazil, April 2020	no
Eta	B.1.525	Multiple countries, December 2020	no
Theta	P.3	Philippines, January 2021	no
Iota	B.1.526	USA, November 2020	no
Kappa	B.1.617.1	India, October 2020	no
Lambda	C.37	Peru, December 2020	no
Mu	B.1.621	Columbia, January 2021	no

Table 4. There are currently two methodologies in use in Canada to track VOCs (and genetic diversity of SARS-CoV-2 in general) through wastewater and estimate their prevalence in communities. The basic technology platforms employed are the same as those used for identifying VOCs in clinical specimens, namely RT-qPCR and genomic sequencing. Specialized allele-specific (AS) RT-qPCR assays are needed to quantify the specific mutations diagnostic for VOC, whereas sequencing strategies fish-out and then assemble the recoverable portions of the viral gRNA (consisting of a string of approx. 30,000 nucleotides) and sgRNA (Lightbody et al. 2019). Sequencing can therefore survey the frequency of multiple mutations in an unbiased manner, i.e., these mutations do not need to be known in advance. Furthermore, retrospective interrogation of these genomic data is possible, allowing newly identified mutations to be investigated in past samples.

4.5.3. Wastewater-based VOC tracking

Multiple waves of COVID-19 have been driven by the emergence of VOCs. A pan-Canadian strategy to survey and track the genetic evolution of SARS-CoV-2 through clinical genomic surveillance started early in the pandemic through the creation of the CanCOGeN consortium in April 2020 (Gooderham 2021). While clinical genomic surveillance was an established method to follow genetic mutations in populations via testing of nasopharyngeal samples, recovery of SARS-CoV-2 genomes

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from wastewater samples connected to multiple different COVID-19 infections is much more complex. Successful recovery of a consensus genome from wastewater was reported in the summer of 2020 (Nemudryi et al. 2020) and served as a proof-of-concept study, illustrating the potential of using this 'metagenomic' surveillance approach method to follow viral variation at the municipal level. This study used long-read sequencing of amplified genomic fragments, while others have used short-read sequencing strategies to pursue the same objectives. An early example of the latter approach demonstrated significant genetic diversity in wastewater consensus SARS-CoV-2 genomes in California that were correlated to the genetic diversity found in corresponding clinical samples (Crits-Cristoph et al. 2021).

In Canada, Lin et al. (2021) undertook metagenomic sequencing to monitor VOCs by analyzing RNA fragments present in wastewater at the British Columbia Centres for Disease Control in Vancouver. In Québec, N'Guessan et al. (2022) reported prevalent SARS-CoV-2 variant lineages in wastewater and clinical sequences from three cities. At the National Microbiology Laboratories (NML) in Winnipeg Landgraff et al. (2021) operationalized metagenomic sequencing wastewaters across Canada in Winter 2021, and then continued routinely sequencing wastewater from a number of municipalities and facilities across Canada to track of SARS-CoV-2 variants. This genomic information is relayed to public health units in an ad hoc manner as there is currently no formal mechanism for this kind of information sharing and processing.

In contrast with metagenomic sequencing, AS RT-qPCR, requires that a diagnostic mutation (allele) be known for the targeted VOC. So far, these have been identified thanks to rapid depositing of genomic information via GISAID and subsequent analysis and crowd-sourced interpretation via the Github community. This highlights the relative utility of both clinical and wastewater-based genomic sequencing vs. RT-qPCR; sequencing requires more effort but is a pre-requisite for PCR assay development. Once a diagnostic mutation is identified, development of the AS RT-qPCR assay may need a lead time of up to two weeks prior to implementation for reagents to be received and minimal validation to be carried out. Currently there are a growing number of verified wastewater-based AS RT-qPCR assays available (Graber et al. 2021; Peterson et al. 2022; Fuzzen et al. 2022), with new ones becoming available typically in response to a new variant or a new wave, as described below. Variant-specific assays can be applied in different combinations to probe for increases in emerging VOCs and (or) decreases in endemic (existing) variants. Although a single mutation may not be 100% specific for a given viral lineage in a clinical sample, owing to the defining characteristics of a given VOC (e.g., ability to spread more rapidly than existing variants in a given population; Hubert et al. 2022), any increase in frequency of this mutation in a wastewater sample is an excellent proxy marker for the emerging VOC. As with clinical samples, confirmation of the presence of a putative VOC and estimation of its proportion relative to all SARS-CoV-2 in wastewater can also be performed by sequencing, by adopting a metagenomic strategy.

The main strengths of AS RT-qPCR are: (1) An ability to probe a sampling site at high frequency to generate real-time information; (2) ease of implementation by any laboratory running standard SARS-CoV-2 RT-qPCR assays on RNA samples from wastewater; (3) short turn-around time (equal to that of standard SARS-CoV-2 RT-qPCR) and, (4) affordability (on average twice the cost of the standard SARS-CoV-2 RT-qPCR on a per reaction basis). Different technical approaches are used in AS RT-qPCR. These have been used for many years in basic biological research laboratories and are also employed in clinical diagnostics. As with any PCR assay development, methods and results must be carefully scrutinized to minimize the chance of false positives or over-interpretation. The same QC measures used for standard RT-qPCR assays are also employed with AS RT-qPCR experiments, which can similarly be performed on raw influent, raw solids, or primary sludge and can be expected to achieve similar sensitivities as standard wastewater-based RT-qPCR.

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In mid-December 2020, Public Health England (now the UK Health Security Agency; UKHSA) published a technical report describing a SARS-CoV-2 variant that emerged from Southeast England (Kent) in fall 2020 and that was rapidly spreading (PHE 2020). A more detailed analysis was also posted (Rambaut et al. 2020b). The WHO was also notified of the identified VOC, now known as Alpha (WHO 2020). By the end of January 2021, the first Canadian cases of Alpha infections were identified by targeted clinical genomic surveillance of travelers. Detection and tracking of this variant was made possible owing to a substantial clinical genomic surveillance program in England coupled with the ability to survey Alpha incidence by using a faster PCR-based proxy test made possible by a mutation that leads to "*S-gene dropout*" (i.e., one of the PCR tests no longer could detect this variant, because its mutation profile changed the genomic region that the S-gene PCR assay was diagnostic for). Public Health Ontario and other Canadian jurisdictions also employed AS RT-qPCR VOC screening to determine variant prevalence, with complementary genomic sequencing of a subset of clinical specimens to confirm the PCR results. When used in conjunction with other PCR assays unaffected by the mutation, this and other AS RT-qPCR assays (for various VOCs) allow PCR testing to reveal information about the presence and proportions of different variants in wastewater (e.g., Peterson et al. 2022; Hubert et al. 2022; Fuzzen et al. 2022), in lieu of more comprehensive and conclusive genome sequencing.

Canadian researchers were among the first in the world to establish robust and accurate wastewater-based methods to not only detect but also quantify the proportions of SARS-CoV-2 RNA signals attributable to different VOCs beginning with the Alpha variant in January 2021. The fragmented nature of RNA in this matrix, (i.e., as gRNA and smaller sgRNA) from faeces mixing and transiting sewer pipes downstream to the sampling points necessitates a different strategy. This need arises because PCR assays being used on nasopharyngeal samples for clinical diagnoses may not demonstrate the same sensitivity and allele specificity (ability to distinguish the mutation from background) to reliably measure the VOC signal as a proportion of total SARS-CoV-2 signal in wastewater. The sensitive and specific tests developed for clinical samples should not be expected, a priori, to perform similarly in the wastewater context. Moreover, Canadian researchers have observed poor allele specificity (cross-talk) and analytical sensitivity of commercially available assays that have been made available more recently, leading to overestimation of VOC prevalence in wastewater (personal communication, Dr. Shelley Peterson, PHAC). An Alpha variant-specific qRT-PCR assay which targets a mutation (N:D3L) close to the N1 region of the genome was developed by Graber et al. (2021) and applied to wastewater in early January 2021 to detect one of the first Alpha outbreaks in Canada at a long-term care facility in Barrie, Ontario. As Alpha spread in communities such as Ottawa, researchers were able to follow its incidence (how quickly it was supplanting the prevailing variant) in near real-time with results being provided to Ottawa Public Health within a day or two of sampling. Retrospective analysis of that period found that the estimates of Alpha incidence and prevalence derived from wastewater by RT-qPCR closely correlated with the estimates provided by clinical testing (via RT-qPCR screening for S:N501Y+/E484- allele positivity, which was a proxy marker for Alpha at the time) but that the wastewater results did not suffer from the same data reporting lags as the clinical testing did (Graber et al. 2021). Throughout the COVID-19 pandemic a variety of RT-qPCR assays have been applied across Canada to detect and monitor emerging variants of concern, including Delta and Omicron (e.g., Fuzzen et al. 2022; Hubert et al. 2022).

4.5.4. Challenges and limitations

The wastewater sample matrix poses unique challenges that have made methods development key to now-established wastewater-based AS RT-qPCR and metagenomic sequencing capacity throughout Canada. Unlike clinical samples where there is (with the exception of co-infections) a single viral variant represented at relatively high concentration and with a putatively intact genome, SARS-CoV-2 RNA in wastewater is present at relatively low concentration and is fragmented. These fragments

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represent contributions from multiple infections, each of which could in theory represent a different variant, making detection and re-assembly of viral genomes from wastewater technically challenging and an ongoing area of research in the context of SARS-CoV-2. The mixture of variants in wastewater and corresponding constellation of mutations that can be identified by sequencing do not necessarily derive from the same SARS-CoV-2 viral genomes present in the viruses circulating in the community. Sophisticated bioinformatics tools are needed to decipher and disentangle this information. This current state of progress highlights the various technical challenges and knowledge gaps that wastewater-based metagenomic sequencing and AS RT-qPCR testing and research continue to address. Both approaches require significant expertise and knowledge to deliver reliable results.

Accordingly, wastewater-based sequencing and PCR analysis methods are rapidly evolving such that analysis and interpretation are not standardized. There is potential to over-interpret findings or arrive at erroneous conclusions regarding the absence/presence of a given viral lineage. Metagenomic SARS-CoV-2 sequencing in wastewater today can provide higher specificity than AS RT-qPCR but can suffer from lower analytical sensitivity. Because of the relatively high cost of sequencing (compared to PCR), multiple samples are generally run as a batch, prior to subsequent computational analysis. These factors generally preclude high frequency reporting of sequencing results from wastewater (currently every week or fortnightly in Canadian jurisdictions). There is currently a lack of properly benchmarked studies comparing sequencing to AS RT-qPCR. The lower sensitivity for sequencing may be lineage dependent and might also be affected by the viral diversity in the samples vis-a-vis the number of different variant lineages contributing to a wastewater sample. Notwithstanding these limitations, tremendous advances in wastewater-based sequencing of SARS-CoV-2 RNA have been achieved since 2020 and continued innovations are expected. Overall, the complementary nature of metagenomic sequencing and AS RT-qPCR in wastewater surveillance means the two strategies can be deployed effectively in a context- or question-specific manner.

4.5.5. Today: enhancing situational awareness by strategically employing complementary VOC assays

Complementary AS RT-qPCR assays and metagenomic sequencing are being strategically used today in Canada to identify the emergence of known variants, and to act as a proxy estimate of their incidence and prevalence in a sampled population (how quickly it spreads in a population) but also have the potential to identify unknown variants. Metagenomic sequencing can be used to confirm AS RT-qPCR results as viral sub-lineages may not be easily distinguished using AS RT-qPCR. Because of its relative ease of implementation and fast time-to-reporting, AS RT-qPCR can be used to identify the likely presence of a particular VOC at the facility-, neighbourhood-, or city-level in near real-time (within eight hours of sampling). Metagenomic sequencing can identify diagnostic mutations, knowledge of which can be used to design new AS RT-qPCR assays should clinical genomic surveillance be insufficient for this objective or miss new mutations. Metagenomic sequencing can also be strategically located at facility-, neighbourhood-, or city -level to monitor for emerging, unknown variants (i.e., variants that have not yet been identified clinically) and known variants at country or provincial entry points. Ad hoc monitoring of airplane toilet pump-outs in Australia (Ahmed et al. 2022a, 2022b) and airport wastewaters in Germany (Agrawal et al. 2022) have successfully detected VOC in travellers prior to the detection of community transmission using both metagenomic sequencing and AS qRT-PCR. It could also be possible to infer the emergence of an unknown variant through monitoring signal drop-out for a given allele using AS RT-qPCR.

Since late 2021, Ontario research groups, through the province's Wastewater Surveillance Initiative (WSI), have been performing metagenomic sequencing in many regions of Ontario including transportation hubs. Low frequency sequencing at sentinel sites is used strategically together with high frequency AS RT-qPCR at multiple locations. The Ontario VOC data and trends are routinely reported

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to the affected public health units. VOC signatures in wastewater collected from all major urban centres in Canada and other strategic locations are monitored on a regular basis through NML. Furthermore, Canada's Coronavirus Variants Rapid Response Network (CoVaRRNet) – a network of interdisciplinary researchers from institutions across the country – includes a wastewater surveillance priority area and is performing metagenomic sequencing of wastewater samples provided from across the country. AS RT-qPCR assays are being used in several locations across Canada (AB, SK, ON) to monitor VOC prevalence. Some jurisdictions have reported "*cryptic*" SARS-CoV-2 variants in wastewater (Smyth et al. 2022), although there has not yet been an instance of a VOC first identified through wastewater. It is likely this will happen given the reductions in clinical genomic surveillance around the world. Indeed, researchers in Québec have shown that it is more likely that a variant will be detected through wastewater than through an equivalent clinical sampling effort (N'Guessan et al. 2022). The strategic use of wastewater-based VOC tracking capacity in both Alberta (Hubert et al. 2022) and Ontario (Arts et al. 2022) as part of the relatively advanced surveillance programs in these provinces, enabled tracking of the emergence of Omicron from the time of the first cases identified in November 2021, through to the peak in infections of sub-lineages BA.1 as clinical testing in both provinces became severely restricted. This showcased the scalability of the wastewater testing platform, giving both a high-level overview of Omicron attack rates at the provincial level, as well as more regional- and municipal-level situational awareness. In both instances, wastewater closely reflected available clinical estimates of VOC over time. Because new variants sometimes contain similar diagnostic mutations to prior variants (e.g., Omicron BA.1 and Alpha B.1.1.7 share a common mutation used in diagnostic PCR assays), these nuances must be accounted for (Hubert et al. 2022). Designing and implementing multi-plex assays that truly allow different variants to be disentangled is essential for wastewater monitoring using AS RT-qPCR.

5. Public health applications of wastewater surveillance and communication needs

5.1. Introduction – challenges and opportunities

The ultimate justification for substantial investment in wastewater surveillance for SARS-CoV-2 is that it can likely provide evidence that is in some way useful to the public health management of the pandemic. This rationale is critical to the ethical justification for this type of surveillance, as discussed in Section 5.7. Although there are many interesting scientific questions that can be addressed by wastewater surveillance, curiosity-driven research alone does not necessarily justify the level of activity that has been invested over the past two years, but in any case, research must be subject to ethical justification. The potential for useful evidence from wastewater surveillance is clear, but this potential has not universally resulted in public health decision-makers embracing the value of such evidence. As noted by Vogel (2022), despite having what is arguably the most comprehensive national wastewater surveillance programs in the world, Dutch researchers have acknowledged that this work had limited impact on national public health policies. However, some local officials have made use of the wastewater data for increasing clinical testing in neighborhoods where wastewater data suggested COVID-19 cases were not being captured by clinical testing. Of course, the speed and scale of the pandemic has stressed the capabilities of public health systems to cope, particularly before vaccines had become available. Under these circumstances, it was not realistic to expect rapid adoption by public health managers of data generated from relatively new and unfamiliar wastewater surveillance systems being concurrently created largely from the ground up. The experience gained over the past two years with wastewater surveillance for SARS-CoV-2 RNA can provide a basis for rapidly implementing a novel type of surveillance in Canada for current and future events (e.g., other pandemics) including the VOCs that overwhelm clinical testing capacity as Omicron has done.

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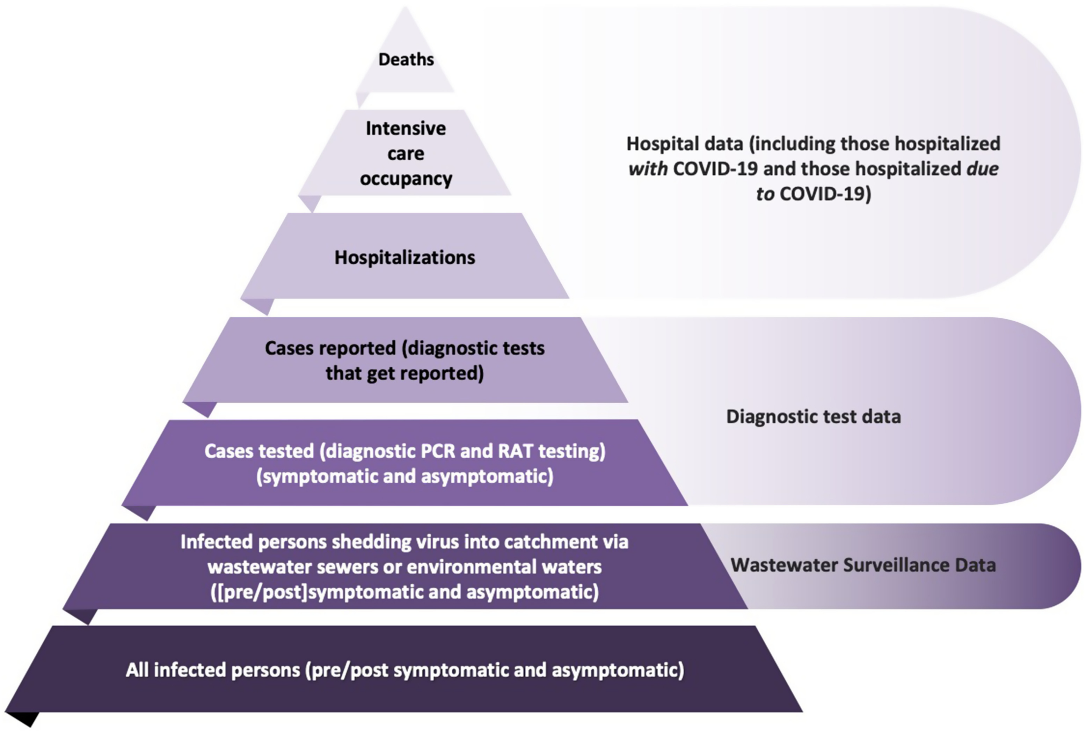

Fig. 8. Integration of case location of wastewater surveillance data into the overall public health surveillance evidence pyramid for COVID-19 (after WHO 2022c).

In Canada, it is not possible to generalize about the degree of uptake and use by public health professionals of wastewater surveillance data because of the substantial differences from one provincial/territorial jurisdiction to another concerning the organization of their healthcare systems and how public health functions within them. Canada's geography with many remote, small communities provides a challenge for performing wastewater surveillance. However, there have clearly been some success stories and some of these are captured in the case studies provided in Part 1 of the **Supplementary Information**.

Figure 8 (WHO 2022c) provides a generic representation of how wastewater surveillance data can fit within the overall public health surveillance pyramid. By its nature, severity of illness rises as one progresses up the pyramid, but a smaller proportion of the total disease burden is represented at higher levels. Wastewater surveillance for SARS-CoV-2 RNA captures closest to the entire population infected.

5.2. Detectability of COVID-19 cases by surveillance of SARS-CoV-2 RNA in wastewater

Black et al. (2021) undertook a major Australian study of 46 sewer catchments in Melbourne and surrounding regions with weekly sampling from late August to late October 2020 to identify the presence of COVID-19 cases residing in regions of the sewer network. This study benefitted from relatively low COVID-19 prevalence combined with an effective case identification and quarantine program that documented the specific geographic locations of identified confirmed cases (354 155 person-days of confirmed cases at known locations). That said, case counts likely underestimated

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the true number of infections that might be detected by wastewater (no information was given about the percentage of cases that were deemed pre-symptomatic and asymptomatic). In addition, sensitivities are likely underestimated in the study because of reliance on grab sampling which is prone to under-sampling bias relative to composite or passive sampling (discussed in Section 4) which are now favoured to increase probability of detection. Nevertheless, in this study context, Black et al. (2021) found that early detection of a single infected person in a sewer catchment was possible, but unlikely (10% probability estimate). Their analysis suggests very high chances of wastewater-based detection of SARS-CoV-2 RNA when 20 cases or more are present within a 34 km radius of the catchment within one week of sampling. They concluded that SARS-CoV-2 RNA detection for a sewer sample would justify further investigation in an area where clinical surveillance shows low or no COVID-19 prevalence.

Campbell et al. (2021) reported experience with wastewater surveillance from New South Wales (NSW), Australia, in 2020 when COVID-19 prevalence was comparatively low (mostly single to occasional double digit daily cases) in the state (over 8 million population) with most cases attributable to overseas visitors or returnees. After conducting a pilot project to establish viability of wastewater surveillance in December 2020, NSW Health was able to match wastewater detection of SARS-CoV-2 with two identified clinical cases in a suburban area of Sydney at a time when the statewide 7 d average was only 5–7 new cases per day. As a result, clinical testing was increased from 1 per 1,000 residents to 90 per 1,000 residents within three days and the specificity of the data allowed half of the region to be subjected to movement restrictions. Continued wastewater surveillance was relied upon to subsequently relax movement restrictions. Also in NSW, Camphor et al. (2022) performed a retrospective analysis of the metropolitan Sydney wastewater surveillance program based on 100 24-hr composite samples collected between March and July 2020, concluding that the odds of detecting a SARS-CoV-2 signal in a wastewater sample increased by 5.68 (95% CI: 1.51–32.1, P: 0.004) with rates of one or more cases in the sewer catchment sampled. The diagnostic specificity of SARS-CoV-2 detection in wastewater was 88% (95% CI: 69–97%) while the overall diagnostic sensitivity was only 44% (95% CI: 33–56%). This analysis found that the probability of detecting SARS-CoV-2 in wastewater sample exceeded 50% (95% CI: 36–64%) for case rates within a catchment that exceeded 10.5 notified cases per 100 000 population.

Jørgensen et al. (2020) reported on a program to evaluate 10 different wastewater protocols applied to 78 individual samples from 18 sites (12 WWTPs and 6 hospitals) in Denmark, France, and Belgium. Based on a number of assumptions (that authors admit reduced precision in their estimates) they conclude that it should be possible to detect cases by means of wastewater surveillance at between 2 and 10 cases per 10 000 of surveyed population.

Wurtzer et al. (2022) performed a retrospective analysis of 16 months of wastewater surveillance data since March 2020 from five Paris WWTPs and multiple sewer sampling sites in concert with clinical data for the same period. This study found good concordance of the wastewater data with identified clinical case data and an average of about three days lead time in the wastewater data compared to clinical data for their specific circumstances. They estimate that their situation allowed them to detect COVID-19 cases at a rate of about seven cases per 100 000.

Wolfe et al. (2021) found strong correlations between wastewater settled solids (primary sludge) collected daily at eight WWTPs in California and COVID-19 clinical incidence rates in the associated sewersheds. The method sensitivity indicated potential detection of COVID-19 incidence rates of approximately one case in 100 000 (range, 0.8 to 2.3 cases per 100 000). Kim et al. (2022) conducted a retrospective assessment of both wastewater influent and primary sludge surveillance data from five WWTPs in the USA alongside COVID-19 incidence in the associated sampling zones. Analysis included a total of 216 pairs of matched data from primary clarifiers and raw wastewater influent.

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Detection limits reported in terms of incidence rate ranged from 0.7 to 20 out of 100 000 for samples of primary clarifier sludge, and from 0.9 to 18 out of 100 000 for samples from the influent. Incident rates observed over the duration of the study ranged from 0.4 to 12 cases per 100 000 population.

The first refereed publication reporting detectability for wastewater surveillance for SARS-CoV-2 in Canada (D'Aoust et al. 2021a) relied on quantitative analysis of wastewater solids (settled solids and primary clarified sludge solids) in Ottawa and Gatineau between early April and early June 2020. During this period these locations were experiencing low COVID-19 prevalence (~57 cases per 100 000 population).

Daigle et al. (2022) reported on successful field deployment of molecular testing to Yellowknife (population of 20 000 capital of Northwest Territories) with GeneXpert™ equipment which was able, with sample preconcentration to detect and rapidly report a consistent SARS-CoV-2 signal in community wastewater that was subsequently confirmed in samples shipped to the PHAC National Microbiology Laboratory in Winnipeg 1 745 km away. After a detection on 16 April 2021, daily sampling was implemented leading to increased clinical testing focused on recent travelers to Yellowknife that allowed location of a cluster of six COVID-19 cases over the period April 20 to 26. Samples sent to Winnipeg were received on April 21 and SARS-CoV-2 detection confirmed on April 23, illustrating the benefits of local testing capacity.

Li et al. (2023) performed a Probit analysis on data from over 1 800 wastewater samples collected from 12 Alberta WWTPs over 14 months from the beginning of May 2020 and spanning three waves of the COVID-19 pandemic to estimate the detection sensitivity of SARS-CoV-2 RNA in wastewater in relation to size of community population served. This study benefited from a high per capita level of clinical testing over the study period. Alberta had conducted 4.7 million clinical tests on Alberta's population of 4.4 million up to 1 July 2021. The clinical test number includes multiple tests on a single individual, so these numbers do not mean that every resident of the province was tested, however, they do reflect a high level of clinical testing over the period of this study. For communities serving more than 150 000, seven cases per 100 000 population could be detected at 50% probability rising to 21 cases per 100 000 at 99% probability. In this category, one new case could be detected in 4 762 population at 99% probability and in 14 286 population at 50% probability. For communities with less than 50,000 population, 16 cases per 100 000 population could be detected at 50% probability rising to 71 cases per 100 000 at 99% probability. For these smaller communities, one new case could be detected in 1 408 population at 99% probability and in 6 250 population at 50% probability.

WHO (2022c) have noted that the ability of wastewater surveillance to detect those infected with COVID-19 depends on a number of specific factors, most of which are likely not known for a specific surveillance site:

- "the variant-dependent quantity of virus shed by an infected person;
- the timing of personal hygiene and sanitation activities and the usage patterns (e.g., weekdays vs. weekends) of sewers or sanitation systems within the sampled catchment relative to the time window represented by the sampling;
- the extent of dilution and degradation of viral RNA in the water matrix due to inflow and infiltration into the sewer (rainwater and runoff, groundwater, industrial and commercial discharges), and the influence of wastewater quality and potentially some forms of treatment or chemical additives before the sampling point;
- PCR assay inhibition due to inhibitory substances in the water matrix; and
- the recovery efficiency of the method used."

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The foregoing are a compilation of well-experienced expert views. Specific citations supporting some of these views were not provided by WHO (2022c) (e.g., the reasonable expectation that shedding rates for SARS-CoV-2 RNA are likely to differ among VOCs as they may also differ after different kinds of vaccination and the possibility that shedding rates are likely different according to immunization status). Concerns expressed about the time window of sampling for accurately capturing excreted SARS-CoV-2 RNA can be reduced by using frequent 24-hour composite wastewater sampling where feasible. Inhibition is certainly an issue that needs to be addressed (and is addressed in Section 4.3) and different levels of recovery will pose less of a concern when results from a single laboratory using effective QA/QC procedures are being compared for different dates or sites.

5.3. Early warning and protecting high risk populations

Arguably, the prospect of wastewater surveillance being able to provide an early warning of an impending outbreak is one of the cases for adoption that was most often cited by proponents of wastewater surveillance. Some of the optimism for this possibility was justifiably based on the reality that pre-symptomatic individuals are known to shed SARS-CoV-2 before they display symptoms that would make it likely for them to receive clinical testing. Certainly, as discussed in Section 3 there have been some reports demonstrating that useful early warnings can be achieved, most clearly for surveillance in sewers with a known population catchment (e.g., on-campus student residences). Many of the initial international reports of early warnings provided by wastewater surveillance at WWTPs were in fact based on retrospective analyses of archived samples (Hrudey and Conant 2022). Likewise, the wide range of clinical testing and reporting practices in different jurisdictions made some apparent cases of early wastewater warning a consequence of slow clinical test reporting. Clearly, as more transmissible VOCs have become dominant, clinical testing has been unable to cope with the levels of COVID-19 infection making home testing that is not generally collected or reported and wastewater surveillance as major sources of evidence.

Any expectation of meaningful early warning requires sufficient sampling frequency (several times a week) combined with rapid sample processing, analysis, and reporting. Likewise, an expectation of an actionable early warning would depend on being clearly distinguishable from background, making an early warning only likely to be discernable in a situation of low COVID-19 prevalence. The reality that recovered COVID-19 patients continue to shed SARS-CoV-2 for days to weeks after the secession of symptoms, although there is likely variation in these details among VOCs and apparent recovery means that detection of small numbers of new cases for an effective early warning will require the prevalence of active and recovering cases to be low. The impact of high prevalence is somewhat reduced by the expectation that new cases will exhibit the highest rates of SARS-CoV-2 RNA shedding.

In any case, it should be clear that generalizations about wastewater surveillance being able to always provide early warning of COVID-19 cases cannot be justified across the wide range of circumstances that may exist. Every situation must be judged based on what is the prevalence of active and recovering COVID-19 cases who will be contributing a SARS-CoV-2 signal to wastewater and what is the lag time between wastewater sample collection, processing, analysis, and reporting in relation to the population coverage and turn-around of clinical cases. The detectability of SARS-CoV-2 signals in wastewater described in the previous section illustrates that actions based on obtaining a detectable signal are entirely context-specific and call for close collaboration between those generating the wastewater data and those who need to interpret its meaning and take actions. In any case, the persistence of COVID-19 suggests that there will likely be a constant, detectable background SARS-CoV-2 RNA background signal in WWTPs for medium and large size communities making the subject of simple detection early warning somewhat moot in those circumstances.

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5.4. Tracking trends and concordance of wastewater data with waves of COVID-19 cases

Reliance on wastewater surveillance by public health professionals requires confidence in the signals that are provided. Correlations of wastewater surveillance for SARS-CoV-2 with COVID-19 incidence or prevalence has been demonstrated in many circumstances. Consistent tracking of meaningful measures of COVID-19 (e.g., confirmed cases, case positivity, hospitalizations), often with some lead time, all make a case for wastewater data being useful. Beyond trend classification and analysis of correlations, predictive models offer the potential to translate wastewater data into absolute measures of COVID-19 incidence or prevalence. While there is not a firm basis for such models to date, promising progress is being made (see Nourbakhsh et al. (2022) Section 5.6).

Fernandez-Cassi (2021) used wastewater surveillance data for three WWTPs (Lugano, Lausanne, Zürich, combined population over 600 000) during the first pandemic wave in Switzerland (February to April 2020) to produce model incidence predictions that were compared with clinical testing confirmed cases. During this period, clinical test positivity reached as high as 26%. Fernandez-Cassi (2021) concluded that when clinical test positivity was high, wastewater model predictions better tracked the timing and shape of the infection peak than estimates based on clinically confirmed cases. However, the opposite was true during declines in clinically confirmed cases, which provided a better estimate than wastewater model predictions during those periods. These findings are consistent with clinical testing under-reporting when clinical test positivity is high and asymptomatic cases are not being tested, while wastewater surveillance over-reports when COVID-19 cases are recovering, but shedding of SARS-CoV-2 is still taking place. Zhang et al. (2021) performed a systematic review on faecal and respiratory shedding and reported that, on average, shedding of faecal RNA lasted more than three weeks after clinical case presentation and a week after the last detectable respiratory RNA. Wu et al. (2022) found among 97 confirmed COVID-19 patients that faecal shedding was detectable in 35% of cases and lasted a median of 25 days with a maximum of 33 days duration.

Hillary et al. (2021) performed a longitudinal analysis of weekly wastewater surveillance data from six WWTPs from major urban centres (equivalent total population of ~6 million) in the UK over the period from March to July 2020. They found that wastewater results generally correlated with clinically confirmed cases for the corresponding urban centres. They also observed a marked decline in abundance of SARS-CoV-2 in wastewater following implementation of public health lockdown measures. More reports about this important feature will be needed to judge how well wastewater evidence can document benefits from public health interventions. Such reports will likely have to be retrospective in nature because substantial public health interventions have been removed in most Canadian jurisdictions.

Weidhaas et al. (2021) reported findings from a study of wastewater surveillance conducted in April and May of 2020 involving 10 WWTPs in Utah (combined population of 1.26 million residents, almost 40% of the state population). They detected SARS-CoV-2 in 61% of the 126 wastewater samples collected with communities greater than 100 000 population having higher wastewater positivity rates (median 89%, range 40-100%, n = 4) than smaller communities (median 33.5%, range 13-56%, n = 4) except for two tourist destinations that did not follow this pattern. Moab had 60% detection frequency, but with low gene copy concentrations detected, while the WWTP serving the popular ski resort at Summit County had 91% detection frequency and also had the second highest gene copy concentrations. The latter was in an area where Utah's first COVID-19 cases were reported. Only two of the 10 communities (populations 94 000 and 9 100) showed a significant correlation between case counts and the wastewater signals for SARS-CoV-2, despite this paper being titled "*Correlation of SARS-CoV-2 RNA in wastewater with COVID-19 disease burden in sewersheds*".

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Fitzgerald et al. (2021) reported a study of wastewater surveillance based on a survey of 28 WWTPs serving about 50% of the total population of Scotland from late May 2020 to the end of January 2021. These WWTPs represented catchment areas ranging from large urban centres to low density rural and remote areas (six sites with <21 samples, three sites >80 samples). They evaluated a range of statistical models of their data, finding the strongest correlation for wastewater by using influent flow to provide an influent viral RNA load with clinical COVID-19 cases in the catchment area. Large WWTPs ($\gtrapprox$ 200,000) were able to detect as low as 25 cases versus small WWTPs being able to detect a single case.

Wurtz et al. (2021) reported on wastewater surveillance involving daily sampling at the Marseille WWTP (serves a population of ~615 000) from July 1 to 15 December 2020, providing an important database because of a high level of clinical testing (~20% of the population) over the study period. This work found a high level of concordance (correlation significant at $p = 0.013$) between the wastewater signal and clinical case results for the second wave of their study period (October to December 2020). There was much less concordance evident in the first wave from July to mid-September when wastewater showed a much earlier rise than clinical cases, followed by a decline while clinical cases continued to rise. A variety of explanations are discussed, including the role of tourists in the summer who may have contributed to the wastewater signal, but whose cases were subsequently not reported in this jurisdiction. Overall, Wurtz et al. (2021) found little evidence in either wastewater surveillance or clinical case occurrence to demonstrate beneficial impacts from public health interventions.

Safford et al. (2022b) reported on wastewater surveillance data in Davis, CA (total population ~67 000) collected between September 2020 and June 2021 to evaluate agreement concordance between the wastewater signal and COVID-19 case data at the sub-community level for 16 sampling zones isolating city sub-regions, and in seven zones isolating high-priority building complexes or neighborhoods. This program is described in a case study in Part 1 of the **Supplementary Information**. They found reasonable agreement between the wastewater signal and imputed case counts at all geographic scales, including often matching isolated spikes in clinical case counts.

WHO (2022c) has noted that tracking and interpretation of wastewater surveillance for following trends in COVID-19 differs in high prevalence vs. low prevalence settings because detection of SARS-CoV-2 RNA in the former circumstances is expected. The value of wastewater evidence for high prevalence scenario is most useful in showing trends, particularly for VOCs (section 5.5). The insights about VOCs are clearly important to public health understanding of circumstances because of differing degrees of transmissibility and severity of symptoms among different VOCs. In low prevalence situations, detection in wastewater can signal cases of COVID-19 that have not been detected clinically, whether because of true low prevalence or policies that limit clinical testing. Furthermore, WHO (2022c) noted factors that make either clinical or wastewater surveillance approximate. For wastewater surveillance:

- *"infected people may move between wastewater catchments (e.g., between home and work; for shopping, tourism and recreation);*
- *members of the population using on-site sanitation (e.g., septic tanks, pits) will not be captured in sewer-based sampling programs;*
- *wastewater catchment may not be accurately defined and (or) may not match the population area observed by epidemiological and clinical surveillance and;*
- *wastewater and sludge from on-site systems may be transferred to other systems at periodic intervals."*

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Some of these factors are readily recognized and readily factored into the interpretation of wastewater data.

Clinical surveillance inevitably experiences capacity limits such as: "*factors that influence the consistency of public health surveillance, and the willingness and ability of potentially infected people to get tested, such as:*

- *availability and recommendations of use of specific tests with different sensitivity, specificity and predictive values such as nasopharyngeal or saliva specimens analyzed with PCR tests, rapid antigen tests or other;*
- *availability of testing stations and personal tests within a reasonable distance;*
- *cost of tests – both at testing stations and for personal tests;*
- *wait times in queues for testing;*
- *opening hours of testing stations;*
- *concerns about the potential implications of a positive test result for freedom of movement;*
- *cultural and behavioural factors encouraging or discouraging testing;*
- *policies encouraging, requiring or discouraging testing; and*
- *capacity of testing and reporting systems.*"

Substantial uncertainties in both approaches to COVID-19 surveillance make correlating them a challenge, but their respective, objective strengths and limitations suggest the value in using both in a complementary manner. No matter how advanced wastewater surveillance is likely to become, it cannot replace clinical testing on individuals who become infected with COVID-19 and need to provide evidence specific to themselves to healthcare providers who must treat them.

5.5. Tracking of variants of concern (VOCs)

The emergence of VOCs of SARS-CoV-2 has caused a number of serious waves of COVID-19 in Canada, despite relatively high levels of vaccination with very effective vaccines. This aspect of the pandemic and the ability of wastewater surveillance to be able to rapidly inform public health professionals about the dynamics of VOCs and their contribution to waves of infection is likely one of the most important contributions provided by wastewater surveillance because it has been able to do so more effectively than capacity-limited, universal clinical testing. The WHO labels for VOCs and variants of interest (VOIs) were summarized in **Table 4** (Section 4).

The first VOC that was successfully tracked via wastewater surveillance was Alpha (see **Table 4**). Bar-Or et al. (2021) retrospectively evaluated nine once-per-month wastewater sampling sites (58 samples) representing ~50% of the population of Israel from August 2020 to February 2021 and were able to identify the appearance of the Alpha VOC in December 2020 and its spread to additional regions in January and February 2021. Meanwhile, VOC Gamma and VOIs Epsilon, Iota, and Eta did not show increased frequency of detection.

Jahn et al. (2021) were able to track Alpha and Beta VOCs using wastewater surveillance in Switzerland between July and December 2020 and were able to detect the Alpha VOC at a ski resort two weeks before it was first verified in a clinical sample. Goncalves Cabecinhas et al. (2021) acknowledged that early wastewater VOC detection suggested the presence of Alpha in Switzerland in early December while describing a national Swiss rapid diagnostic screening and whole genome sequencing

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program of clinical samples that identified 13 387 VOC cases consisting of predominantly Alpha, with limited Beta and Gamma. This program detected VOCs rising rapidly with detections between 6 % and 46% from January 25 to 31, increasing to 41% to 82% between February 22 to 28.

Carcereny et al. (2022) described VOC surveillance for 14 WWTPs in Spain that were sampled weekly from November 2020 to April 2021. They found over a 6-week period that Alpha VOC was detected in all 14 WWTPs and it had become dominant, on average, within 11 weeks. Rios et al. (2021) described a wastewater surveillance program with 20 sewer sites in addition to the WWTP for Nice, France (population 550 000) concerning VOCs between October 2020 and March 2021. They detected a spike of Alpha in January 2021 in one neighbourhood from which it rapidly spread to become dominant across the city. Beta and Gamma VOCs were also detected, but with low frequency. The VOCs identified in wastewater compared well with clinical case data, leading the authors to conclude that wastewater surveillance of VOCs was useful for tracking the progression of VOCs geographically and trends over time.

As described in Section 3.3.4, Ahmed et al. (2022a) reported detecting the Omicron VOC in aircraft wastewater in Australia and Agrawal et al. (2022) reported detecting it in Frankfurt, Germany airport wastewater, confirming the expectations that such easily transmissible VOCs would be expected to spread rapidly to other countries

As part of their larger COVID-19 wastewater surveillance effort that started November 2020—described in Section 5.4—Wolfe et al. (2022) developed and applied mutation-specific assays for variants Mu, Beta, Gamma, Lambda, Delta, Alpha, and Omicron in wastewater settled solids in a California WWTP that serves approximately 1 500 000 people. Retrospective analysis of wastewater over a 16-month period showed consecutive replacement of variants in circulation. Despite limitations noted including data availability at a more resolved geographic scale, significant positive associations with clinical variant data were observed for Alpha, Delta, Omicron, and Mu. The authors noted procurement of assay reagents as a bottleneck for assay implementation. Rapid implementation of variant-specific assays for the SARS-CoV-2 B.1.1.529 (Omicron) variant by this and other groups provided early warning of variant entry into several cities in the USA (Kirby et al 2022).

As outlined in Section 4.5.3, Graber et al. (2021) were able to detect an early Alpha outbreak at a long-term care facility in the Canadian city of Barrie, Ontario and then follow its rapid progression in Ottawa, Canada's capital in the same province. Likewise, following the developments of Lin et al. (2021) at the B.C. Centres of Disease Control and Landgraff et al. (2021) at the National Microbiology Laboratory in Winnipeg, wastewater surveillance programs across Canada have been able to track VOCs (Alpha, Beta, Delta, Mu and Omicron, since January 2021) with results reported to public health personnel.

Hubert et al. (2022) reported on a province-wide wastewater surveillance program that used an assay developed by Fuzzen et al. (2022) to determine relative proportions of Delta and Omicron VOCs in wastewater from 30 municipalities representing more than 75% of the Alberta population of 4.5 million. This study showed over the period from November 2021 through January 2022 the time course of how Omicron displaced Delta in each community. With two explainable exceptions, the displacement began earlier and was completed sooner in the major cities of Calgary and Edmonton compared to smaller, remote communities. Exceptions were the tourist destination of Banff and the remote northern city of Fort McMurray which hosts a large fly-in worker population. As would be expected, there was a demonstrable relationship between distance from Calgary having the largest airport with greatest number of international flights and delay of the Omicron wave overtaking Delta in more distant communities.

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N'Guessan et al. (2022) retroactively sequenced 936 wastewater samples together with thousands of matched clinical sample sequences from Montreal, Québec City, and Laval, comprising ~50% of Québec's provincial population to evaluate the merits of wastewater surveillance for tracking VOCs. They concluded that wastewater sequencing is highly efficient and able to detect more variants for a given sampling effort than genomic sequencing of clinical samples. The potential for sequencing of RNA signals in wastewater to identify novel variants, in addition to known VOCs, while not without challenges for interpretation, has been suggested (Smyth et al. 2022)

5.6. Modelling to estimate epidemiological indicators

A number of relevant epidemiological indicators are defined in the Terminology section of this report. The ability of modelling to predict some of them follows.

Hart and Holden (2020) provided an early publication that used a number of assumptions and computer simulations to predict that wastewater surveillance could prove very sensitive for detecting cases of COVID-19 in a large population and could be very cost-effective vs. individual clinical testing, acknowledging that the evidence would be complementary to clinical testing.

Modelling the COVID-19 incidence (new cases) and prevalence (cumulative cases) using RNA signals in wastewater remains challenging, but progress is being made on analytical approaches to this problem. Vallejo et al. (2022) used a variety of regression models to relate wastewater surveillance data collected from a WWTP in Coruña, northwest Spain, (~370 000 population) from 22 April to 14 May 2020 to COVID-19 clinical case data (PCR-confirmed active cases) and estimated total cases based on national sero-prevalence data that suggested actual cases over five times higher. Their regression models were able to achieve up to an R^2 of 90% suggesting good correspondence. Li et al. (2021) curated a multi-national wastewater dataset to investigate three modelling approaches— multiple linear regression (MLR), artificial neural network (ANN), and adaptive neuro fuzzy inference system (ANFIS)—for COVID-19 community prevalence. The ANN model reasonably estimated prevalence of COVID-19 at the initial phase of the outbreak and offered a 2-4 day forecast of post-peak levels.

Cao and Francis (2021) analyzed weekly variations on the SARS-CoV-2 wastewater concentrations and COVID-19 cases for the borough of Indiana County, Pennsylvania, USA between 29 April 2020 and 17 February 2021. The study evaluated the ability of a statistical model to predict future trends in cases based on time series from one week to three weeks, but case forecast accuracies were only between 12% and 22% of actual confirmed cases, a level that the authors acknowledged to be low.

McMahan et al. (2021) developed a classical compartment epidemiological SEIR model (i.e., susceptible – exposed – infected – recovered) based on clinical case data and wastewater surveillance data from weekly or twice-weekly samples from three sewer sheds (including Clemson University WWTP) in South Carolina between the end of May and the end of August 2020. They noted multiple limitations to their database including the absence of knowledge about the true number of active cases of COVID-19 in the study area because of limited clinical testing and uncertainty about whether reported cases from students were registered to their county of permanent residence rather than being on-campus. The authors maintain that their model provides a framework that could allow wastewater SARS-CoV-2 data to provide cost-effective, useful insights about progress of COVID-19.

Petala et al. (2022) tackled the challenge of estimating SARS-CoV-2 shedding rates to allow wastewater surveillance data to be used to predict COVID-19 cases based on three times per week WWTP sampling for Thessaloniki, Greece (population ~700 000) from early October 2020 to early January 2021. Using a theoretical faecal shedding model assuming an exponential increase in

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SARS-CoV-2 shedding from time of infection to the day of symptom onset and an exponential decay in SARS-CoV-2 shedding until the end of the disease, they mathematically related wastewater SARS-CoV-2 data to reported clinical cases. These authors concluded, considering all factors, that their data suggested about a two-day advance warning of the wastewater signal to the clinical case data.

Nourbakhsh et al. (2022) provided a comprehensive evaluation of modelling to incorporate wastewater surveillance data together with clinical COVID-19 data to explore key epidemiologic aspects of the pandemic. They used their simulations to provide "*wastewater-informed estimates*" for the COVID-19 prevalence, the effective reproduction number (R_{eff}) and COVID-19 incidence forecasts. This evaluation involved a Canadian national collaboration of investigators and substantial wastewater surveillance data obtained from one WWTP in each of Edmonton and Ottawa and four WWTPs in Toronto serving a combined total of over 4.9 million residents covering the period from September 2020 through June 2021. Nourbakhsh et al. (2022) employed an expansion of the classical epidemiological compartment SEIR model as their framework to represent SARS-Cov-2 at the population level. Specifically, they considered multiple compartments representing: (*S*) individuals can be susceptible; (*E*) exposed (infected but not yet infectious; (*J*) symptomatically infected who will later become hospitalized; or (*I*) recovered without hospitalization during active COVID-19; (*A*) asymptomatically infected; (*H*) hospitalized; (*Z*) those recovered and were no longer infectious but still shedding virus in faeces; (*R*) fully recovered and "*permanently*" immune but not shedding anymore; and (*D*) deceased. They also incorporated modelling of the fate of SARS-CoV-2 particles in the sewer system based on an "*advection-dispersion-decay*" model to predict the fate of SARS-CoV-2 in wastewater in transit from the points of excretion to the WWTP sampling site.

Nourbakhsh et al. (2022) concluded that it is encouraging to demonstrate that wastewater surveillance data can be used to provide reasonable estimates of important epidemiological parameters, albeit with greater uncertainty than from extensive clinical data. The latter is substantially more resource intensive, making wastewater surveillance an attractive alternative in terms of investment. Realistically, wastewater surveillance needs to be seen as a complementary data source that is useful for triangulating with more conventional public health data sources (e.g., clinical test data) to model and estimate critical parameters that can support actionable public health metrics.

The reproduction number (R_0) is a fundamental parameter characterizing the dynamics of an epidemic. Although there are various explanations, Annunziato and Asikainen (2020) have defined the basic reproduction number R_0 as describing "*how many persons an infectious person infects totally in average during his or her time being infectious in a population where nobody is assumed to have any protection against the disease, so in most situations it describes what happens if a new disease enters a population*". This accurately describes the spread of COVID-19 in early 2020. The effective reproduction number R_{eff}, or R(t) in their model, describes "*how many persons an infectious person infects totally in average during his or her time being infectious in a population where some individuals can have protection against the disease*." Annunziato and Asikainen (2020) described a number of mathematical methods for estimating the reproduction number using epidemiological data based on case numbers over time.

Kaplan et al. (2021) performed a retrospective analysis of hospitalization data and the wastewater (primary sludge) surveillance performed by Peccia et al. (2020) in New Haven, CT WWTP (serving a population ~200 000) at the outset of the COVID-19 pandemic. They developed a model that predicted the reproduction number (R_0) as being ~2.4 during this period and that the detection of SARS-CoV-2 in sludge was only able to shorten the time from infection to detected signal by three to five days relative to hospital admissions. Kaplan explained this finding by noting that their analysis occurred during a period of lockdown and physical distancing mandate that split the total population

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into two groups: one demonstrating an unmitigated outbreak among an estimated 11% of the population who remained exposed to infections versus the remaining 89% who complied with the public health restrictions. In total, Kaplan et al. (2021) estimated that about 9.3% of the entire population, i.e., most of those who had not adopted public health mitigations, became infected during this period.

Huisman et al. (2021) estimated the *effective* reproductive rate, R_{eff}, using longitudinal WBS data in Zürich, Switzerland and San Jose, California, USA finding their R_{eff} estimates to be as similar to those estimated from case report data as R_{eff} estimates based on observed cases, hospitalizations, and deaths are among each other.

5.7. Ethical considerations

Perhaps an aspect of the COVID-19 pandemic that has been most surprising is how much and how intense public controversies have been about seemingly obvious measures to reduce the risk of individuals becoming infected. The reality of what has happened is that even a cautious, conventional approach to ensuring that all public health measures in response to the pandemic meet the highest ethical standards is likely to be challenged by some segments of our society as it has been influenced by the pandemic. Even the terminology, wastewater surveillance, as a means of complementing conventional public health surveillance has been noted to be conveying a negative message. Specifically, Joh (2021) argues from a U.S. perspective that wastewater surveillance will become a routine part of police surveillance infrastructure with justification drawn from applications of wastewater surveillance being used for tracking illicit drug use before the pandemic. Even Statistics Canada (Werschler and Brennan 2019) had been engaged in a pilot program of wastewater surveillance since March 2018 for five Canadian cities to track the use of cannabis following its legalization. This program also included monitoring for a dozen illicit drugs (opioids and stimulants), and it has become the platform for the PHAC's National Wastewater Surveillance Program. Van der Sloot (2021) has argued that such practices in the U.S. and Europe have been essentially "*flushing privacy down the drain*".

If the COVID-19 pandemic has taught us nothing else, it should be clear that public confidence and support for public health interventions is essential for their widespread adoption. Undertaking programs that are perceived to be unethical even by fair-minded citizens will be problematic. These circumstances make it essential for those proposing and adopting wastewater surveillance for SARS-CoV-2 to ensure that such programs meet the highest public health ethical standards. Of course, the challenge is to determine what ethical standards are relevant and actually apply to wastewater surveillance for SARS-CoV-2. Ethical considerations for other applications of wastewater surveillance for matters such as law enforcement are beyond the scope of this report.

The Code of Ethics for the American Public Health Association (APHA 2019) includes four components that are required for the ethical analysis of any proposed public health action:

- *"Determination of the public health goals of the proposed action*
- *Identification of the ethically relevant facts and uncertainties*
- *Analysis of the meaning and implications of the action for the health and rights of affected individuals and communities*
- *Analysis of how the proposed action fits with core public health values."*

However, among 12 domains outlining ethical action guidance, surveillance is mentioned only once in one **domain: "***Investigate health problems and environmental public health hazards to protect the community***.** *When investigating health problems and environmental hazards, it is necessary to collect* *the information most relevant to characterizing the problem in question and implementing control measures. There are several methods for doing so, all involving some form of active surveillance such as outbreak investigations or surveys of populations and individuals."* A search of the Canadian Public Health Association (CPHA) website and an internet search did not locate an equivalent document for CPHA.

The Public Health Agency of Canada developed an public health ethics framework to be used as a guide for use in response to the COVID-19 pandemic in Canada (PHAC 2022). This framework listed major values and principles as: "*Trust, Justice, Respect for persons, communities and human rights, Promoting well-being, Minimizing harm, and Working together*". Procedural considerations for implementing this ethics framework included hallmarks of: "*Accountability, Openness and Transparency, Inclusiveness, Responsiveness, and Intersectionality*." While valuable in a broad sense to set an appropriate context, this ethics guidance is not explicit for the details of wastewater surveillance.

Literature related to ethical guidance for wastewater surveillance for SARS-Cov-2 has been limited, despite calls for development of such guidance (Coffman et al. 2021). The Canadian Water Network (CWN) recognized from the outset that wastewater surveillance for SARS-CoV-2 needed to support a clearly articulated public health purpose. CWN formed a public health advisory group to develop ethical guidance for this purpose (CWN 2020a). These guidelines were developed based on WHO guidelines for public health surveillance generally (WHO 2017) and were refined specifically for the kinds of activities that wastewater surveillance entails (Hrudey et al. 2021). This guidance has been acknowledged by the European Commission Joint Research Centre (Gawlik et al. 2021) in its feasibility assessment for a European sentinel system based on wastewater surveillance for SARS-CoV-2.

The guidelines proposed by Hrudey et al. (2021) from WHO (2017) as being clearly applicable to wastewater surveillance for SARS-CoV-2 are:

- *"Countries have an obligation to develop appropriate, feasible, sustainable public health surveillance systems. Surveillance systems should have a clear purpose and a plan for data collection, analysis, use and dissemination based on relevant public health priorities.*
- *Surveillance data should be collected only for a legitimate public health purpose.*
- *Countries have an obligation to ensure that the data collected are of sufficient quality, including being timely, reliable and valid, to achieve public health goals.*
- *The values and concerns of communities should be taken into account in planning, implementing and using data from surveillance.*
- *Those responsible for surveillance should identify, evaluate, minimize and disclose risks for harm before surveillance is conducted. Monitoring for harm should be continuous, and, when any identified, appropriate action should be taken to mitigate it.*
- *Surveillance of individuals or groups who are particularly susceptible to disease, harm or injustice is critical and demands careful scrutiny to avoid the imposition of unnecessary additional burdens.*
- *Governments and others who hold surveillance data must ensure that identifiable data are appropriately secured.*
- *Under certain circumstances, the collection of names or identifiable data is justified.*
- *Individuals have an obligation to contribute to surveillance when reliable, valid, complete data sets are required and relevant protection is in place. Under these circumstances, informed consent is not ethically required.*

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- *Results of surveillance must be effectively communicated to relevant target audiences.*
- *With appropriate safeguards and justification, those responsible for public health surveillance have an obligation to share data with other national and international public health agencies.*
- *During a public health emergency, it is imperative that all parties involved in surveillance share data in a timely fashion.*
- *With appropriate justification and safeguards, public health agencies may use or share surveillance data for research purposes.*
- *Personally-identifiable surveillance data should not be shared with agencies that are likely to use them to take action against individuals or for uses unrelated to public health."*

Hrudey et al. (2021) have elaborated how each of these WHO (2017) generic public health surveillance guidelines apply specifically to wastewater surveillance for SARS-CoV-2.

Finally, in addition to these obligations, specific considerations will apply in conducting wastewater surveillance in First Nations communities to reflect the Assembly of First Nations ethics policy and principles of OCAP™ (ownership, control, access, possession) on research (AFN 2009) even though the surveillance may not be classified as "*research*" by some institutions.

5.8. Public health decision-making

WHO (2022b) assembled a team of international experts with direct experience initiating, implementing and interpreting wastewater surveillance for SARS-CoV-2. They have developed an overview (**Table 5**) of how different applications of wastewater surveillance ("*use cases*") can inform public health decisions with a consensus, generic semi-quantitative rating of how useful each can be. This WHO expert summary rating is very informative because the current literature is not very helpful in addressing this critical question at a high level.

Nourbakhsh et al. (2022) have noted that wastewater surveillance is less influenced by sampling bias than clinical surveillance, particularly when clinical testing policies are shifting because of capacity limits, participant reluctance and other considerations, and by the inability of clinical testing to capture asymptomatic cases unless wide coverage random testing is practiced. However, wastewater surveillance may be less able to closely track downward trends in clinical cases because it may be capturing signals from recovering patients who continue to shed some virus. Nourbakhsh et al. (2022) note that vaccination may contribute to this issue with tracking downward trends because it may result in a greater number of sub-clinical and asymptomatic patients who will still shed SARS-CoV-2 RNA, thereby altering the relationship with active clinical cases. Fernandez-Cassi (2021) reported wastewater, based on monitoring two WWTPs, to provide a more accurate measure of new cases rising than clinical testing, but the opposite was found true for when cases were declining. Contrary to concerns expressed by Nourbakhsh et al. (2022), wastewater declined faster than clinical case counts. These differing concerns suggest the importance of site-specific details for both wastewater surveillance and clinical test policies and resulting data. Such details will need to be resolved to reliably use wastewater data for evaluating the effectiveness of NPIs, for example.

5.9. Communications and relations among participants

Fundamental elements of communication include knowing who the audience is to be reached and ensuring there can be functional two-way communication. The former is essential to planning any communication strategy while the latter needs to be fostered at all stages of communication. For the purposes of wastewater surveillance for SARS-CoV-2, the main audiences for those who are planning such programs and generating data are:

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Table 5. Summary of use cases and their benefits in COVID-19 response strategies in various settings (adapted from WHO 2022c).

Application of Wastewater Water Surveillance (use cases)	Description	Benefits for COVID-19 response strategy (Legend +++ = primary benefit, ++ = secondary benefit, + = ancillary benefit)							Setting or level where surveillance application has greatest benefit, with comments on benefits
		Provides early warning	Encourages diagnostic clinical testing	Informs decisions on control interventions	Encourages compliance with control interventions	Informs decisions on hospital care capacity	Informs decisions on targeted clinical testing	Improves vaccine uptake	
Tracking increasing and decreasing **Trends** at community level to help **target COVID-19 responses and interventions**	Observing increasing and decreasing trends at community level to, once confirmed, provide an early indication (4-7 days) of changes in incidence & levels of virus circulation for timely decisions on strategies and interventions	++	+	+++		+++	+++		Regional & local/city level planning. Applies to all prevalence levels. Communities with low uptake of clinical testing, failing reporting or increased reliance on self-testing. Larger populations sizes
Finding outbreaks in places thought to be COVID-19 free	Involves testing for SARS-CoV-2 in areas where it is not expected, to provide early warning of its emergence and enable earlier intervention	+++		+++		++	+	+	Locations where COVID-19 is thought to have been eliminated or locations where COVID-19 cases have not been identified
Augmenting risk communication to help **promote safer behaviours**	Publicizing data on detection in wastewater reminds community that the virus is still circulating, may encourage people to seek clinical testing and may reduce complacency about control interventions	+	+++	+	++			++	Low to moderate prevalence
Cost-effective **targeting of public health surveillance** (clinical test resources)	Allows deployment of limited clinical testing resources in hot spot areas with higher signals	+	++	++			+++		Spatially differentiated, low to moderate prevalence. Larger population sizes
Informing early and localized restrictions in pockets of (re-) emergence by helping **detect outbreaks**	Informs more targeted rapid interventions to minimize the extent and economic impact of restrictions (e.g., service closures, travel limits)	+	+++	+++					Spatially differentiated, low prevalence.

(*continued*)

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Table 5. *(concluded)*

Application of Wastewater Water Surveillance (use cases)	Description	Benefits for COVID-19 response strategy (Legend +++ = primary benefit, ++ = secondary benefit, + = ancillary benefit): Provides early warning	Encourages diagnostic clinical testing	Informs decisions on control interventions	Encourages compliance with control interventions	Informs decisions on hospital care capacity	Informs decisions on targeted clinical testing	Improves vaccine uptake	Setting or level where surveillance application has greatest benefit, with comments on benefits
Identifying existing **known Variants of Interest or Concern**	Involves testing for known gene targets where proportions of VOCs in circulation are uncertain or higher resolution of information is needed	++		++		++	+		Locations where occurrence of VOCs have not been adequately characterized
Detecting emergence of VOCs	Involves specialized PCR targets for VOCs or whole-genome sequencing to identify VOCs emerging in the sampled system	+++							Needs development of specific probes for VOC
Biobanking and Retrospective **analysis**	Involves retrospective analysis of data to provide intelligence on introduction, evolution & dissemination of the virus to inform future pandemics			++					Global, but particularly for areas more vulnerable to future pandemics
Targeted surveillance for early warning of circulation:	Allows early warning to inform earlier intervention to help limit COVID-19 spread in targeted settings	+++		+++			++	+	
-vulnerable or high-risk settings	-managed isolation facilities, aged care facilities, schools, prisons, informal settlements, refugees & displaced persons	+++		+++			++	+	Ensure equity & protect vulnerable groups
-isolated communities	-remote & indigenous communities, industrial, mining & research facilities; quarantine facilities; student residences	+++		+++			++	+	Enable "*bubbles*" or groups to be contained. Augment data in areas with low uptake of diagnostic, clinical testing
-transport vessels	-sewage tanks of arriving ships & aircraft	+++		+++			++	+	Test before passengers disembark or disperse
-multi-day events or gatherings	-meetings, events, or festivals spanning days or weeks	+++		+++			++	+	Evidence to inform continuation of events or gatherings

Note: Ratings (+, ++, +++) are Interim guidance from WHO, based on experience to early 2022.

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1. Frontline public health practitioners and their epidemiological advisors
2. Policymakers, including government decision-makers
3. The public

Communication with these groups is necessary to honour the need to translate surveillance data into action. Foege et al. (1976) state that for surveillance programs "*…collection and analysis should not be allowed to consume resources if action does not follow*". Actions that may result from effective SARS-CoV-2 wastewater surveillance include: providing early warning indicators of an increase in numbers of cases in communities, identification of "*hot spots*" or institutional outbreaks, identifying a need for increased clinical testing in communities or institutions, informing public messaging about the need to wear masks, maintaining physical distancing, washing hands, alerting the public about rising case numbers, and the need for lockdowns and quarantine measures (O'Keeffe 2021; CDC 2020; PHO 2021). Experience shows that there can often be a gap in translating surveillance data into public health action (Orton et al. 2011). For public health to effectively adopt and act on SARS-CoV-2 wastewater surveillance data there needs to be strong partnerships between the players along with clear, concise, and effective communication.

Translating knowledge into action is best facilitated by having the decision-makers involved in the early stages of surveillance development (Lemire et al. 2013; Innvaer 2002). The previous sections and following Section 6 case studies provide examples where SARS-CoV-2 wastewater surveillance has informed action by frontline public health personnel to investigate potential outbreaks and take control measures to reduce COVID-19 transmission.

The examples that have been most successful in translating wastewater data into action have been cases where there has been extraordinary collaboration between researchers, wastewater utilities, laboratories, and public health. This type of collaboration has also been observed in other aspects of the COVID-19 response, such as the international collaboration on vaccine development (Druedahl et al. 2021). These early examples demonstrate that effective collaboration is possible, but a concerted effort will be needed to ensure these relationships will continue and will be adopted by other jurisdictions.

The relationship between public health departments and water/wastewater utilities is often not well established and may only become active at times of crisis (Gelting and Miller 2004; Jalba et al. 2010, 2014). Where relationships are established, it is more likely related to drinking water. In fact, in many municipalities, health departments may never have had cause to be involved in wastewater activities before. For the continuing application of wastewater surveillance data across jurisdictions, simply collecting the data and presenting it to public health will not be enough to facilitate action. As wastewater monitoring becomes more routine, there will be a need to create lines of communication and a common language for discussing and understanding wastewater surveillance results. This will then be reflected in more formal agreements, frameworks, and reporting relationships among stakeholders for sustainable collaboration.

Experience from the USA about what worked and did not work in establishing wastewater surveillance was summarized by Hoar et al. (2022). This account led us to develop a similar summary about the experience of investigators in Canada (**Table 6**).

Lemire et al. (2013) found that for public health managers to make decisions based on data they need clear, concise, and consistent information and, when possible, information that can show concrete applications. Safford and Brown (2019) outline strategies to address the particular challenge of communicating effectively with policymakers, particularly political decision-makers. The COVID-19

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Table 6. Lessons learned for establishing effective wastewater surveillance in Canada.

Effective Actions	Challenges
Establishing early partnerships between the academic research laboratories, municipal utilities, and public health units was critical. e.g., CWN role	Mechanisms to initiate partnerships, especially early in the pandemic.
Regular and sustained interaction, reporting and dialogue (interpersonal relationships and trust)	Limitation on human resources to sustain the interactions.
Early initiative by academics to test concept and provide early proof of concept.	University laboratory access/ COVID restrictions, lack of personnel, infrastructure and resources.
Engaging the public health laboratories at the national and provincial level (e.g., NML, Alberta Provincial Laboratory, BC CDC).	Securing and sustaining commitment and resources early on when agencies were challenged in dealing with the emerging pandemic.
Establishing national method leadership at NML (working groups, leadership in method development and application)	Establishing the structure and commitment of Federal Department and Agencies and many provincial governments.
Facilitation of regular communication among laboratories and public health agencies nationally to share developments (e.g., informal "*coffee club*", PHAC and MECP working groups, CanCOVID)	Need for champions to lead and sustain these initiatives.
Open collaboration and sharing of information across laboratories.	Avoiding traditional academic competition and priority concerns for publications, commercial IP, etc.
Establishing interlaboratory sample exchanges and studies targeted at improving and contrasting methods	Logistically very difficult and required commitment of resources and personnel. Lack of pre-existing consistent minimum requirements for level QA/QC across laboratories
Making data publicly available in almost real time	Concerns about confidence in the interpretation and possible misunderstanding or misuse of complex data by the media or the public
Open sharing of data in a common format (e.g.: PHES-ODM; github.com/Big-Life-Lab/PHES-ODM)	Concern over data ownership and rights. Establishing appropriate data-level QA/QC across laboratories. Need for cross disciplinary conversations between laboratories, data scientists/engineers, and data users (epidemiologists) Establishing right data products to serve end user needs
Eventual provision of sustained funding from governments to enable the laboratories to have the infrastructure, human resources and material to conduct analysis.	Short term funding because of the uncertainty of scope of the pandemic. Retention of qualified personnel. Moving from academic-based initiative to public health or commercial laboratories
Flexibility for research within programs to ensure quality and program development	Early focus of funding surveillance and lack of research funding avenues for academia
Provincial data infrastructure and sharing capacity	Logistic, expertise and securing resources
Having a champion in public health. (e.g., Peter Juni, the Ontario Science Table.	Finding a champion and a forum within public health agencies.
Leveraging existing laboratory quality management Infrastructure (accreditation)	Not in best interest for academic laboratories to seek accreditation, no impetus for commercial laboratories to seek accreditation without a viable, sustainable business case
Engaging in open science. Sharing primary data and information and foregoing concerns of institutional advancements.	Difficulties of established institutions to openly share primary data and information can stymie overall progress.
High level of public support and media interest. Public engagement with research results, real time questions and answers on social media. High trust of researchers engaging in science communication.	Data transparency and ethical oversight are essential for continued public support.

pandemic has revealed political conflicts about public health interventions that may have been difficult to foresee before March 2020, making such communication more important and challenging than ever. Despite those evident difficulties, the advice is very basic. Recommended strategies for success include knowing whom you want and need to reach, having clear and actionable recommendations, repackaging your work (i.e., not presented in the style and form of an academic paper), writing well (concise, organized and clear), presenting your case at an opportune time, sustaining and amplifying your engagement.

There is currently limited experience and understanding from public health as to the interpretation and use of wastewater surveillance for SARS-CoV-2 RNA data as it relates to action (PHO 2021), although the growing coverage of this topic in 2022 is likely to have increased awareness.

Public health decision-makers will seek answers to questions such as:

- At what detectable level of SARS-CoV-2 RNA should action take place?
- What should that action look like?
- Can decisions be consistently applied across communities?

Such answers would allow decision makers to understand the surveillance data, helping them use it to make informed decisions. That said, an action level will depend on many specific factors that will need to be determined locally.

However, decision-making in a new and emerging field, such as SARS-CoV-2 wastewater surveillance, is not as easy and straightforward as the users of that information would demand. Standard methods for testing and reporting from the scientific community would allow public health to compare results across jurisdictions and have greater trust in the data that decisions would be made on, however, the challenges for providing meaningful standardization should not be underestimated (Ahmed et al. 2020c). The longer these systems are in place, the more likely we are to reach that point. Furthermore, it is also important for public health decision makers to recognize that SARS-CoV-2 wastewater data is one of several pieces of information that can be used, and that, in general, no surveillance indicator should be used in isolation (Nsubuga et al. 2006). The goal of using SARS-CoV-2 wastewater data in public health decision-making should be to supplement other epidemiological data related to COVID-19, not replace it (CDC 2020). A correlation between wastewater data, sampling data, and hospital data will be most meaningful.

A natural step as we make the shift from COVID-19 being considered a pandemic to being more endemic is to consider developing sentinel surveillance sites for SARS-CoV-2 wastewater monitoring across Canada. Sentinel surveillance sites are predetermined locations, where data is gathered to inform programs and policies, using defined geographical areas. Sentinel surveillance is not designed to provide comprehensive data on community cases (PHAC 2015). Rather it is intended to describe trends of disease overtime, estimates of case numbers and description of patterns without having to sample all locations (Colman et al. 2019).

In Canada, sentinel surveillance is conducted for a variety of pathogens, and can include (clinically-based) from sampling of individuals for illnesses like influenza, to environmental sampling. Canada's sentinel surveillance system for enteric pathogens in the environments, FoodNet Canada, has four sites located across the country (Alberta, British Columbia, Ontario, and Québec) that are comprised of public health units, private and public health laboratories, farms, retail food outlets, and sources of drinking water (PHAC 2015). The criteria for choosing their four sites have been:

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- *"a population of 500,000 to 1,000,000 residents;*
- *an urban/rural mix representative of major geographic areas of Canada;*
- *private and public health laboratory capacity;*
- *innovation in local public health and water services; and*
- *willingness to participate"*

This could be a starting point for considering where SARS-CoV-2 wastewater surveillance sentinel sites could be located or considering if this existing network can be expanded or developed to include ongoing wastewater surveillance. Determining which types of locations should be targeted for sentinel sites for SARS-CoV-2 wastewater surveillance will require great thought as sentinel surveillance allows only a fraction, but necessarily a representative fraction, of the population to be monitored. Deciding exactly which areas will be targeted will be critical but could have great benefit in informing public health policy. Choosing sites near international travel hubs would be a consideration, based on the findings of Hubert et al. (2022) about Omicron dynamics in Alberta communities.

Public health decision making is often more complex than it may appear (Orton et al. 2011). Expertise and understanding of COVID-19 wastewater sampling methodology and metrics will be needed in public health, but it is unrealistic to expect that all health departments will achieve the same levels of that expertise. A framework that would support both the translation of wastewater results into a usable format for all public health decision makers and enable collaboration between the various parties is needed.

Data presentation will be a key factor in successful communication. WHO (2022c) have provided a summary depiction (**Fig. 9**) of how hypothetical wastewater surveillance data and clinical case data may relate and provide some basis for interpreting public health intervention options.

Public-facing dashboards (Part 2 of **Supplementary Information**) have become a common means of presenting wastewater surveillance data, usually together with public health surveillance data for appropriate public access. WHO (2022c) have recommended that the minimum information to be included in a dashboard for it to be useful for the public and for public health agencies should include:

- *"physical location of sample collection and catchment (represented spatially and by name);*
- *population monitored as represented by each sample;*
- *historical results from the same location;*
- *current and historical results from nearby and comparable locations;*
- *reported COVID-19 cases from the same location for the same period as sample collection;*
- *trends (rising, falling or steady); and*
- *implications of high, medium or low levels relative to a benchmark (e.g., using traffic light indicators).*
- *gene target*
- *assay detection limits; and*
- *quality assurance and quality control process and performance on method sensitivity and specificity."*

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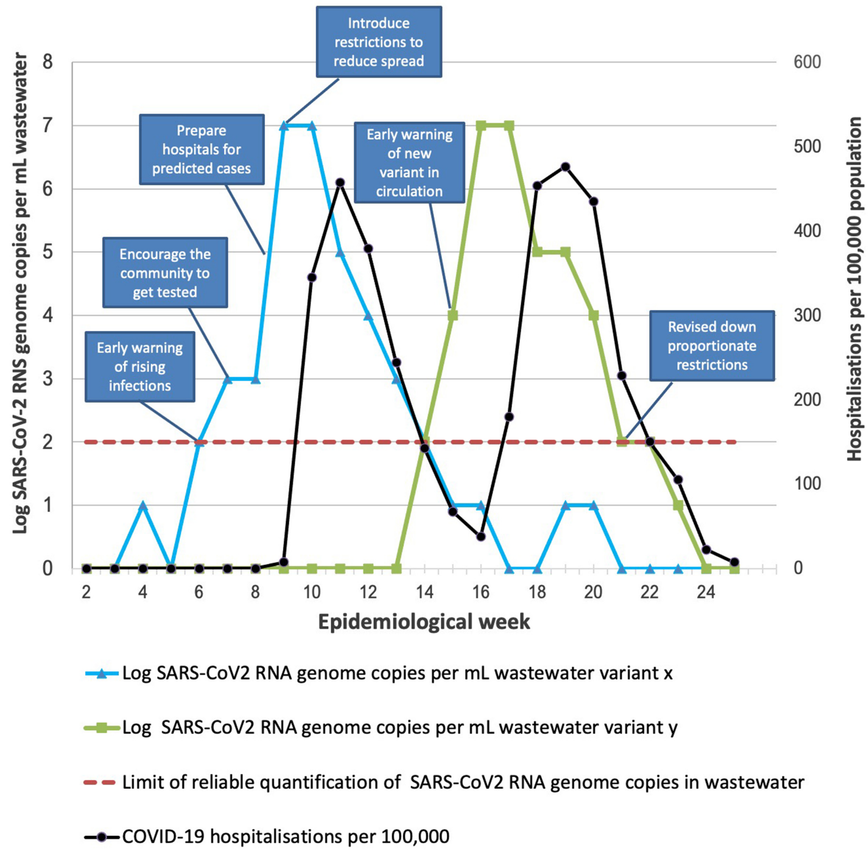

Fig. 9. Hypothetical depiction comparing wastewater surveillance data in relation to public communications and public health decision-making (after WHO 2022c).

These points have merit at a high level, of course the challenge comes with trying to define what levels and criteria should be used.

5.10. Some Canadian wastewater surveillance success stories

This report cannot do justice to all of the initiatives to implement wastewater surveillance for SARS-CoV-2 RNA in Canada that have occurred over the past two years. Part 1 of the **Supplementary Information** provides a selection of case studies of wastewater surveillance implementation that the authors were aware of and invited, but we do not claim that our selection is exhaustive in its coverage. Part 3 of the **Supplementary Information** provides a listing of 48 publications accepted or published to date, authored by Canadian investigators who were mostly pre-occupied with developing wastewater surveillance programs rather than writing papers. This collection of publications provides another window on what has been achieved.

The CWN facilitated networking by means of the Wastewater Coalition in the spring of 2020 among Canadian researchers who had advised the Coalition that they had established a laboratory capability by the summer of 2020 to detect SARS-CoV-2 RNA in wastewater. Participation by this initial group led to the first Canadian interlaboratory study CWN (2020b) that used the capability of the NML of PHAC to prepare and ship the samples, containing known spikes, to seven other laboratories

(BCCDC, Alberta Public Health Laboratory, University of Saskatchewan, University of Ottawa, University of Waterloo, University of Windsor, and Polytechnique Montréal.

Interested readers are encouraged to read the case studies (Part 1 of the **Supplementary Information**) prepared by groups of collaborators working with the resources they could muster to respond to Canada's needs in the face of the COVID-19 pandemic. Although some of the details are provided in each case and the publications they reference in the case studies in Part 1 of the **Supplementary Information**, a few summary highlights follow.

5.10.1. British Columbia

The British Columbia Centres for Disease Control (BCCDC) Public Health Laboratory (PHL) leveraged an existing collaboration with Metro Vancouver focusing on enteric viruses in wastewater since 2018 so that methods for the quantification of SARS-CoV-2 in wastewater were developed in May 2020. Following participation in the CWN inter-laboratory study (Chik et al. 2021), the BCCDC PHL team further optimized its methods for detecting and quantifying SARS-CoV-2 in wastewater. Starting in October 2020, wastewater samples were collected weekly from five WWTPs in the metro-Vancouver area capturing close to 50% of BC's population and spanned its two largest health authorities. The wastewater results have been integrated with clinical case counts by a medical geographer at the sewershed level. Public health epidemiologists have compared the concentration of SARS-CoV-2 in wastewater at each WWTP to the incidence of COVID-19 cases in the corresponding wastewater catchment area.

On a weekly basis, the wastewater data, epidemiological graphs, and key messages are compiled and reported weekly for Medical Officers of Health and epidemiologists at the regional health authorities since March 30, 2021. Wastewater data is also incorporated into the bi-weekly BC COVID-19 Data Summaries since August 14, 2021 and in the BC Situation Report (BCCDC 2022) since November 28, 2021. To make the data and information available to the general public and to help facilitate the dissemination of the SARS-CoV-2 in wastewater data, Metro Vancouver launched an online page providing an interactive map that allows the public to view SARS-CoV-2 concentrations at each WWTP over time.

In collaboration with Dr. Ziels and Xuan Lin at UBC, methods were quickly developed to test wastewater samples (Lin et al. 2022) for variants of concern (VOCs). These methods have been deployed for both Metro Vancouver WWTPs (since January 2021) and the UBC project (since September 2021) and successfully detected Alpha, Gamma, Delta, and Omicron VOCs.

5.10.2. Alberta

In the first half of 2020, two experienced Alberta research teams began implementing SARS-CoV-2 wastewater monitoring programs. Dr. Xiaoli Pang at the Provincial Laboratory of Public Health (cross-appointed to the University of Alberta) began testing for SARS-CoV-2 RNA in WWTP samples from across Alberta, later expanding to include long-term care facilities in Edmonton. In parallel, preliminary studies at the University of Calgary were initiated by an inter-disciplinary team with expertise in environmental microbiology and virology, wastewater engineering and clinical microbiology including Calgary's head of infectious diseases Dr. Michael Parkins. The Edmonton and Calgary teams agreed to submit separate proposals to a Canadian Institutes of Health Research (CIHR June 2020) competition and both secured approximately \$500k each for one year pilot studies. Wastewater testing became established in different Alberta municipalities, urban neighbourhoods, and hospitals by mid 2020, followed by long-term care facilities in Edmonton funded by the COVID-19 Immunity Task Force (covid19immunitytaskforce.ca).

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In Calgary, wastewater monitoring was performed in hospitals, a setting with a high degree of testing of patients and healthcare workers and thus a very good understanding of transmission dynamics. Accordingly, SARS-CoV-2 levels in hospital wastewater enabled differentiation of new nosocomial outbreaks of COVID-19 against a high background of patients admitted to hospital with COVID-19 infection. This indicated that the vast majority of RNA shedding into wastewater was associated with early onset of disease (Acosta et al. 2021).

Testing in several Edmonton long term care facilities studied the cost-effectiveness and early warning potential of wastewater surveillance (Lee et al. 2021) finding that undetected clinical cases could be revealed from wastewater surveillance. Nodal sampling in neighbourhood sub-catchments throughout Calgary demonstrated links between COVID-19 infection and social determinants of health during Alberta's second and third waves in late 2020 and early 2021 (Acosta et al. 2021). Li et al. (2023) reported a Probit analysis of over 1 800 wastewater samples collected from 12 Alberta WWTPs (reported in Section 5.2) for over a year that provided estimates of case detectability in relation to population size.

By 2021, with two successful wastewater monitoring programs up and running, the Edmonton and Calgary teams began collaborating more closely. The groups formally coalesced and secured funding from the Alberta government creating a single PanAlberta monitoring program to cover large and medium sized municipalities throughout the province as well as selected institutions. WWTP samples taken three times per week were sent by courier to either the Edmonton or Calgary groups for RT-qPCR testing with rapid turnaround times of 24 to 48 hours. By late 2021, this program covered more than 80% of the province's population and ~95% of its urban population. Also in 2021, in partnership with data sharing experts from the University of Calgary's Centre for Health Informatics (CHI), wastewater results began being published on CHI's COVID-19 tracker website (covid-tracker.chi-csm.ca/). By 2022, this website's wastewater page was getting up to 8 000 visits per day.

5.10.3. Saskatchewan

A pilot study in Saskatoon was initially funded by the University of Saskatchewan-led Global Water Futures (GWF) program and supported through in-kind contributions of personnel and sampling equipment by the City of Saskatoon, postdoctoral fellows, and students. Based on three weekly samples, viral loads in Saskatoon's wastewater remained low throughout July, August, and September 2020, but began to rise exponentially in October and November 2020 providing a leading indicator of impending surges in case numbers. The team informed Saskatoon's population of upcoming potential increases (and decreases) in positive cases primarily through press releases and media interviews. Wastewater data were shared with the Saskatchewan Health Authority and the Saskatchewan Ministry of Health. Provincial modelling teams used the information from wastewater surveillance to refine their models that helped forecast future health risks associated with COVID-19. A first-of-its-kind study with Indigenous communities was initiated in partnership with the Indigenous Technical Services Co-operative (ITSC), which included five First Nations with one each from Agency Chiefs Tribal Council, File Hills Qu'Appelle Tribal Council, Saskatoon Tribal Council, Touchwood Agency Tribal Council, and Yorkton Tribal Council.

5.10.4. Ontario

The University of Ottawa, in collaboration with the Children's Hospital of Eastern Ontario's Research Institute (CHEO-RI) and the City of Ottawa, performed the first measurement of SARS-CoV-2 viral signal in Canadian wastewaters on April 8, 2020 (D'Aoust et al. 2021a). With sufficient data, SARS-CoV-2 wastewater surveillance was found to provide useful information such as early detection of disease incidence in the community, shown during the beginning of the second resurgence of COVID-19 in Ontario (July 2020). Specifically in the summer of 2020, SARS-CoV-2 wastewater surveillance was shown to forecast increases in clinical cases of COVID-19 by 48 hours, and increases in COVID-19-related hospitalizations by 96 hours (D'Aoust et al. 2021b). Ottawa Public Health rapidly became further involved in the novel surveillance system, ultimately requesting testing seven days a week and an analysis turn-around-time of 24 hours, which was attained in September 2020. In addition, the first public-facing dashboard (613covid.ca) of SARS-CoV-2 surveillance in Canada was put online in Ottawa in September 2020 in collaboration with the University of Ottawa, CHEO-RI, the Ottawa Hospital Research Institute, and Ottawa Public Health.

The daily testing frequency and rapid turn-around-time demonstrated improved understanding of COVID-19 surveillance data in the City of Ottawa with the wastewater data being triangulated with clinical data. In response to the use of wastewater surveillance data, the City of Ottawa, University of Ottawa, and CHEO-RI contributed to an Ontario Science Advisory Table Science Brief (Jüni et al. 2020) which resulted in planning of an Ontario-wide SARS-CoV-2 Wastewater Surveillance Initiative (WSI).

Led by Ontario's Ministry of the Environment, Conservation and Parks (MECP), the Ontario WSI was established as a provincial program that is comprised of a network of 13 academic and research institutions along with involvement of PHAC-NML and now extends to 170 locations capturing over 75% of Ontario's population. This wastewater surveillance network is clearly the largest in Canada. Wastewater surveillance efforts have emerged as a critical measure of community spread of COVID-19 that is independent of clinical testing, attracting considerable media attention and increasing public awareness (December 2021-February 2022). In Ontario, Public Health Units and their respective Medical Officers of Health have since used wastewater surveillance data to assist in planning, public messaging, and directing resources.

At the University of Waterloo, Dr. Mark Servos, a Biology Professor recognized that his laboratory's experience in wastewater and environmental research could be adapted to detect SARS-CoV-2 in wastewater influent. Early in the pandemic (April 2020), he and his team returned to the laboratory to focus on developing methods that could be applied to conduct wastewater surveillance for Ontario communities. Once the methods were developed in the summer of 2020, with the support of municipal and Public Health Unit partners, pilot programs were initiated at several sites to test and validate the approach. Within a few weeks this grew into a formal surveillance program for these regions with multiple sites covering a population of more than 2.6 million people. Eventually, this program has grown under the Ontario WSI to cover the regions of York, Peel and Waterloo. All three Public Health Units (PHUs) /Regions early in the pandemic established mechanisms to disseminate the results to senior management as well as the public by means of dashboards (see Part 2 of the **Supplementary Information**). This group, in collaboration with the Ottawa group and NML developed refinements that could be used with PCR (Fuzzen et al. 2022) to allow tracking of VOCs, a critical advance that has allowed the tracking of the take-over by Omicron from Delta in late 2021 when provinces had largely abandoned wide-spread clinical testing in the population, leaving wastewater surveillance as a more unbiased means of tracking VOC waves.

5.10.5. Québec

Researchers in Québec, using their own research funds, began surveillance of SARS-CoV-2 in wastewater in March 2020 (with the earliest samples collected in February 2020). They did not initially have access to their laboratories because of large outbreaks in Montréal early in the pandemic. A frozen archive (stored at −80 °C) of samples from the early days of the pandemic in the Montréal was created. Once permission was granted to return to the laboratory, researchers joined nation-wide initiatives such as the CWN-led interlaboratory study (Chik et al. 2021) prior to selecting a final protocol for analyzing archived and fresh wastewater samples.

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In December 2020, a grant from the Fonds de recherche du Québec (FRQ), the Trottier family Foundation, the Molson Foundation, and the National Centre for Electrochemistry and Environmental Technologies (CNETE) for a total of $1.7 million enabled the launch of the CentrEau-COVID 6-month pilot project with collaborators from municipalities, seven universities, local public health, and the *Institut national de santé publique du Québec* (INSPQ). Internal funding through McGill's Mi4 program enabled the sequencing of samples collected from Québec's CentrEau-COVID pilot project (N'Guessan et al. 2022) and comparison with clinical samples to find that wastewater sampling was highly efficient for the detection of VOCs.

5.10.6. Nova Scotia

Wastewater monitoring for SARS-CoV-2 in Nova Scotia has been led by Dr. Graham Gagnon and Dr. Amina Stoddart at Dalhousie University's Centre for Water Resources Studies. Their work has been conducted through support and partnership with Research Nova Scotia, Halifax Water, LuminUltra Technologies, Genome Atlantic, and many other municipal and industrial partners. Sustained weekly sampling was undertaken at four WWTPs in Halifax Regional Municipality processing 92% of the wastewater in the region as well as WWTPs in Sydney, Antigonish, and Wolfville contributing weekly samples which were quantified at partner Universities. Passive samplers were also collected up to three times per week from targeted sewershed locations in Halifax Regional Municipality and other communities across the province totalling over 30 sites across Nova Scotia. The research team developed a passive sampling device consisting of a small spherical cage about the size of a softball — named the COVID-19 Sewage Cage (COSCa) — which can be 3D-printed for about $1. The device was ultimately outfitted with an electronegative filter that adsorbs viruses and viral remnants including the biochemical species that is/are associated with SARS-CoV-2 RNA signal derived from wastewater. (Hayes et al. 2021, 2022) and it has been used at sites in France, Australia and across Canada (B.C., Ontario, Northwest Territories).

5.10.7. Newfoundland & Labrador

In November 2020, the Water Resources Management Division of the Department of Environment and Climate Change, and the City of St. John's made a proposal to the Department of Health and Community Services to begin sampling wastewater from the Riverhead WWTP. HCS gave approval for this surveillance in February 2021, when there were known cases in the St. John's area. The province now monitors wastewater for the presence of COVID-19 virus in 17 separate sewershed catchment areas, including residents from 14 communities, representing about 46% of the provincial population. In 2021, wastewater surveillance was useful as an "*early warning*" system for detecting COVID-19. For example, Public Health issued a public advisory for the Town of Deer Lake in November 2021 when the wastewater suddenly showed a strong presence of SARS-CoV-2 RNA. The notification prompted symptomatic residents to seek testing which led to the identification and isolation of previously unknown cases. In September of 2021, the province released its Wastewater Surveillance for COVID-19 dashboard to share wastewater data publicly.

One of the most important lessons learned was to wait until there was buy-in for wastewater surveillance for COVID-19 from public health officials. As the end user of the data, it was vital that the public health decision-makers be part of the conversation. The establishment of a provincial working group that meets every two weeks to discuss results, issues, and new advances was also instrumental in helping guide the development of the wastewater surveillance program in Newfoundland and Labrador.

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5.10.8. Public Health Agency of Canada (PHAC) and National Microbiology Laboratory (NML)

The Federal Fall Economic Statement 2020 allocated $37.4M to support the advancement of innovative approaches to COVID-19 detection from which approximately $12.8 million was allocated over a period of 2.5 years to establish a wastewater monitoring program in Canada. NML had identified Statistics Canada as a key partner early in the pandemic because the existing Canadian Wastewater Survey - CWS covering Metro Vancouver, Edmonton, Toronto, Montreal and Halifax already existed. Through a pilot program between the two organizations, wastewater surveillance covering ~23% of the Canadian population was established by fall 2020. Building on this success, a long-term agreement between the organizations was signed in the spring of 2021 and has formed the core of the national program since then.

NML was vital to the first Canadian interlaboratory study done in partnership with CWN because it provided the logistics for the common sample collection, spiking with known quantities of inactivated virus and internal standards and shipping to seven other Canadian laboratories. NML conducted a second study with the Ontario Ministry of Environment, Conservation and Parks, and the Ontario Clean Water Agency in February 2021 where 29 laboratories across Canada participated in assessing detection of SARS-CoV-2 RNA in wastewater. Since those early days, PHAC has worked with partners to directly support wastewater surveillance at 65 sites (as of April 28, 2022). PHAC estimates that between provincial and other programs, as well their own approximately 60% coverage of the Canadian population was achieved in March 2022. PHAC has set a target of 80% coverage of the Canadian population by the end of 2022.

PHAC arranged with the Government of the Northwest Territories to deploy molecular testing to Yellowknife (Daigle et al. 2022) that was able, with sample preconcentration to detect and rapidly report a consistent SARS-CoV-2 signal in community wastewater that was subsequently confirmed in samples shipped to NML. After a detection on April 16, 2021, daily sampling was implemented leading to increased clinical testing focused on recent travelers to Yellowknife that identified a cluster of six COVID-19 cases over the period April 20 to 26.

6. Strengths and limitations of wastewater surveillance for SARS-Cov-2 RNA

6.1. Strengths of wastewater surveillance for SARS-Cov-2 RNA

6.1.1. Provides objective relevant evidence independent of clinical testing policies

Signals of SARS-CoV-2 RNA measured in wastewater, as long as they are obtained in a rigorous manner of sample collection, preparation and analysis, can be accurate and provide an independent and complementary source of relevant information that can be generally free from inevitable biases arising with clinical test results which are caused mostly by clinical testing policies.

6.1.2. Provides inclusive coverage within a sewershed

Wastewater should capture excretion of SARS-CoV-2 RNA from all residents who are served by the sewer system being sampled, except those who are incontinent or whose faecal waste does not enter the wastewater system. This coverage of wastewater surveillance would include otherwise marginalized populations including those who cannot, or decline being tested for personal reasons. Clinical testing for COVID-19 has not and generally cannot service all groups in society equally. There is a need for surveillance systems to provide equity to marginalized populations, for which wastewater surveillance can contribute to providing more equitable access to community public health data. Widespread and voluntary cooperation of wastewater treatment plants was typically demonstrated,

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though some communities may be under-resourced and unable to participate or are otherwise resistant.

6.1.3. Capable of detecting signal from asymptomatic and pre-symptomatic cases

During the COVID-19 pandemic in Canada, asymptomatic and pre-symptomatic cases in overall communities have not been commonly tested by clinical surveillance, except for potential high risk exposure circumstances and for travel requirements. Asymptomatic and pre-symptomatic cases contributed substantially to community transmission. Evidence that asymptomatic cases can excrete SARS-CoV-2 RNA, and pre-symptomatic cases can excrete SARS-CoV-2 RNA for days before onset of any symptoms, while clinical testing is unlikely to detect such cases raises the expectation that wastewater monitoring done frequently and reported rapidly can provide an early signal of such cases in the population being sampled.

6.1.4. Cost effective sampling

Particularly when sampling at WWTPs, a large population can be monitored with a single daily composite sample. In most Canadian jurisdictions, such samples need to be regularly taken for environmental regulatory monitoring and (or) effective WWTP operations. In such cases, the additional personnel burden for sampling is limited to splitting the sample and preparing it for shipping to the analytical laboratory. The cost per unit of population sampled varies according to the size of the population served but is negligible compared with the costs per individual for clinical testing even after taking account of the slightly higher cost for wastewater sample preparation.

6.1.5. Scalable

Wastewater surveillance for SARS-CoV-2 RNA is inherently scalable for WWTPs, involving the same sampling and analytical cost for a large community as for a small one. Sample shipping costs will be higher for communities distant from the analytical laboratory. Resource demands are higher per sample within sewer networks. The cost-effectiveness is clearly greater in terms of cost per individual for large populations sampled. Ethical issues will inevitably become a greater concern when smaller populations are sampled because of the possibility of identifying infection in small groups of individuals. Wastewater surveillance for SARS-CoV-2 RNA is capable of informing targeted public health interventions at different scales whether implemented at building, neighborhood, or city-scales. Wastewater surveillance also offers future scalability in terms of its potential to monitor other public health risk targets from the same sample. Institutional investments in wastewater sampling infrastructure now will enable adaptation of methods for new and complementary public health surveillance goals. The ability to scale up quickly will always be limited by existing laboratory capacity.

6.1.6. Provides useful information on trends

As long as the procedural quality control and quality assurance measures are satisfied and sufficient wastewater testing frequency is achieved, evidence from many Canadian and international locations has shown the ability of wastewater surveillance for SARS-CoV-2 RNA to show trends for the virus RNA signal in community wastewater. In addition to detecting increases in infections, reporting of trends may provide insight into the success of public health interventions implemented to curb new infections.

6.1.7. Incorporation into high-level public health risk classifications

In some locations, wastewater signals are being incorporated into public health warning systems (e.g., through trend classification, CDC 2022b). While these metrics are not standardized, they demonstrate potential utility of wastewater data for high-level classification systems. As models that relate

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wastewater measurements to other metrics of COVID-19 improve, wastewater data can be more systematically incorporated into informational and decision-making frameworks.

6.1.8. Can detect local hotspots and monitor institutions

Numerous studies in Canada and around the world have demonstrated that wastewater surveillance performed on samples from sewershed nodes and building outflows can provide site-specific information—i.e., by flagging emerging "*hotspots*" when COVID-19 prevalence is otherwise low, and (or) by monitoring specific priority buildings, like residential living complexes. This site-specific information can then in turn justify site-specific interventions, such as targeted communications to a population or mass testing of all individuals residing in a given location and document trends if representative composite sampling is possible. Effectiveness of this approach requires that the frequency of sampling is high enough and sample turn-around-time and reporting is short enough, to inform public health action provided ethical considerations discussed in Sections 5.7 and noted in limitation 6.2.4 are respected.

6.1.9. Tracks dynamics of variants of concern (VOCs)

Several applications reported internationally and in Canada have shown that wastewater surveillance for SARS-CoV-2 RNA can be effectively adapted to detect the proportion of variants in a wastewater sample. These examples have allowed an accurate assessment of how a given VOC is becoming dominant within the system being sampled, information that should be useful to public health officials in knowing what VOCs are going to be showing up in the healthcare system. Although more demanding in terms of analytical facilities, metagenomic sequencing may allow for detection of new variants provided the RNA signal is strong enough to provide a clear signal.

6.1.10. Ability to document spatial and temporal patterns of virus shedding

The nature of wastewater surveillance for SARS-CoV-2 RNA provides it with a demonstrably more efficient ability to track the community dynamics of virus shedding spatially and over time such that reasonable inferences about geographic patterns of disease distribution can be developed.

6.1.11. Ability to deal with rapid increases in cases that overwhelm clinical testing

The emergence of the Omicron variant overwhelmed the ability of clinical testing to track the dynamics of that wave of COVID-19 infection in most jurisdictions. The availability of wastewater surveillance for SARS-CoV-2 RNA provided the only near-real time tracking of the first and second Omicron waves in those locations that were performing such wastewater surveillance. Routine wastewater surveillance can also fill gaps in public health data caused by increasing use of at-home test kits for which results are not reported.

6.1.12. Raises public awareness

Provided that results are made available to the public in a comprehensible manner, wastewater surveillance for SARS-CoV-2 RNA can enhance public understanding about the occurrence of COVID-19 in a community. Effective engagement of the public about the status of the pandemic is important. Particularly as policies such as mask mandates and physical distancing are relaxed, wastewater data can help inform individuals what preventive actions to take given their personal risk profile and tolerance.

6.1.13. Non-Invasive surveillance sampling

Unlike clinical testing of individuals who are asymptomatic and are not seeking medical care, wastewater surveillance for SARS-CoV-2 RNA, itself, is normally, entirely non-invasive to individuals. Only when wastewater provides evidence of undetected infection and individual clinical testing becomes necessary the latter is invasive. At that point the individuals identified are likely at higher risk.

6.1.14. Generates a valuable database for retrospective analyses

The ability of wastewater surveillance for SARS-CoV-2 RNA to generate large quantities of data in a cost-effective manner has created a number of substantial databases in Canada. These can be subjected to retrospective analyses for model development and multivariable assessment to seek better understanding of the dynamics of the COVID-19 pandemic at a given monitored location in relation to public health interventions there. This source of evidence opens the prospects for developing much better evidence-informed insight about public health interventions than have been available for previous pandemics. Likewise, the availability of archived samples in many cases may allow pursuit of new research answers to questions that are not yet evident.

6.2. Limitations of wastewater surveillance for SARS-Cov-2 RNA

6.2.1. Requires accurate knowledge of served population relative to clinical testing

Attempts to relate wastewater surveillance signals to clinical test results have not always been based on assured knowledge about the physical boundaries for each category. The catchment of a given sewer system or WWTP is not likely to be the same physical boundary as may be used for reporting clinical results. Even when locational data are investigated, there are inevitably problems with individuals contributing to wastewater in a different location than where they may have their clinical test result reported. This is particularly a challenge for tourist destinations, educational institutions with students from afar, commuter populations who live and work in different sewersheds, and industrial locations with a fly-in worker population. Similar, but possibly less recognized challenges exist for clinical testing where individual test location or home residence need not represent where the individual has been exposed.

6.2.2. Practical limits of ability to estimate prevalence of COVID-19

Ideally, wastewater surveillance for SARS-CoV-2 RNA would provide data that could reliably be translated into estimates for COVID-19 prevalence in the community. The signal obtained for SARS-CoV-2 RNA in wastewater is dependent on a number of unknown factors such as the rate of SARS-CoV-2 RNA shedding (either as intact virus, viable or not, or as RNA fragments) that can be attributed to a given case. Such numbers will also depend on the stage of infection (initially asymptomatic, active, or recovering), possibly different VOCs and characteristics of the patient (age, health status, vaccination and booster status, etc.). There will also be expected degradation of the RNA signal with sewer travel time to the sampling point and a variety of factors in wastewater that may inhibit the PCR signal. Models that estimate community prevalence may account for many of these factors, but such models would need to be developed and validated across jurisdictions and relevant local factors to facilitate their application.

6.2.3. Achieving early warning depends on surveillance program factors

The reasonable expectation that wastewater surveillance for SARS-CoV-2 RNA provides an early warning of COVID-19 emergence in a community, based in part on viral shedding by asymptomatic patients who will not likely be subject to clinical testing, is absolutely dependent on the frequency of wastewater sampling, rapidity of analysis, and reporting efficiently. Where the necessary resource commitments to achieve these requirements, early detection has been reported. Many initial reports of early warning were based on retrospective analysis of archived wastewater samples and comparison with case data for the time of collection of the archived sample. In simplest terms, a weekly wastewater sampling program, with additional days for sample handling, analysis and reporting cannot be expected to deliver an effective early warning unless clinical testing of such a system is also infrequent

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and delayed in reporting. Because resources were committed early on to achieve sufficient frequency of sampling, rapid analysis and reporting in Ottawa, early warning from wastewater surveillance for SARS-CoV-2 has been demonstrated there and in other Canadian locations that have also committed to satisfying these requirements.

6.2.4. Ethical issues

Consideration of ethical issues was limited in the beginning of wastewater monitoring for SARS-CoV-2 RNA programs in Canada. This is still evident in most international publications. For example, Hoar et al. (2022) present an overview of how academic research can align with a transition of wastewater monitoring to routine public health surveillance in the U.S. with no mention of ethics. Canada has published a set of ethical guidelines for wastewater surveillance for SARS-CoV-2 RNA (CWN 2020a, Hrudey et al. 2021). The smaller the population that is under surveillance, the greater the likelihood that ethical considerations will arise.

6.2.5. Homes that are on septic systems are generally not covered

In addition to institutions (hospitals, prisons, and universities) that treat their own waste, households that have their own private holding tanks or septic system will not necessarily be covered unless their septic tank is pumped out and delivered to a community wastewater system that is under surveillance. Pump-outs are only done intermittently and the RNA signal may be degraded, so there may not be timely nor accurate representation of such households in the community system sample. Although this can include many rural communities, these could be high risk (based primarily on risks of serious outcomes, but also applies to risks of high exposure) or marginalized populations that would benefit from this type of surveillance, such as remote indigenous communities, or migrant farm workers. The same populations that are not being served by clinical testing, due to access issues, may also be missing out on wastewater surveillance. The current ability to cover small and remote communities, or individual homes not on sewerage systems, is limited by logistics, capacity, and cost, leaving a gap in total population coverage.

6.2.6. Variant tracking requires some specialized analytical capability

Although tracking of VOCs has proven to be a major positive feature of wastewater surveillance for SARS-CoV-2 RNA in Canada, not every laboratory that has been involved has necessarily been able to adopt the modified procedures necessary to track VOCs even though Canadian researchers have been very creative and collaborative in sharing the necessary knowledge to be able to track recent VOCs.

6.2.7. No common metric for reporting results

As documented by Canadian inter-laboratory studies, quantitative differences will be found across different laboratories analyzing the same wastewater sample because of differences in procedures used in sample processing and analysis. Likewise, there is not yet a national consensus on how to present quantitative wastewater surveillance data including a lack of consensus about means for normalizing faecal strength of wastewater. These factors mean that results for different communities produced by different laboratories are not readily comparable. Policymakers need to be provided an opportunity to understand the inherent variability and uncertainty in the analytical results while also appreciating the timeliness of such surveillance.

6.2.8. Communication gaps exist among relevant disciplines

The existing "*silos*" among academic researchers, many in environmental science and engineering, public health professionals and wastewater utility personnel have interfered with achieving common understanding about the meaning of results from wastewater surveillance for SARS-CoV-2 RNA.

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Important progress has been made at some locations and efforts by the CWN and NML have helped in some cases, but further work is needed.

6.2.9. Practical limitations for sampling sites in sewer networks

Sampling wastewater from manholes or other sewer access points is much more challenging and resource-intensive than sampling at WWTPs. Manholes are potentially dangerous, confined space locations that require professional occupational health and safety supervision for sampling. These sites are often in public spaces (roadways) that make security of samplers a problem. Difficulties also arise in winter with factors such as: freezing, low or intermittent flow as spatial granularity increases (e.g., wastewater outflow from a single building), presence of materials flushed into sewers that can clog samplers etc. Passive samplers can block smaller sewer pipes or be lost to the monitoring system if not anchored properly

6.2.10. Reticence to participate by some municipalities

In a number of cases, municipal or institutional personnel have been reticent about allowing sampling for the purposes of wastewater surveillance for SARS-CoV-2 RNA to be performed on their premises. Reasons have differed but having a clear level of support for this activity by municipal, provincial/territorial and federal governments might assist in recruiting such sites.

6.2.11. High levels of variability in quantitative RNA signals within and between sites

Wastewater SARS-CoV-2 data seen to date, within Canada and internationally, show a high level of variability from one day to the next and between sites, even when analyzed by the same laboratory. Trends are generally discerned by using three to seven day rolling averages to smooth out the day-to-day fluctuations. To date there is not a clear understanding of what are the most important factors driving the observed variability.

6.2.12. Lack of a coherent strategy for sampling location selection

Sampling at a WWTP is generally and successfully done at the entrance to primary treatment or using primary sludge. Likewise, choosing municipalities according to population coverage is reasonably logical. Beyond that, choosing sampling sites for sampling within a sewer network or to characterize institutions has been more challenging to rationalize. Sampling within building sewers will raise even more challenges.

7. Emerging opportunities and research needs

7.1. Expanding public health applications beyond COVID-19

Besides specific improvements related to detection and use of SARS-CoV-2, the overall experience gained with applying these techniques to the current pandemic should be expanded to cover other pathogens and biomarkers of health state targets. There is much broader and extensive beneficial public health potential, i.e., multiplexing known variants of concern with other pathogens (respiratory viruses, enteric pathogens, antimicrobial resistance determinants, other health biomarkers). Continued development of methods that are easily adapted to monitor new variants of concern (e.g., lower cost sequencing tools, rapid on-site development of targeted assays, mass spectrometry screening tools, etc.) will help to identify more cost-effective approaches to monitoring changing public health targets.

There is a need to better understand the degree to which communities are protected from disease. More direct biomarkers of COVID-19 immunity (e.g., antibodies) are normally surveyed clinically but these population surveys are logistically complex, expensive, and have turn-around times

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measured in weeks and months. COVID-19 immunity biomarkers might be quantifiable in wastewater matrices. Tracking these together with the etiological agent has the potential to identify communities that are under-protected in real-time (e.g., due to waning vaccine efficacy). Non-targeted or suspect screening analysis of organic small molecules in wastewater could also assist in identifying biomarkers that correlate with measures of immunity, disease severity, or other public health metrics of interest. Proteomics analysis could offer similar insights into potential measures of disease metrics beyond case counts.

7.2. Improving analytical methodology

The full potential of wastewater surveillance for pathogens to provide actionable information to public health personnel demands achieving the highest possible consistency of procedures to promote confidence in the accuracy of the sampling and analytical techniques being employed. Academic researchers outside the health sciences may not be familiar with the concepts (Hrudey and Leiss 2003) of diagnostic sensitivity and diagnostic specificity that allow determination of positive predictive value (PPV) and negative predictive value (NPV), but these characteristics are vital to achieving confidence and uptake of results by public health professionals. There is considerable scope to develop optimized QA/QC procedures and gold standard reference materials, including digital PCR and alternate detection methods (protein markers) with enhanced diagnostic sensitivity and diagnostic specificity of detection of chosen targets. This kind of standardization activity is not the normal purview of individual academic research laboratories, so there is a need for support and coordination by appropriate standardization agencies. Without effective QA/QC procedures in place, confidence in analytical results must be low. Without some means of achieving standardization of analytical methods, or at least assessing the quantitative consequences of different analytical methods, comparing quantitative results for SARS-CoV-2 RNA in wastewater among different laboratories cannot be considered reliable. Demonstrable rigour akin to that required for clinical testing is needed.

7.3. Developing applications for incorporating wastewater surveillance for SARS-CoV-2 RNA into routine public health surveillance

Going forward, it is not realistic to expect that academic research institutions sustain routine public health surveillance, so the conditions for effectively establishing wastewater surveillance into institutional public health surveillance are needed. A discussion about the challenges facing this transition in the U.S. has been provided by Hoar et al. (2022). Building meaningful, effective and sustainable collaboration among public health, wastewater utility and analytical laboratory professionals is vital to this goal. A transition into routine public health surveillance does not mean that there are not many viable research questions that can and should be pursued by academic researchers. However, policy-makers need to realize that academic research always requires sources of external funding. The Canadian national research granting councils maintain their largest and most accessible sources of funding for "*discovery*" research. Research activities in support of "*surveillance*" are typically viewed by grant selection panels as a lower, if not entirely excluded, priority. Fortunately, under the exceptionally serious circumstances that arose in 2020 with the COVID-19 pandemic, some research grants were awarded that played a major role in facilitating capacity-development for wastewater surveillance for SARS-CoV-2 RNA in Canada. Designation of applied and translational research funds in support of public health issues and questions, that would be awarded using equal rigour to awarding of basic research grants should be considered. A natural step as we move away from monitoring as many WWTP as possible and COVID-19 becomes endemic, is to move towards sentinel surveillance sites for SARS-CoV-2 wastewater monitoring in Canada. There will be a need to determine which locations should be targeted for sentinel sites and will require great thought to determine if large communities, specific locations like airports and long-term care homes, or a combination of both would provide the most valuable data. Section 5 discusses Canada's FoodNet program and whether this existing network can be expanded to include wastewater surveillance.

7.4. Retrospective analyses of wastewater surveillance concerning public health outcomes

During the past two years of the COVID-19 pandemic, effort has had to be focused on rapid turnaround of evidence that may provide useful insight for justifying public health interventions. These priority demands have limited the scope for pursuit of in-depth and critical analysis to gain insights about what has been the effectiveness of public health intervention measures. The massive efforts that have been devoted to wastewater surveillance for SARS-CoV-2 RNA potentially provide (including archived samples) extensive research opportunities for better understanding the evolution of community transmission. Studying wastewater data may provide valuable insights for understanding the nature and character of successive waves of COVID-19 as well as seeking objective evidence concerning benefits/liabilities of non-pharmaceutical public health interventions and the role and effectiveness of vaccination. Retrospective assessment of increasingly long-term datasets will enable modelers to refine approaches to account for changes through time regarding circulating variants, population vaccination status, etc. Meaningful pursuit of such research will require full engagement by public health professionals who seek to understand the nature of wastewater surveillance data to pose research questions that can conceivably be addressed by the kind of evidence that wastewater surveillance for SARS-CoV-2 RNA can provide.

7.5. Retrospective analyses of site-specific applications

With the large number of cases where wastewater surveillance was implemented on specific sites, particularly on campuses of educational institutions, there should be a viable dataset to evaluate how many cases may have been avoided in comparison with institutions that did not practice any surveillance, provided that all of the key comparative variables (details of sampling, frequency, analysis methods, rapidity of reporting, etc.) can be addressed. Canadian researchers should be encouraged to seek collaborations nationally and internationally with institutions that invested substantially in such studies. There is also a wealth of lessons learned from distributed surveillance programs, provided adequate recognition is made to account for differences in methods deployed and local circumstances. Concerted effort to synthesize lessons learned and assess program costs is needed to harness collective learnings, to develop recommendations, and to align on protocols that may be applied in future public health responses. This effort should include a retrospective analysis of sampling and analytical protocols with a view to future guidelines for standardization.

7.6. Improved methods for normalizing wastewater strength

The most commonly used substances adopted for normalizing wastewater strength to compensate for measured concentration of SARS-CoV-2 RNA for dilution with non-sanitary sewage (i.e., stormwater, groundwater infiltration, non-sanitary institutional water use that is unrelated to the sources of faecal excretion) have been PMMoV, crAssphage and indicators like artificial sweeteners (e.g., acesulfame). PMMoV is commonly found in human faeces and is easily measured, but it is inherently a measure of dietary intake as well as the prevalence and concentration of PMMoV in vegetables which can change according to the origin of such vegetables in local food supplies. These factors do not detract from PMMoV (and acesulfame) being useful indicators of the wastewater impact on natural waters receiving wastewater discharges. Usage of PMMoV for this purpose has become prominent. However, the dietary rather than metabolic link of PMMoV and artificial sweeteners to human faeces undermines

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their potential value for normalizing the dilution of the sanitary sewage component of wastewater. Upon a review, the indicator chemicals most closely tied to faecal content of sewage are the faecal sterols and bile acids. As well, craAssphage, being a virus that infects the common faecal bacteria *Bacteroides* has been proposed by some as a basis for normalizing wastewater data. Because it is functionally tied to faecal bacterial content and is not tied to diet, it may be more conceptually similar to the faecal sterols and bile acids. Different indicators of faecal strength may also be affected differently by inhibiting substances present in wastewater. The performance of suites of indicators should be evaluated under broad contexts (e.g., in facilities where local industrial or agricultural discharges represent a larger proportion of the flows). The matter of non-faecal sources of SARS-CoV-2 (e.g., sputum) in wastewater is discussed next.

7.7. Quantitative evaluation of SARS-CoV-2 load and dynamics from sputum vs. faeces

The prospects that sputum, which is known to contain high concentrations of SARS-CoV-2 in active cases of COVID-19, being an important contributor to wastewater concentrations needs to be understood better. Knowledge of what relative contributions sputum can make to wastewater, considering the expectations of sputum having a higher content SARS-CoV-2 RNA per unit mass than faeces, needs to be better understood. There are also likely to be substantial variations in this contribution from household to household which may also be subject to cultural factors. All sources, human and non-human, of emerging pathogens ending up in wastewater samples for surveillance must be considered.

7.8. Attempts at estimating disease prevalence from wastewater require

Shedding Rates Wastewater data for any pathogen will be precluded from producing meaningfully accurate estimates of disease prevalence without site-specific knowledge about the time course of pathogen shedding and quantitative estimates of faecal (and potentially sputum for SARS-CoV-2 RNA) shedding rates per person. Likewise, there is no current knowledge about any substantial differences in shedding behaviour for VOCs and for any impact that vaccination has upon these factors. The consensus among current investigators using wastewater surveillance is likely that these gaps in knowledge are too substantial and variable to provide much hope that wastewater surveillance for SARS-CoV-2 will be able to make very precise, accurate predictions of actual COVID-19 prevalence in a monitored population. Yet evolving and adaptable modelling approaches that account for co-variates and utilize complementary public health data (i.e., case counts) for calibration can help account for uncertainties in faecal shedding and variations in local context. As waves of more readily transmissible VOCs have overwhelmed clinical testing capacity and more individual testing is being done in the home with identified cases much less likely to be reported unless symptoms become serious enough to cause affected individuals to seek healthcare, accurate evidence of COVID-19 case prevalence is weakened and wastewater data becomes increasingly valuable.

7.9. Review, evaluation, and development of various models proposed for using wastewater surveillance

Notwithstanding the many unknown input values for critical variables, there have been a number of excellent attempts to model important epidemiologic parameters using wastewater surveillance for SARS-CoV-2 RNA in combination with clinical case data. Given the many unknowns involved, it is likely that efforts to build models with some mechanistic structure that incorporate other environmental, demographic, and public health data sources will be more generally successful and useful than non-specific, data fitting models. Effective model development will require meaningful,

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interdisciplinary collaboration to ensure that models for this topic can deal with the quote attributed to famous statistician George E.P. Box (Box et al. 2005), "*All models are essentially wrong, but some are useful.*" The message intended by this quote was that all models are inherently a simplification of reality and therefore "*wrong*", but a good model will be one that can represent and predict outcomes for parameters that matter in sufficient detail to be useful.

7.10. Improving communication and interaction between water utilities and public health

Wastewater surveillance necessarily starts with investigators having access to the WWTPs and (or) the sewer collection system. The remarkable level of adoption of wastewater surveillance for SARS-CoV-2 in Canada is a tribute to laudable levels of cooperation and collaboration among the numerous and normally disparate parties involved. Some of the success has depended on commitment and investment by larger utilities to encourage cooperation from smaller ones. There is scope for documenting success stories and translating these actions into future programs. In particular, the wastewater utility people who "*know their system*" should be encouraged, wherever their role is not fully appreciated, to recognize that utilities are also guardians of public and environmental health. The lack of understanding may be a problem with utility owners, whether municipal governments or private corporations, who may regard a utility as being like any other business or service. Utility experts need to be thinking about how they can apply and share their knowledge of their system(s) to help other stakeholders (i.e., public health officials and researchers) to ultimately benefit their customers and public-at-large.

7.11. Value for informing the public for personal risk management

Those who have been involved in wastewater surveillance programs and who regularly follow the dashboards for their region have been able to form judgements about the state of the pandemic in their region. That information has been useful for informing personal risk management decisions, such as mask-wearing and engagement with larger groups. That said, most of the design of public-facing communications has had to be based both on experience and intuition. There is certainly some scope for well-focussed social science research to gauge and evaluate public perceptions about the use of wastewater surveillance so that dashboards and other communication mechanisms can be improved to maximize their public use and benefit.

7.12. Development and validation of new methods for surveillance of travel

The remarkable rapidity with which the COVID-19 pandemic engaged the world is a reflection of unprecedented levels of human travel around the globe. Recognition of this reality led to imposition by most countries of some form of international travel ban, largely without the benefit of useful evidence to guide or amend such policies. There has been limited work to evaluate the utility of monitoring wastewater from passenger aircraft, these efforts have been resource-intensive and they provide low confidence that they are able represent all passengers on a given aircraft. Once it became known that SARS-CoV-2 is efficiently transmitted via fine aerosols, the security of air travel has relied upon the high degree of cabin air circulation in a vertical direction through high efficiency particulate air (HEPA) filters capable of removing such fine particles. This reality raises the prospect of how feasible it would be to analyze onboard aircraft HEPA filters to evaluate the loading of SARS-CoV-2 captured on a given flight although adequate detectability would need to be established experimentally. Such technology would ensure that all persons breathing on an aircraft would be sampled to some degree during a flight. As complementary environmental surveillance modalities such as

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HEPA filter monitoring are evaluated at smaller spatial scales, ethical considerations for human participation and consent are critical.

7.13. Applications to vulnerable communities

COVID-19 has posed a higher threat to a variety of communities including remote and otherwise vulnerable communities, such as some First Nations communities. There is scope for meaningful research to address how overcoming the challenges to implement wastewater surveillance in these circumstances can be used to provide broader insights into the delivery of healthcare and public health services, such as safe drinking water in these situations.

7.14. Equitable and representative sampling designs

There is a need for equity to be considered when determining locations for environmental surveillance. Considering the potential value of broad and routine wastewater surveillance to provide meaningful public health data, design of representative sampling networks should consider ways to reduce inequities in access to public health data. Research should address to what extent comprehensive wastewater surveillance is needed and how an environmental surveillance program can ensure equitable access to public health data. An equity framework can also be used to guide roll-out of new surveillance programs, providing targeted program funding where needs are greatest. Analysis of the costs to implement sampling designs should consider relative access the public has to equivalent data sources, historical resource constraints and inequities, and added costs associated with program implementation in different regions (e.g., implementation in rural areas is more expensive per person than in urban areas). Research into how to reduce current limitations of sampling and analysis may help to make wastewater surveillance more feasible for most communities in the future.

7.15. Impact of community water use

Community water use practices will affect the degree of dilution of SARS-CoV-2 RNA in wastewater which may dictate how detectable in wastewater the signal from those shedding the virus will be. This may include seasonal issues in wastewater quality or quantity that may affect viral detection. These differences make quantitative comparisons between communities difficult, even if being monitored by the same laboratory. There is a need to validate approaches to better standardize how SARS-CoV-2 signals are modified/normalized to address differences in the sewersheds so that comparisons can be reliably made across site or regions

7.16. Creation of a framework for the use of wastewater surveillance results

There is a need for the creation of a framework to support public health practice as it relates to SARS-CoV-2 wastewater surveillance. This should establish common operational policies, data standards, and reporting processes that would support on-going engagement of public health and the translation of data into action.

8. Conclusions and recommendations

8.1. Conclusions

Evaluation of wastewater surveillance should be performed with full appreciation of the context of COVID-19 being the first truly global pandemic in a century. This historic reality is combined with the realization that uncertainty of knowledge about the pandemic and its evolution has been enormous, notwithstanding the remarkable speed with which the infective agent (SARS-CoV-2) was identified and genetically coded. Although medical analytical procedures can be extremely

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informative, evidence about the effectiveness of non-pharmaceutical interventions (NPI) for public health measures to reduce transmission of COVID-19 has been very uncertain. Assessment of the merits and limitations of wastewater surveillance for providing valuable evidence for informing public health intervention policies should be judged in the foregoing context, not against a hypothetical, non-existent body of robust evidence and certain knowledge.

Experts from all relevant disciplines involved in wastewater surveillance for SARS-CoV-2 RNA can debate details of how useful aspects of such surveillance have been for generating evidence about public health relevant understanding of the COVID-19 pandemic. However, at least across most of Canada during the Omicron wave of late 2021 and as public pressure has grown for reducing public health restrictions to varying degrees, wastewater surveillance for SARS-CoV-2 RNA has provided an objective and independent window on the persistence of COVID-19 infection as indicated by virus-shedding in the population. Many Canadian locations where wastewater surveillance became well-established and trust between laboratory investigators and public health decision-makers was developed were able to make effective use of wastewater surveillance evidence to reveal apparent local trends in community COVID-19 infection. The value of such insights has only grown in response to a decline in the evidence from clinical testing that occurred with increasing reliance on home testing and reductions in reportable clinical testing

Based on evidence to date, prospects for understanding what wastewater evidence of virus shedding in a population is able to predict about objective public health indicators like hospitalizations, intensive care cases and deaths is promising. Deeper interpretation of and reliance on wastewater evidence remains a work in progress. At the very minimum, this evidence, where it is publicly accessible, has provided motivated individual Canadians with insights that they can use to guide their individual risk management decisions like mask-wearing, physical distancing, and public activity choices. With more comprehensive implementation, routine wastewater surveillance data appears to be able to inform public health interventions and public health communications, to provide signals of changing public health conditions that may require new resources (e.g., laboratory capacity), direct clinical testing resources towards regions where they are needed most within a jurisdiction, track emergence of variants of concern (VOCs), and to fill gaps in public health data more broadly for indicators of a future range of disease agents.

As the COVID-19 pandemic progresses with subsequent waves of infection caused by VOCs, extent and effectiveness of vaccination and other measures, the more apparent it has become that insights from wastewater surveillance for SARS-CoV-2 have provided evidence of these waves of infection that could not have been obtained as rapidly and extensively as has been possible through wastewater surveillance. This approach cannot replace individual clinical testing because individual-specific knowledge will always be necessary to guide medical care for individual patients. However, the potential for rapidly and cost-effectively providing evidence about the occurrence of SARS-CoV-2 excretion within the population being served, including the dynamics of VOC infections, has been clearly demonstrated and achieved with evident cost effectiveness. Although the individual analysis cost of a wastewater sample may be marginally higher than the analysis cost of a clinical sample, a wastewater sample represents the whole community that can include hundreds to many thousands of symptomatic or asymptotic individuals that would each require separate clinical samples.

Canadians and their governments (Provincial and Federal) should understand that the rapid pace of the achievements documented in this report were not accomplished because of any centralized oversight or prior, high level, pandemic response plan. Most of what was achieved in the first six to nine months of the pandemic (i.e., in 2020) relied on Canadian researchers who were able to apply their prior knowledge, analytical capacity and international collaboration to initiate pilot, proof-of-concept studies to demonstrate what could be done. In most cases, these initiatives had to be funded by

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creatively diverting resources from other sources and employing a high level of volunteer time and effort to initiate these pilot programs. An unprecedented level of national and international collaboration among researchers allowed them to share experiences and fine tune procedures in their own laboratories to be able to generate useful results. In many cases, these researchers had not previously collaborated. While committing personal time towards establishing capability, most researchers faced a challenge with convincing public health decision-makers and governments that wastewater surveillance for SARS-CoV-2 was worthy of investigation, let alone commitment of longer-term funding.

Those who believe that research funding needs are adequately met by provincial and national research grant-funding agencies need to understand that most competitive public research dollars are dedicated to discovery (basic rather than applied) research. Because surveillance is clearly a scientific activity—one that research grant selection panels may predictably regard as being a government responsibility—principal investigators often encounter challenges securing funding for research related to surveillance. If Canadian governments wish to establish wastewater surveillance as a pillar of the public health system, they must facilitate sustained investments into the necessary research, infrastructure, and trained personnel.

The need for such sustained investments in public health has long been recognized. Almost 20 years ago, Canada undertook a major review of its public health infrastructure (HC 2003). That review led to the creation of the Public Health Agency of Canada, in the aftermath of perceived Canadian failures to manage the first SARS epidemic effectively in 2003. Section 5 of that report recommends in some detail the need to improve Canada's capability to perform surveillance of communicable (infectious) diseases. Those recommendations remain applicable in direction, if not in similar scope, for addressing the aftermath of the COVID-19 pandemic in Canada. Wastewater surveillance for SARS–CoV-2 along with wastewater surveillance for other disease-causing agents has demonstrated sufficient capability to be included in planning for improvement of Canada's public health surveillance infrastructure. We provide the following recommendations to help guide such plans.

8.2. Recommendations

1. Capture useful lessons from wastewater surveillance for SARS-CoV-2.

As Canada considers how to integrate wastewater surveillance into its public health ecosystem, it can draw on extensive and diverse expertise that has been accumulated from deploying wastewater surveillance for SARS-CoV-2 RNA. Our report seeks to capture that experience and expertise while it is still evolving, but it was an entirely volunteer initiative for the Royal Society of Canada, with some limited support staff resources generously provided by the Canadian Water Network. Future progress could be achieved by identifying an appropriate receptor for a targeted, analytical, systematic review. The Council of Chief Medical Officers of Health (CCMOH), which provides a national forum for federal, provincial, territorial and First Nations public health decision-makers to exchange ideas and establish public health policy would be an appropriate policy body to consider future roles for wastewater-focused public health surveillance in Canada. The review should constitute a well-resourced investigation (including with professional support staff) to gather and analyze relevant data with an eye towards conclusively identifying what did and did not work with respect to wastewater surveillance of SARS-CoV-2 and determining how Canada can build on experience to use wastewater surveillance most effectively in the future. Our report should provide a running start for an exercise, fully accountable to CCMOH, that will ensure development and adoption of a fully informed and durable surveillance policy going forward.

Canadian experience with wastewater surveillance for SARS-CoV-2 RNA has produced a valuable dataset that can be exploited by means of thorough retrospective, interdisciplinary analyses to achieve

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an insightful understanding of the dynamics of the successive waves of the COVID-19 pandemic in Canada. Better planning for effective public health interventions for future pandemics needs to be based on such comprehensive analyses of all sources of evidence.

2. Create structures and capacity to sustain capability and develop rapid response to future public health threats

While the societal disruption caused by the pandemic remains fresh in our collective conscience, we need to create tangible innovative capacity to deal with future threats. A public health emergency response research and development program, reporting annually to CCMOH and effectively linked into targeted programs at academic research granting councils (CIHR, NSERC, SSHRC) and into relevant international initiatives could be a successful model to build on what has been achieved with wastewater surveillance. Ultimately, support will be needed for a sustainable baseline of activity that can be rapidly expanded to meet the needs of future public health threats. Although government and commercial laboratories will be more equipped and viable to support long-term wastewater surveillance, it will be important to maintain research/academic engagement in monitoring programs with a medium term (e.g., 3–5-year period) funding mechanism for continued data collection during the current pandemic.

3. Develop frameworks for surveillance program design

Well-designed pandemic response strategies will integrate clinical surveillance and wastewater surveillance approaches in ways that are complementary. Wastewater surveillance cannot replace diagnostic testing, but it can inform deployment of clinical testing programs and prepare diagnostic laboratories for expected increases in testing loads. Given that wastewater surveillance is much less expensive on a per capita basis and is much more scalable than mass diagnostic testing for tracking broad disease trends, it offers to be a cost-effective strategy for long-term surveillance. Establishing broad and routine wastewater surveillance programs, with accepted guidelines/standards, will institutionalize knowledge gained during the COVID-19 pandemic to help maintain preparedness for future public health threats.

Elements of an ongoing Pan-Canadian Framework could include:

- Categorizing different surveillance situations and outlining approaches for defining new ones. Defining within each defined category, as well as for the specific agreed-upon collaborative purpose, the frequency and optimal sampling locations, analytical methods and approaches.
- Adopting ethical program design and review
- Developing a wastewater surveillance quality assurance program that can work across provincial jurisdictions to "*certify*" that consensus QA/QC procedures are being used
- Developing and maintaining an up-to-date list of laboratories who are "*certified*" and who can report results of this testing reliably to public health.

4. Develop frameworks for interpretation of surveillance program results

Investments in thoughtful design of wastewater-sampling schemes that optimize information gained relative to resources devoted to data collection and analysis will prove more cost effective in the long run. Optimization of methods for obtaining, organizing, analyzing, and presenting data is needed to gain the most value from wastewater surveillance. Public-private partnerships that engage and leverage expertise in the private sector could provide value. Working together with international bodies to optimize interpretation of surveillance data could include:

- Defining steps for interpreting results
- Defining action thresholds for differing circumstances with appropriate cautions about uncertainty
- Defining sampling frequency appropriate to specific applications such as early warning or trend definition
- Standardizing results reporting
- Defining who needs to receive results
- Determining where data will be stored and who needs to have access
- Defining links to clinical data including policies regarding clinical testing coverage
- Continuing development of public-facing dash boards with focus group evaluation and surveys to determine effectiveness of content and design

5. Maintain and promote academic partnerships and communication networks that will help identify new opportunities and threats.

Researchers in Canada and internationally continue to develop and apply advanced analytical techniques that will broaden the public health value of wastewater surveillance. For instance, genomic sequencing of wastewater can potentially provide information on the introduction of new viral strains in a region before those strains are detected by clinical sequencing in that jurisdiction. Similarly, wastewater samples can, in principle, also deliver evidence of novel genetic sequences that have not yet been identified. Developing PCR assays diagnostic for emerging strains depends on this genomic information. In this regard, VOC surveillance in Canada has benefited greatly from inter-provincial collaboration on PCR assays developed in one province which have been shared with surveillance teams in other provinces. Regular communication among academic researchers, wastewater surveillance laboratories, epidemiologists, and public-health officials will facilitate pathways for novel wastewater findings to inform broader policy responses and valuable changes in scope and approach. Tangible support for such a network would increase the likelihood of it being sustained.

6. Build upon existing infrastructure and programs

Wastewater treatment systems routinely collect influent samples to measure a range of physical, chemical, and biological water quality indicators. Leveraging existing sample collection processing when creating wastewater surveillance programs can reduce start-up costs and time. Investments in local capacity (e.g., public- and private-sector laboratories possessing instrumentation, personnel, and expertise) may be a cost-effective approach. Development of capacity in public health laboratories (in addition to or in collaboration with environmental laboratories) may facilitate integration of wastewater with other public health data and enable surge capacity in response to risk-driven spikes. Existing wastewater infrastructure in Canada mostly offers an opportunity to provide public health surveillance that can be made equitable and inclusive for most, including disadvantaged, communities. Canadian water and wastewater utilities have developed sophisticated expertise about occurrence of harmful agents in water over recent decades that can and should be better integrated with public health agencies.

Competing interests

The authors have declared that no competing interests exist.

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SEH, HNB, JC, AHSC, BC, RD, SD, TEG, CH, JI-R, WP, HS, MS, and CS conceived and designed the study. SEH, HNB, JC, AHSC, BC, RD, SD, TEG, CH, JI-R, WP, HS, MS, and CS performed the experiments/collected the data. SEH, HNB, JC, AHSC, BC, RD, SD, TEG, CH, JI-R, WP, HS, MS, and CS analyzed and interpreted the data. SEH, HNB, JC, AHSC, BC, RD, SD, TEG, CH, JI-R, WP, HS, MS, and CS contributed resources. SEH, HNB, JC, AHSC, BC, RD, SD, TEG, CH, JI-R, WP, HS, MS, and CS drafted or revised the manuscript.

Supplementary material

The following Supplementary Material is available with the article through the journal website at doi:10.1139/facets-2022-0148.

Supplementary Material 1

List of Abbreviations and Terminology

Allele	An allele is one of two or more versions of a nucleotide sequence (e.g., a single nucleotide variation in the SARS-CoV-2 genome).
(AS)-(RT)-(q)PCR	(Allele-specific) (reverse transcriptase) (quantitative) polymerase chain reaction. Allele-specificity can be added to RT-qPCR which is useful for detection and quantification of VOCs and VOIs.
Biomarker	A measurable substance (generally a molecule) that is (or has been) present in an organism and that can be used as an indicator of biological processes, disease processes, or pharmacologic responses to a therapy. Certain biomarkers in environmental samples can detect changes in the degree or extent of disease in a population.
Copies	RNA representing a single copy of the viral RNA.
crAssphage	Cross-assembly bacteriophage. A bacterial virus (bacteriophage) that infects *Bacteroides*, a bacterial genus found in the human intestinal tract.
CT or Ct	The number of amplification cycles using quantitative reverse transcription Polymerase Chain Reaction (qRT-PCR) technology required for the signal associated with a PCR product (i.e., the target/amplicon) to be detected above a baseline signal that would be present in the assay regardless of whether the target is present (CWN 2020b).
dd/d PCR	Digital droplet/digital polymerase chain reaction. A newer iteration of qPCR that relies on partitioning of the sample such that large numbers of small PCR reactions are carried out in parallel that allows more precise calculation of absolute copies of starting material. Analogous to RT-qPCR, RT-dd/dPCR denotes the iteration of the assay used to quantify RNA in a sample.
DNA	Deoxyribonucleic acid. The molecule that carries genetic information for the development and functioning of an organism. DNA is made of two covalently linked polymers of nucleic acid that wind around each other to resemble a twisted ladder — a shape known as a double helix (CWN 2020b).

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gBlock	A synthetic DNA double helix used as a reference material in q/dPCR assays.
Incidence	The number of new cases of illness commencing, or of persons falling ill, during a given period in a specified population (Porta 2008).
NPI	Non-pharmaceutical Interventions refer to measures such as quarantine, physical distancing, mask-wearing, avoidance of crowds, etc, that do not involved reliance of administration of medicines, as elaborated in **Table 1**, Section 2.2
PCR Inhibition	Inhibitory substances may be present that impede or prevent PCR from running efficiently or effectively, ultimately resulting in delayed Ct quantification (higher Ct) for the actual target of the analysis. Inhibition effects can be monitored by comparing the number of cycles required for detecting a target in a spiked sample matrix compared to that of a distilled water control spiked at the same concentration. Alternatively, inhibition can be inferred if non-linearity of calculated copy number is observed with sample dilution (i.e, no PCR inhibition is present if a sample diluted by ½ results in half the calculated copy number) (CWN 2020b).
Prevalence	A measure of disease occurrence: the total number of individuals who have an attribute or disease at a particular time (Porta 2008)
Positivity Rate	The percentage of diagnostic tests of a given population tested who test positive for the infection or illness under study (e.g., SARS-CoV-2).
Reproductive Number	The basic reproductive number (R0) is used to measure the transmission potential of a disease. It is the average number of secondary infections produced by a typical case of an infection in a population where everyone is susceptible. The effective reproductive number (R, Rt or Re) is the average number of secondary cases per infectious case in a population made up of both susceptible (e.g., unvaccinated) and non-susceptible (e.g., vaccinated) hosts. If R>1, the number of cases will increase, such as at the start of an epidemic. Where R=1, the disease is endemic, and where R<1 there will be a decline in the number of cases. healthknowledge.org.uk/public-health-textbook/research-methods/1a-epidemiology/epidemic-theory
N1, N2	Refers to the nucleotide sequences that are commonly amplified by PCR in both clinical and wastewater diagnostic testing for the presence of SARS-CoV-2. It can also refer to the US CDC-designed N1 and N2 PCR assays which are the gold standard diagnostic assays that target the sequences of the nucleocapsid gene of the virus.
N-gene	A gene that is present in SARS-CoV-2 genomic RNA and which encodes the nucleocapsid protein that surrounds and protects the genomic RNA present in each infectious viral particle.
Normalization	Transformation of the raw SARS-CoV-2 RNA PCR signal to account for systematic variability. Usually, by dividing by another measured factor (e.g., faecal indicator such as PMMoV, flow, TSS, etc).
NPV	Negative predictive value (NPV) is the conditional probability: Given that if there is no true case of COVID-19 that the wastewater will correctly report no detectable signal for SARS-CoV-2 RNA.
NRT	No-RT control. Used to ensure the observed PCR signal is RNA-dependent (generally used to troubleshoot contamination issues).

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NTC	No-template control. Used to detect RNA or DNA contamination in the PCR reaction.
Outliers	Samples that are statistically not consistent with the other data
PCR	Polymerase chain reaction - The process through which genetic material (DNA) can be amplified exponentially through multiple cycles of denaturing, annealing and extension that allows the DNA to self-replicate.
PCR Efficiency	One of several performance measures and quality controls for qPCR assays. A low efficiency assay risks under- or over-estimating copies of starting material.
PMMoV	Pepper Mild Mottle Virus, a plant virus that infects peppers and other vegeables and is found in feces of humans because it is in the human diet
PPV	Positive predictive value (PPV) is the conditional probability given that if there is a true case of COVID-19 that the wastewater will correctly report a positive signal for SARS-CoV-2 RNA.
QA/QC	Quality assurance/quality control: A component of quality management focused on providing confidence that specific quality requirements will be fulfilled, and the fulfillment of the requirements specified; relates to how a process is performed to ensure quality requirements are met and the subsequent inspection aspect of quality management.
RNA	Ribonucleic acid. A biological molecule that has structural similarities to DNA and serves to direct and enable gene expression.
RT	Reverse transcriptase – see explanation below for RT-qPCR
RT-qPCR	PCR is a technology platform by which genetic material (DNA) can be amplified exponentially through multiple cycles of denaturing, annealing and extension that allows the DNA to self-replicate. qPCR is an iteration that allows for absolute measurement of copies of starting material. "RT-qPCR" refers to an iteration that includes reverse transcriptase and can thereby measure RNA as a starting material. healthknowledge.org.uk/public-health-textbook/research-methods/1a-epidemiology/epidemic-theory
Sensitivity	The proportion of truly diseased people in a population who are identified as diseased by a screening test. Also known as a true positive rate. For an analytical procedure, sensitivity is the conditional probability: that when the target analyte is present, the screening test will detect the analyte's presence.
Sewershed	A segment of a sewer network defined that has the sewers draining into it defined sufficiently well that wastewater samples taken from it can be assumed to represent a defined segment of the sewer drainage system
Specificity	The proportion of truly non-diseased people in a population who are identified as non-diseased with a screening test. Also known as a true negative rate. For an analytical procedure, specificity is the conditional probability: that when the target analyte is absent, the procedure will report it as non-detectable.
Surrogate	A spike of a related virus (i.e., HCoV-229E or MHV) used as a whole process control
VOC	Variant of concern: A SARS-CoV-2 variant that meets the definition of a VOI (see below) and, through a comparative assessment, has been demonstrated to be associated with one or more of the following changes at a degree of global public health significance: Increase in transmissibility or detrimental change in COVID-19 epidemiology; OR Increase in virulence

	or change in clinical disease presentation; OR Decrease in effectiveness of public health and social measures or available diagnostics, vaccines, therapeutics (WHO 2022b)
VOI	Variant of interest: A SARS-CoV-2 variant : with genetic changes that are predicted or known to affect virus characteristics such as transmissibility, disease severity, immune escape, diagnostic or therapeutic escape; AND Identified to cause significant community transmission or multiple COVID-19 clusters, in multiple countries with increasing relative prevalence alongside increasing number of cases over time, or other apparent epidemiological impacts to suggest an emerging risk to global public health (WHO 2022b).
WWTP	wastewater (sewage) treatment plant

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