



SARS-CoV-2 known and unknowns, implications for the water sector and wastewater-based epidemiology to support national responses worldwide: early review of global experiences with the COVID-19 pandemic

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ABSTRACT

Wastewater surveillance of pathogens may be a useful tool to help determine whether clinical surveillance of disease is effective or inadequate due to under-reporting and under-detection. In addition, tracking of pathogen concentrations over time could potentially provide a measure of the effectiveness of public health control measures and the impact of the gradual relaxation of these controls. Analysis of wastewater using quantitative molecular methods offers a real-time measure of infections in the community, and thus is expected to provide a more sensitive and rapid indication of changes in infection rates before such effects become detectable by clinical health surveillance. Models may help to back-calculate wastewater prevalence to population prevalence or to correct pathogen counts for wastewater catchment-specific and temporal effects. They may also help to design the wastewater sampling strategy. This article provides a brief summary of the history of pathogen wastewater surveillance to help set the context for the SARS-CoV-2 wastewater-based epidemiology (WBE) programmes currently being undertaken globally.

Key words | COVID-19 pandemic, modelling and back-calculation, national response, SARS-CoV-2, wastewater sampling/surveillance, wastewater-based epidemiology (WBE)

HIGHLIGHTS

- A brief history of pathogen wastewater surveillance.
- Context for current SARS-CoV-2 wastewater-based epidemiology (WBE) programmes.
- Recommendations on research and investigation priorities.
- Wide potential of modelling is presented together with remaining knowledge gaps.

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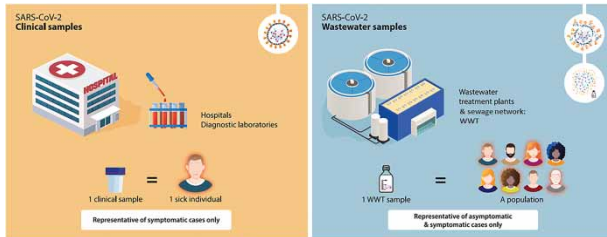
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GRAPHICAL ABSTRACT



INTRODUCTION

Coronaviruses are a large and diverse family of viruses. The name ‘corona’ comes from their round appearance and the spikes on their surface that can be likened to a solar corona (Figure 1(a)). Coronaviruses are enveloped, which means that there is a lipid membrane envelope around the surface of the virus, while ‘naked’ viruses do not have this. The lipid envelope makes coronaviruses more fragile than other viruses (Walls *et al.* 2020) and is hence relevant to understanding their resistance to disinfection, as well as their environmental persistence and transmission. The lipidic structure holds the membrane (M), envelope (E) and spike (S) proteins together, with the spike protein protruding around

the envelope (Figure 1(a)). Since the spike protein is responsible for the connection with the host cells in humans, the virus loses its infectivity if the lipid envelope is destroyed (Figure 1(b)) (Walls *et al.* 2020; Wu *et al.* 2020a, 2020b, 2020c).

Another characteristic relevant to their sensitivity to UV disinfection is that the genome is made up of single-stranded RNA (Figure 1(a)). When looking for the virus in wastewater, scientists look for the genetic information that codes for the key proteins in its structure. Eurosurveillance and Centers for Disease Control and Prevention have provided references listing commonly used primers for the detection of SARS-CoV-2 virus. The Eurosurveillance E primers target regions

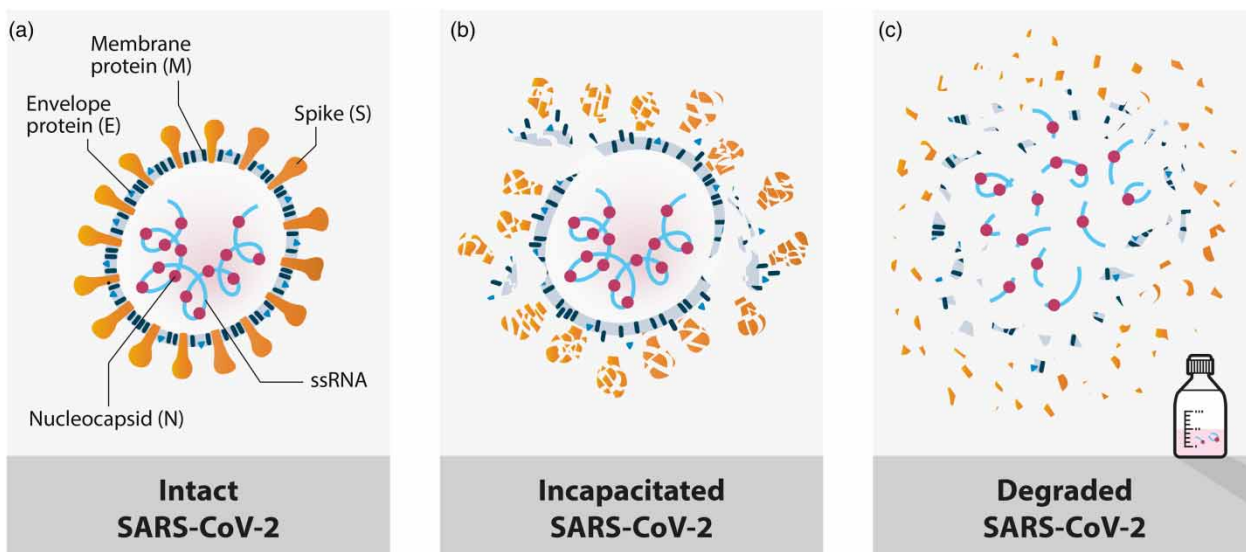


Figure 1 | SARS-CoV-2 (a) key structure includes S, N, M, E and RNA; (b) incapacitation process and (c) degradation. The subsequent analysis of SARS-CoV-2 RNA (typically after conversion to DNA) may follow RNA extraction from intact, incapacitated or degraded virus and combinations thereof.

of RNA that code for the envelope (E), while the CDC N1 and N2 primers detect fragments of RNA that code for the nucleocapsid (N) protein (Figure 1(a)).

The term HCoV is used to represent human coronaviruses. As most human coronavirus infections cause mild symptoms, they may even go unnoticed. Since the beginning of the 21st century, three coronaviruses have crossed the species barrier to cause deadly pneumonia in humans (Drosten *et al.* 2003; Zaki *et al.* 2012). These are:

- Severe Acute Respiratory Syndrome (SARS-nCoV);
- Middle-East Respiratory Syndrome (MERS); and now
- Severe Acute Respiratory Syndrome 2 (SARS-CoV-2).

Six types of human coronavirus were identified before 2019, the seventh (SARS-CoV-2) was revealed after testing of fluid from a patient's lungs on 3 January 2020, following reports of several patients presenting with a strange pneumonia in November and December 2019 in Wuhan Province, China. The first publications about this virus referred to it as the 'novel coronavirus', and the name 2019-nCoV was used to denote it. Since more has become known about the virus, it has been designated SARS-CoV-2 and is associated with the current pandemic of atypical pneumonia (the disease is designated as COVID-19). SARS-CoV-2 is transmitted from person-to-person via the respiratory system through sneezing, coughing and secretions, and by contact with contaminated surfaces (Huang *et al.* 2020; Zhu *et al.* 2020).

Coronaviruses belong to the family of coronaviridae, and the severe acute respiratory syndrome-related (SARS) coronavirus species includes the SARS-CoV-2 strain. This coronavirus is the newest of the family of coronaviruses associated with human infections that are grouped into the beta-CoV genus, with over 70% genetic similarity to SARS-nCoV (Gorbalenya *et al.* 2020).

Wastewater (sewage) monitoring and wastewater-based epidemiology (WBE) have long been used to help inform broader infectious disease epidemiological surveillance and mitigation efforts, such as the Global Polio Eradication Initiative (Humayun *et al.* 2014). Over time, researchers have pooled their expertise to help develop recommendations for standardised methods for testing wastewater for poliovirus, and reviews have compared such methods (Matrajt *et al.* 2018). Environmental surveillance has also

been used and recommended for other infections, such as typhoid (WHO 2018), as well as for antimicrobial resistance (Hendriksen *et al.* 2019), with modelling techniques used to assist both the design and interpretation of those efforts (Wang *et al.* 2020a, 2020b). WBE is also commonly used in the surveillance of licit and illicit drugs and various chemical contaminants which may impact human health (Choi *et al.* 2018).

Evidence of pathogen concentrations in wastewater has been published and is now part of long-term routine monitoring programmes carried out by water utilities, and which utilise accredited laboratories for these analyses. These studies provide the basis for understanding wastewater treatment requirements for safe recycling of water and protection of environmental health. For instance, Australia recently collated and updated the national body of wastewater pathogen monitoring data to revise the Australian Guidelines for Water Recycling (NRMCC *et al.* 2006; Deere & Khan 2016). This provides an example of the value of maintaining an ongoing monitoring programme for pathogens in wastewater to yield a reliable evidence base for public health decision-making.

It is important to note that wastewater testing has been used for long-term monitoring of norovirus and human mastadenovirus, for example, in Sydney and Melbourne (Lun *et al.* 2018, 2019; Fumian *et al.* 2019). At its most basic, this work helps reveal pathogen concentrations and their evolution over time and space; while genetic sequencing permits further characterisation of genetic types, pathogen evolution and the spread of new viral genotypes in human populations. The objectives of this paper are to (1) provide an early review of SARS-CoV-2 'knowns and unknowns', their implications for the water sector and the ability of WBE to support national responses worldwide and (2) share initial lessons from the design and implementation of a national SARS-CoV-2 wastewater monitoring programme, to help inform future research directions.

SIGNIFICANCE TO THE WATER SECTOR

Environmental surveillance implies testing wastewater or other environmental samples for the presence of a virus

or other microorganism or fractions of its structure. On the other hand, clinical surveillance includes systematic collection, analysis and interpretation of direct (e.g. throat swab) and indirect (e.g. symptom observation) health-related data (Ahmed *et al.* 2020; Huang *et al.* 2020). Combinations of both surveillance methods have proven to be useful for the planning, implementation and evaluation of public health practices (Kroiss *et al.* 2018).

In terms of viruses that are significant for the water sector, it is well-established that viruses are commonly found in wastewater, and hence routine testing for viruses often occurs. There are two main groups of viruses commonly found in wastewater. Firstly, viruses that are more resistant to natural and engineered inactivation processes (e.g. UV from sunlight and UV treatment systems, respectively) and have an external protein capsule, but not a lipid envelope. These are classified as having a moderate to high health significance by the WHO and include DNA viruses such as adenovirus, as well as RNA viruses such as hepatitis A and E viruses, norovirus and other enteroviruses, including coxsackieviruses and polioviruses (WHO 2020a, 2020b). The second group of viruses found in wastewater has a lipid envelope. They are known to be more fragile, and while they can also be detected in faeces, they are not normally associated with waterborne disease transmission and outbreaks. These viruses include the influenza virus, coronaviruses like the virus that is now the focus of global attention, SARS-CoV-2, and the herpes virus that can be found in faeces (WHO 2020a, WHO 2020b, 2011; Gall *et al.* 2015; Water Research Australia 2020a; Xu *et al.* 2020). Notably, in the 2020 pandemic, WBE is used to understand the epidemic within the population generating the wastewater. Furthermore, WBE allows scientists and health agencies to monitor the spread of the epidemic even if a large fraction of the population is asymptomatic because this fraction also sheds the virus. However, the extent of shedding by the asymptomatic fraction of the population still requires further investigation.

While focusing on SARS-CoV-2 and the virus in water, it is worth reviewing the disease and its implications for WBE. The most common symptoms include fever, dry cough, fatigue, shortness of breath and atypical symptoms such as headache, loss of taste and smell, sputum and diarrhoea (Wu *et al.* 2020b). Fever and dry cough represent the most

typical symptoms of SARS-CoV-2 infection. The incubation period from infection to display of symptoms is estimated at between 1 and 14 days but is most often between 3 and 7 days (Huang *et al.* 2020; Wu *et al.* 2020b). Given that the symptoms are so common, and relatively mild in most cases, and that the southern hemisphere is entering into the colds and flu season, many of these symptoms are likely to be associated with other respiratory virus infections. To distinguish SARS-CoV-2 from other known viruses, laboratory diagnosis is necessary through SARS-CoV-2 testing.

A review of the stability of SARS-CoV-2 was published in the *Lancet Microbe* in April (Chin *et al.* 2020). The virus was incubated in the virus transport medium at various temperatures for up to 14 days and then tested for infectivity. SARS-CoV-2 virus infectivity was also assayed following incubation on different surfaces, exposure to varying pH values and different disinfectants. Chin *et al.* (2020) found that infectivity was still found on day 14 when the virus was incubated at 4 °C, whereas at 70 °C the virus was inactivated in 5 min. In the same article, the stability of the virus on various surfaces was tested by dropping the cultured virus onto surfaces left at room temperature (22 °C) and a relative humidity of 65%. They found that treated smooth surfaces, particularly steel and plastic, support the persistence of infective virus more than rougher surfaces such as tissue paper, wood and cloth. The virus was stable at a range of pH values (at room temperature).

Chin *et al.* (2020) also noted that the SARS-CoV-2 virus is susceptible to standard disinfection methods and was undetected after 5 min contact with household bleach at various concentrations (1:49 and 1:99 dilution ratios), ethanol (70%), povidone-iodine (7.5%), chloroxylenol (0.05%) and chlorhexidine (0.05%).

A *New England Journal of Medicine* article compared SARS-CoV-2 to the 2005 SARS-nCoV. Researchers found that like SARS-nCoV, SARS-CoV-2 also remains viable in aerosols (testing was for 3 h) (Doremalen *et al.* 2020). Although there is limited data on the survival of SARS-CoV-2 in water because they behave similarly in aerosols, similar behaviour is likely for SARS-nCoV and SARS-CoV-2 in water and wastewater. SARS-nCoV was predicted to be very stable at 4 °C in filtered tap water. SARS-nCoV was found to remain live in stools for 6 days at room temperature, and

fragments of SARS-nCoV continued to be detected in wastewater for up to 3 days, making it less stable in wastewater than polio (Gundy et al. 2009). In laboratories, identification of SARS-CoV-2 mainly includes viral isolation and viral nucleic acid detection. Accurate detection of SARS-CoV-2 RNA is of notable value. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) assays targeting small regions of the SARS-CoV-2 genome have now been developed and are routinely applied in clinical testing (Corman et al. 2020).

Understanding how the disease affects the human body, and how the virus is shed, can give key insights into the virus shedding rates in wastewater. The virus will be shed from the upper respiratory and gastrointestinal systems into wastewater (e.g. from brushing teeth, mouth washing, coughing and sneezing while bathing or showering, washing of hands or clothes, and discarding of tissues and wipes into the toilet). One important question is how much of this virus is excreted in faeces, given that viruses, including coronaviruses, are commonly shed in faeces (Wang et al. 2005; WHO 2011; Mans et al. 2014; Wang et al. 2020b). An article in the *Lancet* by Wu et al. (2020b) found excretion of the SARS-CoV-2 virus after 3 weeks in phlegm and 4 weeks in stools. The same article noted that there was no association between disease severity and the extended duration of the virus in faeces. They also reported an infectious virus in the stool samples of two patients who did not have diarrhoea. Researchers examined clinical specimens from 73 hospitalised patients infected with SARS-CoV-2. Thirty-nine patients tested positive for SARS-CoV-2 RNA in stool

samples and 17 of those patients remained positive for SARS-CoV-2 in stools after becoming negative in respiratory samples, suggesting that viral gastrointestinal infection, or at least shedding, can remain for some time after clearance of the virus in the respiratory tract (Xiao et al. 2020; Xu et al. 2020). This finding has now been repeated in various studies showing the extended duration of shedding of the virus in faecal samples. The *Lancet Gastroenterology & Hepatology* paper by Wu et al. (2020b) provides a clear picture of how long faecal shedding can last (Figure 2). Furthermore, Sethuraman et al. (2020) reported that in some cases, viral RNA can be detected in stool samples by RT-PCR at week 6 following the first positive test.

RESEARCH AND INVESTIGATION PRIORITIES

Two priorities for investigation and research have been identified by the water sector. Firstly, wastewater monitoring can provide a simple means to determine if SARS-CoV-2 is present and which subtypes predominate. Secondly, understanding the resistance of SARS-CoV-2 to water and wastewater treatment processes (noting that SARS-CoV-1 was shown to be sensitive to both chlorination and UV disinfection, and hence, SARS-CoV-2 is predicted to be readily inactivated and otherwise removed through the conventional treatment of water and wastewater, and also by the treatment processes used for the production of high-grade recycled water (Water Research Australia 2020a)).

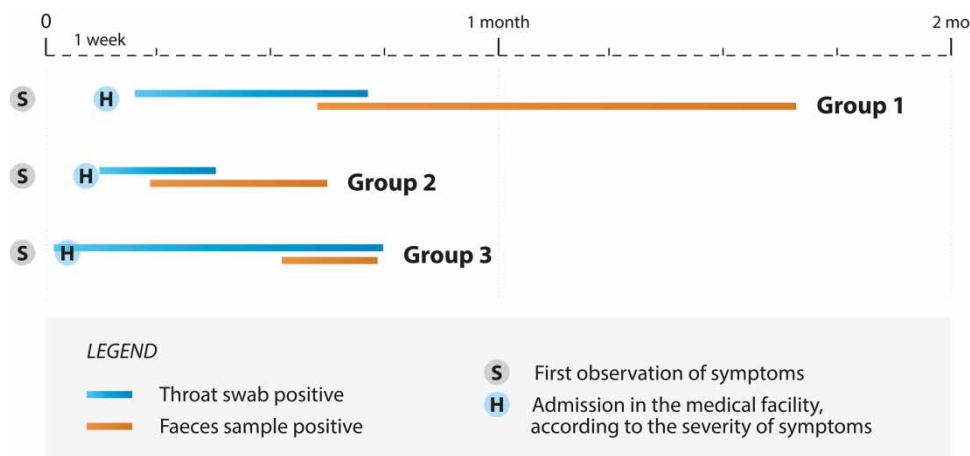


Figure 2 | The extended duration of the virus in faecal samples versus symptom observation and throat swab test results (Adapted from Wu et al. (2020b)).

Recently, leading scientific and medical journals have highlighted the potential benefits of using wastewater surveillance to help inform COVID-19 pandemic response actions (Lodder & Husman 2020; Mallapaty 2020). The objective is to determine whether the viral RNA is present in wastewater and at what concentrations (virions/L) in order to inform those managing the national public health response to the epidemic (one genome is equivalent to one virion).

As noted by Deere *et al.* (2020), virus monitoring in environmental samples (such as water and wastewater) has been practised and continually refined for over 75 years, starting with the isolation of viruses using cultivation-based methods and gradually moving to the complementary use of more rapid and sensitive molecular methods (Metcalf *et al.* 1995). These methods were refined and modernised 25 years ago, providing sensitive and rapid assays for virus concentration, extraction, purification and detection in wastewater, water and sanitary waste (Tsai *et al.* 1993). While this work has focused predominantly on human viruses transmitted by the faecal-oral route (e.g. poliovirus, rotavirus and norovirus), early results suggest that these techniques can be successfully applied to the detection of SARS-CoV-2 in wastewater (Ahmed *et al.* 2020; Lodder & Husman 2020; Medema *et al.* 2020; Wurtzer *et al.* 2020).

While testing for SARS-CoV-2 in wastewater has already been undertaken in several countries, there has not yet been a concerted effort to standardise and coordinate experimental methods and strategies, and there is no precedent for using the results of such surveillance to manage a pandemic. Currently, there is limited quantitative data on the intensity and duration of shedding of the SARS-CoV-2 virus in faeces and respiratory fluids over the course of infection, and there is no simple way to equate a measure of virions/L of wastewater to the number of infected people in the wastewater catchment from which the wastewater is collected. Therefore, it is important to appreciate that it is uncertain how useful such testing will be in providing information to those tasked with managing the COVID-19 pandemic response. At the time of writing, the sampling and testing methods are being refined, controls and quality standards being developed, and their sensitivity and specificity being understood as part of monitoring wastewater for the COVID-19 virus. In addition, the realistic scale of a sewer sampling and wastewater testing programme, particularly when laboratory reagents,

consumables, hardware and staff are in demand for clinical testing, is beginning to be understood. However, given that SARS-CoV-2 is routinely shed in faeces and other secretions that can end up in wastewater (Han *et al.* 2020; Pan *et al.* 2020; Zheng *et al.* 2020), and noting the proven value of historical environmental virus surveillance, the work is expected to provide a valuable information source to help inform COVID-19 control strategies.

At the time of writing, the current SARS-CoV-2 wastewater surveillance programmes are centred around methods that can detect the presence of viral genes. In Australia, a national wastewater monitoring project known as ColoSSoS (Collaboration on Sewage Surveillance of SARS-CoV-2) is being coordinated by Water Research Australia (Water Research Australia 2020b). In Canada, the Canadian Water Network is leading a coalition of municipalities, utilities, researchers, public health organisations and governments supporting public health decisions through wastewater surveillance for COVID-19 (Canadian Water Network 2020). These projects are linked to a global research effort managed by the US-based Water Research Foundation which is developing a coordinated approach to data collection, method development and data interpretation in order to promote best practices, save resources and accelerate progress on SARS-CoV-2 research in the water sector (Water Research Foundation 2020). Similar programmes are underway in other jurisdictions.

MODELLING, BACK-CALCULATION ISSUES AND FUTURE RESEARCH NEEDS

WBE and environmental surveillance benefit from models to back-calculate chemicals (e.g. drugs or other priority contaminants; Choi *et al.* 2018) and their usage patterns, or the prevalence of infection. Epidemiological indicators enter the sewer networks through toilets and other sources of wastewater and are transported for periods of minutes to several hours before collection at a wastewater treatment facility. Back-calculating from the observations at the end of the wastewater catchment to population-relevant information is promising but still under development. Uncertainties that may hamper decision-makers in making the right management decisions on the basis of these

back-calculations include contributing populations, excretion rates, stability of the indicators (including transformation in each unique wastewater system), sampling and sample preparation (Castiglioni *et al.* 2013). Similar to chemical fate models for integrated urban wastewater systems (Schowanek *et al.* 2000; Vezzaro *et al.* 2014), WBE models facilitate calculating the fate of these indicators from population excretion to the inlet of the wastewater treatment plant. These models are becoming increasingly powerful by including such factors as shear rates, chemical conditions (pH and dissolved oxygen) and biological reactions, even to the level of the impact of sewer biofilms (McCall *et al.* 2016).

These models are not only relevant for back-calculation to population-level indicators. During a recent international water research summit on environmental surveillance of COVID-19 indicators in wastewater catchments (Water Research Foundation 2020), three uses for environmental surveillance were identified: (i) trend detection (one direction, up- or downward), (ii) changes in trend (two directions) and (iii) assessment of community infection (tracking disease prevalence). While it was felt that current knowledge is sufficient to advance uses (i) and (ii) by supporting decision-making relating to medical and social interventions, the ultimate objective of back-calculation methods is to assess infection prevalence. For that use, considerable knowledge still needs to be gathered, especially regarding (a) shedding rates and duration, (b) links between the genetic signal and the infection prevalence and (c) fate within wastewater and how this changes with wastewater characteristics (e.g. dilution, temperature, retention time, percentage trade waste, etc.) that may vary with time and season. Models, however, may already be very helpful now for uses (i) and (ii) to normalise the genetic signals for spatial (comparing between wastewater catchments) and temporal (seasonality of fate-affecting conditions) variability in order to maximise the power of the signals obtained in supporting COVID-19 management decisions.

Finally, models have been shown to be useful in the design of environmental surveillance sampling strategies. The above complexity of relating infection rates to surveillance results makes the design of sampling strategies challenging and creates a need for mathematical modelling to guide sampling design (Wang *et al.* 2020a, 2020b). Models are now being proposed not only to design a particular sampling strategy but also

to allow strategies to be adapted to maximise the information gathered during the surveillance effort. Initial knowledge and practical constraints may mean that it is not possible to implement the most optimal sampling design, and changing disease patterns could require the sampling design to be adapted over time (Wang *et al.* 2020a, 2020b).

Note that WBE models have been used for at least the past 20 years, for example, a simulation model to evaluate poliovirus environmental surveillance efficiency was published near the turn of the century (Ranta *et al.* 2001). These models included transmission models (e.g. duration of virus shedding) and impacts of environmental factors (e.g. sewer system fate of the poliovirus) on the surveillance results and, finally, effects of sampling and laboratory analysis. Using such models, the detection probability for small outbreaks can be maximised.

IMPLEMENTATION OF AUSTRALIAN WASTEWATER SURVEILLANCE PROGRAMMES FOR SARS-COV-2

Representatives from the Water Research Australia ColoSSoS project are members of several of the working groups of the global research effort (Water Research Foundation 2020). This ensures that Australia's national approach to wastewater surveillance can be aligned to international best practice guidelines as they are developed.

The key activities of the ColoSSoS project are designed to support the health agencies' response to the current pandemic by providing reliable and robust data on the presence of SARS-CoV-2 in wastewater catchments and by sharing knowledge among the global community.

To respond quickly, Australian water utilities adopted a strongly pragmatic approach upon making the decision to undertake a SARS-CoV-2 wastewater surveillance programme. The implementation of such surveillance in the State of Victoria is illustrative of this pragmatism: sampling commenced on 1 April 2020 at the two large wastewater treatment plants that collectively treat most of Melbourne's wastewater. Victoria had just entered stage three restrictions ('lockdown') and utility and sampling staff movements were tightly controlled because of the need to ensure the ongoing provision of critical water and wastewater services. Observing the decline in the growth of case numbers in the

northern hemisphere following similar lockdown controls (so-called ‘flattening the curve’), it was recognised that the peak number of first-wave COVID-19 infections could be reached in Victoria in a matter of weeks.

In the context of this rapidly changing and highly constrained environment, the objectives for Victoria were to implement a sampling programme before peak case numbers of COVID-19 were reached and to include locations within putatively COVID-19 free wastewater catchments, and sites proximal to large metropolitan hospitals from which the wastewater effluent was expected to be more likely to contain the SARS-CoV-2 virus. The sampling of locations predicted to be relatively enriched with SARS-CoV-2 was given a high priority due to the need to validate methods using both laboratory-generated and actual field samples.

Consequently, Victoria’s initial SARS-CoV-2 sampling programme was designed to:

- Be rapidly implemented at well-established wastewater treatment plant influent and sewerage network sampling locations through grab sampling and auto-sampling without deviating significantly from existing sampling protocols. For the most part, sites were chosen to be within 200 km of Melbourne so that sample bottles could be transported to a centralised storage location (−20 °C facility), resulting in a coverage of approximately 71% of Victoria’s 6.6 million population.
- Place minimal additional burden upon utilities.

Optimisation of the sampling programme will consider the benefits of various stratified sampling designs involving increased sampling frequency and use of inline, flow-weighted autosamplers at strategic locations (Ort *et al.* 2010; Wang *et al.* 2020a, 2020b). The programme may need to run for 2 or more years and should be adapted to improved knowledge, resource availability and changes in virus epidemiology (Moore *et al.* 2020).

A number of communication needs emerged during the development of the project. These include explaining and communicating that there is no evidence that COVID-19 virus is transmitted through wastewater. Another need is communicating the costs, value and benefits of environmental surveillance to agencies leading the COVID-19

control strategies. That means communicating the differences and alignments between clinical and environmental surveillance and how the two are complementary.

CONCLUSION

Analyses to detect and quantify SARS-CoV-2 in wastewater show great promise as efficient and rapid approaches to survey both small and large populations for the presence of SARS-CoV-2 infections in the community. While these analyses are being undertaken for routine surveillance purposes, research work can be undertaken in parallel to improve the value of this approach, i.e. to enhance the sensitivity and specificity of methods, to develop more standardised protocols and to provide the means to relate results to the prevalence of SARS-CoV-2 infection in a wastewater catchment with improved confidence.

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