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Review

SARS-CoV-2 in wastewater: State of the knowledge and research needs

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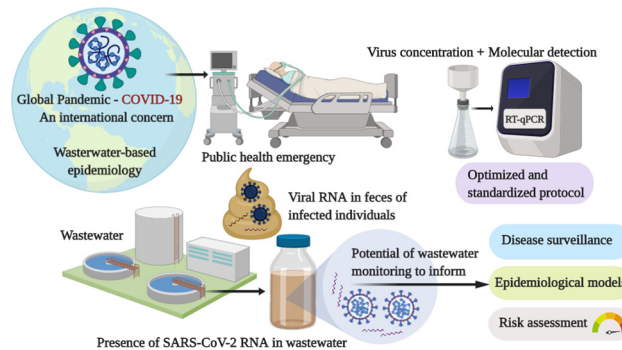
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HIGHLIGHTS

- Presence of SARS-CoV-2 RNA in wastewater has been reported.
- SARS-CoV-2 RNA in wastewater can be used to monitor COVID-19 in a community.
- Effective concentration method is needed for recovery of SARS-CoV-2 from wastewater.
- Surrogate coronavirus data help to predict survival of SARS-CoV-2 in wastewater.
- Data on the infectivity of SARS-CoV-2 in wastewater for risk assessment are limited.

GRAPHICAL ABSTRACT



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ABSTRACT

The ongoing global pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a Public Health Emergency of International Concern, which was officially declared by the World Health Organization. SARS-CoV-2 is a member of the family *Coronaviridae* that consists of a group of enveloped viruses with single-stranded RNA genome, which cause diseases ranging from common colds to acute respiratory distress syndrome. Although the major transmission routes of SARS-CoV-2 are inhalation of aerosol/droplet and person-to-person contact, currently available evidence indicates that the viral RNA is present in wastewater, suggesting the need to better understand wastewater as potential sources of epidemiological data and human health risks. Here, we review the current knowledge related to the potential of wastewater surveillance to understand the epidemiology of COVID-19, methodologies for the detection and quantification of SARS-CoV-2 in wastewater, and information relevant for human health risk assessment of SARS-CoV-2. There has been growing evidence of gastrointestinal symptoms caused by SARS-CoV-2 infections and the presence of viral RNA not only in feces of infected individuals but also in wastewater. One of the major challenges in SARS-CoV-2 detection/quantification in wastewater samples is the lack of an optimized and standardized protocol. Currently available data are also limited for conducting a quantitative microbial risk assessment (QMRA) for SARS-CoV-2 exposure pathways. However, modeling-based approaches have a potential role

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to play in reducing the impact of the ongoing COVID-19 outbreak. Furthermore, QMRA parameters obtained from previous studies on relevant respiratory viruses help to inform risk assessments of SARS-CoV-2. Our understanding on the potential role of wastewater in SARS-CoV-2 transmission is largely limited by knowledge gaps in its occurrence, persistence, and removal in wastewater. There is an urgent need for further research to establish methodologies for wastewater surveillance and understand the implications of the presence of SARS-CoV-2 in wastewater.

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1. Introduction

In December 2019, China reported an outbreak of pneumonia of unknown etiology occurring in Wuhan, Central China's Hubei Province to the World Health Organization (WHO) (WHO, 2020a). Shotgun metagenomic sequencing of bronchoalveolar lavage samples indicated that this outbreak was associated with a novel coronavirus (nCoV) (Zhu et al., 2020). The nCoV was confirmed to have 75–80% nucleotide similarity to severe acute respiratory syndrome coronavirus (SARS-CoV) (Zhu et al., 2020) and was officially designated as SARS-CoV-2 after being provisionally named as 2019-nCoV (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020). SARS-CoV-2 together with SARS-CoV belong to the species *Severe acute respiratory syndrome-related coronavirus* in the subgenus *Sarbecovirus* of the family *Coronaviridae* that consists of a group of enveloped viruses with a single-stranded, positive-sense RNA genome. SARS-CoV and SARS-CoV-2 are distantly related to Middle East respiratory syndrome coronavirus (MERS-CoV), which belongs to the species *Middle East respiratory syndrome-related coronavirus* within the genus *Betacoronavirus* (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020). SARS-CoV-2 is also distantly related to 'classical' human CoV strains (229E, OC43, NL63, and HKU1) belonging to the genus *Alphacoronavirus* or *Betacoronavirus* that have been studied since the 1960s and are estimated to cause 15 to 30% of cases of common colds worldwide (Mesel-Lemoine et al., 2012).

The disease caused by SARS-CoV-2 is referred to as coronavirus disease 2019 (COVID-19). Symptoms of COVID-19 at the onset of illness include fever, myalgia, fatigue, and dry cough, and more than half of patients developed dyspnea (Chen et al., 2020b; Guan et al., 2020; Huang et al., 2020; Wang et al., 2020a, 2020b). On March 11, 2020, WHO declared the current COVID-19 situation a global pandemic on the basis of "alarming levels of spread, severity, and inaction" (Bedford et al., 2020). The WHO then declared the outbreak of COVID-19 a Public Health Emergency of International Concern on January 31, 2020 (WHO, 2020b). As of April 26, 2020, SARS-CoV-2 has further spread to almost all countries and territories around the world with >2,724,809 confirmed cases and 187,847 confirmed deaths, according to WHO (WHO, 2020c). The case fatality rate was estimated as 5.3–8.4% for COVID-19 (Jung et al., 2020), which is lower than SARS (up to 50%) or MERS

(34.4–69.2%) (Park et al., 2018; Wang et al., 2020a, 2020b, 2020c; WHO, 2003). The basic reproduction number (R_0) of SARS-CoV-2 was estimated as 1.4–6.5 (Boldog et al., 2020; Jung et al., 2020; Liu et al., 2020; B. Tang et al., 2020; WHO, 2020c), meaning that each infected individual could transmit the virus to another 1.4–6.5 cases - comparable to that of SARS-CoV (R_0 of 2 to 5) (Lipsitch et al., 2003; Riley et al., 2003; Wallinga and Teunis, 2004).

Both viable SARS-CoV-2 and viral RNA are shed in bodily excreta, including saliva, sputum, and feces, which are subsequently disposed of in wastewater. Although it is believed that the major transmission route of this virus is inhalation via person-to-person aerosol/droplet transmission, and fomite to hand contamination, currently available evidence indicates the need for better understanding of the role of wastewater as potential sources of epidemiological data and as a factor in public health risk. In this paper, we thoroughly reviewed the current knowledge related to the potential of wastewater surveillance for understanding the epidemiology of COVID-19. Given the rapid emergence of SARS-CoV-2, previous studies on human CoVs, SARS-CoV, MERS-CoV, and surrogate viruses can help to inform predictions of the likely environmental fate and subsequent risks of SARS-CoV-2. We also identified critical research needs that will strengthen our understanding on the occurrence, persistence, and potential public health risks associated with SARS-CoV-2 in wastewater. The synthesis of recent findings highlights that the presence of SARS-CoV-2 RNA in wastewater provides an opportunity to use wastewater as a surveillance tool for the invasion, prevalence, molecular epidemiology, and potential eradication of the virus in a community.

2. Gastrointestinal symptoms in COVID-19 and shedding of SARS-CoV-2 in excreta

Human CoVs, including SARS-CoV and MERS-CoV, are known to cause gastrointestinal symptoms in addition to respiratory symptoms (Leung et al., 2003; Memish et al., 2015). In fact, previous studies demonstrated that these viruses replicate in the gastrointestinal tract (Leung et al., 2003; Zhou et al., 2017). Recent reports revealed that 2–10% of COVID-19 patients had gastrointestinal symptoms, including diarrhea (Chen et al., 2020a, 2020b; Gao et al., 2020; Wang et al., 2020a, 2020b, 2020c). Although the exact mechanism of COVID-19-induced

gastrointestinal symptoms largely remains elusive (Gu et al., 2020), a recent study reported that SARS-CoV-2 infects gastrointestinal glandular epithelial cells (Xiao et al., 2020). Angiotensin-converting enzyme 2 (ACE2) is known to be the cellular receptor for SARS-CoV-2 as well as SARS-CoV (Yan et al., 2020), and the receptor ACE2 is abundantly expressed in the small intestine as well as lung and oral mucosa (Hamming et al., 2004; Xu et al., 2020b). This evidence supports the possibility of SARS-CoV-2 replication in gastrointestinal tract.

Previous studies on SARS-CoV and MERS-CoV detected their viral RNA in feces (Corman et al., 2016; Leung et al., 2003). It has been reported that SARS-CoV RNA loads could be as high as 10^7 copies/mL in diarrhea and 2.5×10^4 copies/mL in urine (Hung et al., 2004). A number of recent studies reported the presence of SARS-CoV-2 RNA in human feces (Gu et al., 2020; Holshue et al., 2020; Song et al., 2020). As shown in Table 1, SARS-CoV-2 RNA has been detected in excreta specimens, such as feces and anal/rectal swabs (Gao et al., 2020; Holshue et al., 2020; Jiehao et al., 2020; A. Tang et al., 2020b; Wölfel et al., 2020; Xiao et al., 2020; J. Zhang et al., 2020; W. Zhang et al., 2020b). Wang et al. (2020a, 2020b, 2020c) reported that SARS-CoV-2 RNA was detected by reverse transcription-quantitative PCR (RT-qPCR) in feces in 29% of the patients who were ill, and diarrhea was reported in approximately 2 to 10% of the cases (D. Wang et al., 2020b). Xiao et al. (2020) also found SARS-CoV-2 RNA in 39 (53%) of 73 fecal samples from hospitalized patients. Positive isolation from feces persisted in 23% of the patients even after it disappeared from the respiratory tract (Xiao et al., 2020). Viral RNA concentrations in feces were determined by several studies to be up to 10^8 copies per gram of feces (Lescure et al., 2020; Pan et al., 2020; Wölfel et al., 2020).

Some clinical studies reported prolonged fecal shedding of SARS-CoV-2 RNA for up to seven weeks after first symptom onset (Jiehao et al., 2020; Y. Wu et al., 2020a; Xiao et al., 2020). Another study reported that viral RNA could be detected in the feces of 81.8% cases even with a negative throat swab result (Ling et al., 2020). Recent reports implied that significant proportions (17.9–30.8%) of infected individuals are asymptomatic (Mizumoto et al., 2020; Nishiura et al., 2020), and SARS-CoV-2 RNA was detected in the feces of asymptomatic individuals as well (Tang et al., 2020a). Although two studies demonstrated the presence of culturable SARS-CoV-2 in fecal samples from COVID-19 patients (Wang et al., 2020b; Zhang et al., 2020a), a more recent study reported that culturable virus was not isolated from feces despite high viral RNA concentrations (Wölfel et al., 2020). This discrepancy could

be due to small differences in protocols for virus isolation from feces between laboratories, such as pretreatment method, cell lines, and number of blind passages, as viruses shed in feces are generally fastidious. The use of a recently reported engineered cell line that is highly susceptible to SARS-CoV-2 may enable enhanced virus isolation from feces (Matsuyama et al., 2020). Further research is needed to determine the concentrations of viral RNA and culturable virus particles, if any, in feces of symptomatic and asymptomatic individuals with SARS-CoV-2 infection.

3. Evidence for the presence of SARS-CoV-2 and related CoVs in wastewater

Our knowledge on the presence of CoVs in wastewater is largely limited likely due, at least in part, to the lack of previous environmental investigations focusing on CoVs. As CoVs are an enveloped virus that are thought to be primarily spread via person-to-person contact rather than the fecal-oral route (which has been postulated but not confirmed), their presence in feces requires more nuanced interpretation. In addition, the presence of CoV RNA in wastewater has not garnered widespread use as a disease surveillance tool but is gaining traction in this regard (Ahmed et al., 2020; Lodder and de Roda Husman, 2020). In addition, prior investigations demonstrated that standard virus concentration methods are inefficient to recover enveloped viruses from environmental water samples (Haramoto et al., 2009; Ye et al., 2016). Despite these considerations, one of the first detections of CoVs in wastewater was achieved in 2013 (Wong et al., 2013a). This study reported on detection of DNA and RNA viruses over a 12-month study in the USA and CoVs were found in wastewater in 1 of 12 samples using microarrays. The main focus of the study was on nonenveloped enteric viruses and this detection was not followed up with RT-qPCR to obtain quantitative data. In the same year, a viral metagenomic investigation allowing for untargeted molecular analysis of whole viral community identified the CoV HKU1 genome (a 'common cold' CoV) in sewage sludge (Bibby and Peccia, 2013), providing evidence for CoV presence in wastewater. A more recent study also reported the molecular detection of animal CoV belonging to the genus *Alphacoronavirus* in surface water in Saudi Arabia (Blanco et al., 2019).

During the SARS outbreak in 2004 in China, SARS-CoV RNA was detected in 100% (10/10) of untreated and 30% (3/10) of disinfected wastewater samples collected from a hospital in Beijing, China receiving

Table 1
Detection of SARS-CoV-2 in human excreta specimens.

Specimen	Country	Method	Positive rate ^a	Remarks	Reference	
Feces or anal/rectal swab	China	qPCR	14/31 (45%)	Day 0–11; $550\text{--}1.21 \times 10^5$ gene copies/mL Pediatric patients; Positive for a mean of 21 (range: 5–28) days Day 3–13	(W. Zhang et al., 2020b)	
		qPCR	8/22 (36%)		(J. Zhang et al., 2020)	
		qPCR	9/17 (53%)		(Pan et al., 2020)	
		qPCR	8/10 (80%)		(Y. Xu et al., 2020a)	
		qPCR	5/6 (83%)		(Jiehao et al., 2020)	
		qPCR	54/66 (82%)		(Ling et al., 2020)	
		qPCR	39/73 (53%)		(Xiao et al., 2020)	
		qPCR	1/1 (100%)		Asymptomatic	(A. Tang et al., 2020a)
		qPCR	41/74 (55%)		Positive for a mean of 27.9 days (range: 8–48)	(Y. Wu et al., 2020a)
		qPCR	12/19 (63%)			(Chen et al., 2020c)
	qPCR	10/10 (100%)		(Lo et al., 2020)		
	Cell culture	1/1 (100%)	Culturable virus isolated	(Zhang et al., 2020a)		
	qPCR	44/153 (29%)		(W. Wang et al., 2020c)		
	Cell culture	2/4 (50%)	Culturable virus isolated			
	qPCR	1/1 (100%)	Day 7	(Holshue et al., 2020)		
	USA	qPCR	4/8 (50%)		(Young et al., 2020)	
	Singapore	qPCR	8/9 (89%)	Up to 10^8 copies/g-feces	(Wölfel et al., 2020)	
Germany	Cell culture	0/4 (0%)	No culturable virus isolated			
Urine	France	qPCR	2/5 (40%)	$6.3 \times 10^5\text{--}1.3 \times 10^8$ gene copies/g-feces	(Lescure et al., 2020)	
	China	qPCR	4/58 (7%)		(Ling et al., 2020)	
		qPCR	0/10 (0%)		(Lo et al., 2020)	
	Germany	qPCR	0/9 (0%)		(Wölfel et al., 2020)	
	France	qPCR	0/5 (0%)		(Lescure et al., 2020)	

^a Based on number of patients tested.

SARS patients (Wang et al., 2005). Wastewater was also believed to be at least partly responsible for previous SARS outbreak due to a faulty ventilation and plumbing system (McKinney et al., 2006). There have been initial reports of the molecular detection of SARS-CoV-2 in wastewater in the Netherlands, USA, France, and Australia (Ahmed et al., 2020; Lodder and de Roda Husman, 2020; Medema et al., 2020; Nemudryi et al., 2020; F. Wu et al., 2020b; Wurtzer et al., 2020). These studies reported the detection of SARS-CoV-2 RNA in untreated wastewater with maximum concentrations over 10^6 copies per liter. The study in France detected SARS-CoV-2 RNA in treated wastewater as well, with concentrations of up to nearly 10^5 copies per liter (Wurtzer et al., 2020). Details of these reports on molecular detection of SARS-CoV-2 RNA in wastewater are summarized in Table 2. Beyond these initial reports, continuous monitoring of SARS-CoV-2 in wastewater in multiple geographical regions is underway.

4. Understanding COVID-19 epidemiology through wastewater surveillance

Wastewater-based epidemiology (WBE) serves as an important tool to trace the circulation of viruses in a community, providing opportunities to estimate their prevalence, genetic diversity, and geographic distribution (Sinclair et al., 2008; Xagorarakis and O'Brien, 2020). Wastewater systems offer a practical approach to identify viruses excreted in the feces of an entire region (Carducci et al., 2006; La Rosa and Muscillo, 2013). Using this approach, it becomes possible to monitor the epidemiology of virus infections even if they are not evident by clinical surveillance, especially because traditional epidemiological approaches may be limited by the asymptomatic nature of many viral infections and underdiagnosis of clinical cases (Johansson et al., 2014; Qi et al., 2018). These limitations are applicable for not only fecally-shed viruses such as adenovirus, norovirus, sapovirus, enterovirus, rotavirus, and hepatitis A virus (Okabayashi et al., 2008; Rodríguez-Lázaro et al., 2012; Yoshida et al., 2009), but also for other viruses that are rarely or never reported by epidemiological surveillance systems, such as Saffold virus, cosavirus, and salivirus/klassevirus (Bonanno Ferraro et al., 2020; Kitajima et al., 2014, 2015; Thongprachum et al., 2018).

SARS-CoV-2 is known to cause asymptomatic or pauci-symptomatic infections (Lai et al., 2020; Mizumoto et al., 2020; Nishiura et al., 2020; A. Tang et al., 2020a) making it difficult to determine the actual degree

of viral circulation in a community and in making comparisons among different countries that have different clinical diagnostic testing capabilities with even different diagnostic methods/assays (Ortiz-Ospina and Hasell, 2020). Meanwhile, wastewater surveillance could provide an unbiased method of evaluating the spread of infection in different areas, even where resources for clinical diagnosis are limited and when reporting systems are unavailable or not feasible, such as in developing countries. Moreover, wastewater monitoring can help to detect variations in the circulating strains through phylogenetic analysis, allowing for comparisons between regions and assessment of evolution of the virus genome over time as demonstrated previously for enteric viruses (Bisseux et al., 2018; La Rosa et al., 2014; Lodder et al., 2013), and more recently for SARS-CoV-2 (Nemudryi et al., 2020).

The importance of wastewater surveillance is also highlighted by its ability to detect low levels of viruses; this can happen when the number of infected cases is decreasing following public health interventions, which has been the case for successful examples such as poliovirus eradication programs (Asgar et al., 2014). It is also useful to determine when a new virus is introduced into a population (Savolainen-Kopra et al., 2011; Sinclair et al., 2008) or when fluctuations occur due to changes in seasons or precipitation (Hellmér et al., 2014; Prevost et al., 2015; Sedmak et al., 2003). Thus, such a surveillance strategy can be useful as an "early warning" system (Xagorarakis and O'Brien, 2020) to determine if re-introduction of SARS-CoV-2 had occurred in a community, or conversely as an assessment of whether exposures have decreased sufficiently following public health interventions, such as lockdown, social isolation, and social distancing. Virome analysis of wastewater opens up further possibilities of detecting novel viruses before their clinical recognition in a community, allowing for preventative measures and allocation of resources to potentially affected areas (Bibby, 2013; Bibby et al., 2019; Fernandez-Cassi et al., 2018; Ng et al., 2012). A recent study explored the numbers of SARS-CoV-2 RNA copies observed in untreated wastewater that could estimate the number of infected individuals in the catchment via Monte Carlo simulation. The model estimated a median range of 171 to 1090 infected persons in the catchment, which was in reasonable agreement with clinical observations (Ahmed et al., 2020). The authors identified the need for further methodological and molecular assay validation for enveloped viruses in wastewater in order to enhance the accuracy of wastewater surveillance. While a number of applications of wastewater surveillance are

Table 2
Details of reported molecular detection of SARS-CoV-2 in wastewater.

Sampling location		Water type	Virus detection methods			Detection results		Reference
Country	State/city		Virus concentration method	qPCR assay ^a	Sequence confirmation	Positive rate	Maximum concentration (copies/L)	
Australia	Brisbane, Queensland	Untreated wastewater	Electronegative membrane-direct RNA extraction; ultrafiltration	N_Sarbeco NIID_2019-nCoV	Direct sequence of qPCR products (Sanger + MiSeq)	2/9 (22%)	1.2×10^2	(Ahmed et al., 2020)
The Netherlands	Amsterdam, The Hague, Utrecht, Apeldoorn, Amersfoort, Schiphol, Tilburg	Untreated wastewater	Ultrafiltration	CDC N1, N2, N3 E_Sarbeco	Not done	14/24 (58%)	Not available	(Medema et al., 2020)
USA	Massachusetts	Untreated wastewater	PEG precipitation	CDC N1, N2, N3	Direct sequence of qPCR products (Sanger)	10/14 (71%)	$>2 \times 10^5$	(F. Wu et al., 2020b)
France	Paris	Untreated wastewater	Ultracentrifugation	E_Sarbeco	Not done	23/23 (100%)	$>10^{6.5}$	(Wurtzer et al., 2020)
		Treated wastewater	Ultracentrifugation	E_Sarbeco	Not done	6/8 (75%)	$\sim 10^5$	
USA	Bozeman, Montana	Untreated wastewater	Ultrafiltration	CDC N1, N2	Re-amplification by regular PCR followed by Sanger sequencing	7/7 (100%)	$>3 \times 10^4$	(Nemudryi et al., 2020)

^a See Table 3 for details of each qPCR assay.

obvious, there are limitations. Correlating levels of viruses with a specific number of cases identified epidemiologically may be challenging because of differences in excretion rates of viruses during the course of infection, temporal delays and the inconsistent capture of spatial variability due to travel and use of multiple wastewater systems in time, and dilution due to precipitation, inactivation during the wastewater transport process, and/or infrequent or absent clinical testing (La Rosa and Muscillo, 2013). Also, stability of the genome in wastewater, low efficiency of virus concentration methods, sampling variability (e.g., grab vs. composite) and lack of sensitive detection assays especially at low virus concentrations may collectively limit virus detection and quantification.

Despite these challenges, multiple efforts are underway to develop environmental surveillance programs for SARS-CoV-2. As noted above, there have been initial reports of the molecular detection of SARS-CoV-2 in wastewater in the Netherlands, USA, France, and Australia (Ahmed et al., 2020; Lodder and de Roda Husman, 2020; Medema et al., 2020; Nemudryi et al., 2020; F. Wu et al., 2020b; Wurtzer et al., 2020). One of these recent studies carried out in the USA adopted a wastewater surveillance approach to reveal phylogeny of circulating SARS-CoV-2 strains, infer viral ancestry, and observe the efficacy of public health interventions to contain the outbreak (i.e., mandated social isolation) (Nemudryi et al., 2020). These recent studies and other potentially ongoing efforts in many parts of the world may help to inform epidemiological modeling of the prevalence of SARS-CoV-2 in communities, as well as serve as a warning signal to communities attempting to mitigate the spread of the infection. To gain public acceptance of wastewater surveillance, a framework highlighting ethical issues related to basic access to sanitation, privacy, and rights may be required. It should be widely understood that one of the advantages of WBE is that this approach provides epidemiological information on disease prevalence in a community by circumventing individual stigmatization, which often results from clinical diagnosis in the ongoing COVID-19 outbreak (Murakami et al., 2020).

5. Methods for SARS-CoV-2 detection in wastewater

Although viral loads in the feces of COVID-19 patients are variable (Table 1), SARS-CoV-2 RNA can be sometimes detected with comparable concentrations to many enteric viruses ($\sim 10^8$ viruses per gram of feces) (Bosch, 1998; Prüss et al., 2002; Wyn-Jones and Sellwood, 2001). Nevertheless, it will likely be necessary to perform a virus concentration step(s) prior to subsequent detection of SARS-CoV-2, even in untreated wastewater, as conducted previously (Ahmed et al., 2020; Lodder and de Roda Husman, 2020; Medema et al., 2020; Nemudryi et al., 2020; F. Wu et al., 2020b; Wurtzer et al., 2020) (Table 2).

Numerous types of methods have been developed for concentrating viruses in wastewater; however, most of those studies aimed to establish concentration methods for nonenveloped enteric viruses such as norovirus, enterovirus, adenovirus, and hepatitis A virus, using culturable viruses and/or bacteriophages as model viruses (Haramoto et al., 2018). Electropositive or electronegative membranes have been widely used to concentrate enteric viruses in untreated and treated wastewater samples (Cashdollar and Wymer, 2013; Haramoto et al., 2018; Ikner et al., 2012). These methods were developed based on electrostatic interactions between filters and viruses, utilizing the fact that a majority of enteric viruses have a net negative electrostatic charge in environmental water near neutral pH. In this method, negatively charged virus particles directly adsorb onto electropositive filter or adsorb onto electronegative filter via salt-bridging with a multivalent cation (Ikner et al., 2012; Michen and Graule, 2010). Another commonly used membrane-based method for concentrating viruses in environmental water samples is ultrafiltration, which is based on size exclusion (Hill et al., 2005, 2007). Other methods including polyethylene glycol (PEG) precipitation (Lewis and Metcalf, 1988), ultracentrifugation

(Fumian et al., 2010), and skimmed-milk flocculation (Calgua et al., 2013) have also been used for concentrating viruses from wastewater samples.

The effectiveness of these virus concentration methods has been well demonstrated by successful detection of various types of indigenous enteric viruses which were not used as a model virus during the method development (Fong and Lipp, 2005; Haramoto et al., 2018). However, limited knowledge is available on recovery efficiencies of enveloped viruses, including CoVs, with the existing virus concentration methods. Ye et al. (2016) reported greater adsorption of enveloped viruses (mouse hepatitis virus [MHV] and *Pseudomonas* phage $\Phi 6$) to the solid fraction of wastewater compared to nonenveloped viruses. Haramoto et al. (2009) reported that enveloped koi herpesvirus showed high enough adsorption efficiency to an electronegative filter (87% or higher). Taken together, these results suggest that virus concentration methods using filters may potentially be used to recover SARS-CoV-2 from water and wastewater and requires further investigation. Even within enteric viruses, recovery efficiencies of viruses can vary greatly depending on virus and water types (Haramoto et al., 2018). Therefore, little scientific evidence is available to inform judgments of the usefulness of these existing virus concentration methods for enveloped SARS-CoV-2, which has quite different characteristics in structural and physical properties from enteric viruses. For example, Wang et al. (Xin Wei Wang et al., 2005b) reported that recovery of SARS-CoV from wastewater was only 1% using an electropositive membrane filter method, a significant decrease in performance compared to that observed for many types of enteroviruses (Li et al., 1998).

Nevertheless, virus concentration will likely be necessary to increase the chance of detection of SARS-CoV-2 in wastewater and research is needed to evaluate the recovery efficiency. Meanwhile, efforts are needed to evaluate the applicability of these existing methods to concentrate SARS-CoV-2. For method evaluation and development, low-pathogenic CoV strains (such as MHV and classical human CoVs) and/or *Pseudomonas* phage $\Phi 6$ may be used as models of SARS-CoV-2 for biosafety reasons. Recent studies in Australia, France, the Netherlands, and USA reported that SARS-CoV-2 RNA was successfully detected in wastewater using different concentration methods, such as ultrafiltration, PEG precipitation, and electronegative membrane adsorption followed by direct RNA extraction (Ahmed et al., 2020; Medema et al., 2020; Nemudryi et al., 2020; F. Wu et al., 2020b; Wurtzer et al., 2020).

Concentration volumes of water are one of the important factors that can affect the results of detection of viruses; normally, concentrating <100 mL of untreated wastewater samples is sufficient to detect enteric viruses (Haramoto et al., 2018). The initial studies reporting molecular detection of SARS-CoV-2 in wastewater concentrated up to 200 mL of raw wastewater samples (Ahmed et al., 2020; Medema et al., 2020; Nemudryi et al., 2020; F. Wu et al., 2020b; Wurtzer et al., 2020). However, a larger volume of wastewater sample may need to be processed for the detection of SARS-CoV-2 in regions where COVID-19 is less prevalent in the community.

Currently, detection of SARS-CoV-2 primarily relies on RT-qPCR or (nested) RT-PCR (CDC, 2020a; China CDC, 2020; Corman et al., 2020; Department of Medical Sciences, Ministry of Public Health, T, 2020; Institut Pasteur, 2020; Poon et al., 2020; Shirato et al., 2020). Currently available RT-qPCR and nested RT-PCR assays for SARS-CoV-2 are summarized in Table 3. Corman et al. (2020) developed three TaqMan-based qPCR assays targeting RNA-dependent RNA polymerase (RdRp), envelope (E), and nucleocapsid (N) protein genes, with an absolute limit of detection (ALOD) of 3.8, 5.2, and 8.3 RNA copies per reaction, respectively (Corman et al., 2020). The RdRp gene-RT-qPCR assay uses two fluorescent probes to discriminate SARS-CoV-2 from SARS-CoV and bat-SARS-related CoVs, while the E gene-RT-qPCR assay can react with both SARS-CoV-2 and SARS-CoV. Because of its slightly higher ALOD than the other two assays, the performance of the N gene-RT-qPCR assay was not assessed in detail in that study. In contrast, Shirato et al. (2020) reported that among the three assays, only the N

Table 3
Oligonucleotide sequences for selected SARS-CoV-2 RT-qPCR and nested RT-PCR assays.

Type of PCR	Target gene	Function	Name	Sequence (5'–3') ^a	Product length (bp)	Reference
RT-qPCR	RdRp	Forward primer	RdRp_SARSr-F	GTGARATGGTCATGTG TGGCGG	100	(Corman et al., 2020)
		Reverse primer	RdRp_SARSr-R	CARATGTAAASACAC TATTAGCATA		
		TaqMan probe (specific for SARS-CoV-2)	RdRp_SARSr-P2	FAM-CAGGTGGAACC TCATCAGGAGATG C-BBQ		
RT-qPCR	RdRp	TaqMan probe (reactive with SARS-CoV-2, SARS-CoV, and bat-SARS-related CoVs)	RdRp_SARSr-P1	FAM-CCAGGTGG WACRTCATCMGGTGAT GC-BBQ	108	(Institut Pasteur, 2020)
		Forward primer	nCoV_IP2-12669Fw	ATGAGCTTAGTCCTGT TG		
		Reverse primer TaqMan probe	nCoV_IP2-12759Rv nCoV_IP2-12696bProbe(+)	CTCCCTTTGTTGTGTGT HEX-AGATGTCTTGT GCTGCCGTA-BHQ1		
RT-qPCR	RdRp	Forward primer	nCoV_IP4-14059Fw	GGTAACTGGTATGATT TCG	107	(Institut Pasteur, 2020)
		Reverse primer	nCoV_IP4-14146Rv	CTGGTCAAGGTTAATA TAGG		
		TaqMan probe	nCoV_IP4-14084Probe(+)	FAM-TCATACAAACC ACGCCAGG-BHQ1 CCCTGTGGGTTTTACA CTTAA		
RT-qPCR	ORF1ab	Forward primer	Not provided	ACGATTGTGCATCAGC TGA	119	(China CDC, 2020)
		Reverse primer	Not provided	FAM-CCGTCTGCGGT ATGTGGAAGGTTAT GG-BHQ1		
		TaqMan probe	Not provided	TGGGGYTTTACRGGTA ACCT		
RT-qPCR	ORF1b-nonstructural protein 14	Forward primer	HKU-ORF1b-nsp14F	AACRCGCTTAACAAA GCACTC	132	(Poon et al., 2020)
		Reverse primer	HKU-ORF1b-nsp14R	FAM-TAGTTGTGA TGCWATCATGACT AG-TAMRA		
		TaqMan probe	HKU-ORF1b-nsp141P	ACAGGTACGTTAATAG TTAATAGCGT ATATTGCAGCAGTACG CACACA		
RT-qPCR	E protein	Forward primer	E_Sarbeco_F	FAM-ACACTAGCCAT CCTTACTGCGCTT CG-BBQ	113	(Corman et al., 2020)
		Reverse primer	E_Sarbeco_R	CACATTGGCACCCGCA ATC		
		TaqMan probe	E_Sarbeco_P1	GAGGAACGAGAAGAGG CTTG FAM-ACTTCCTCAAG GAACAACATT GCCA-BBQ		
RT-qPCR	N protein	Forward primer	N_Sarbeco_F	GACCCAAAATCAGCG AAAT	72	(CDC, 2020a)
		Reverse primer	N_Sarbeco_R	TCTGGTTACTGCCAGT TGAATCTG		
		TaqMan probe	N_Sarbeco_P	FAM-ACCCCGCATTA CGTTTGGTGG ACC-BHQ1		
RT-qPCR	N protein	Forward primer	2019-nCoV_N1-F	TTACAACATTGGCCG CAAA	67	(CDC, 2020a)
		Reverse primer	2019-nCoV_N1-R	GCGCGACATCCGAAG AA		
		TaqMan probe	2019-nCoV_N1-P	FAM-ACAATTTGCC CCAGCGCTCAG-BHQ1 GGGAGCCTTGAATACA CCAAAA		
RT-qPCR	N protein	Forward primer	2019-nCoV_N2-F	TGTAGCAGCATTGCAG CATTG	72	(CDC, 2020a)
		Reverse primer	2019-nCoV_N2-R	FAM-AYCACATTGGCAC CCGCAATCCTG-BHQ1 GGGGAACCTTCTCTGC TAGAAT		
		TaqMan probe	2019-nCoV_N2-P	(not provided)		
RT-qPCR	N protein	Forward primer	(not provided)	CAGACATTTGCTCTC AAGCTG	99	(China CDC, 2020)
		Reverse primer	(not provided)			
		TaqMan probe	(not provided)			

Table 3 (continued)

Type of PCR	Target gene	Function	Name	Sequence (5'–3') ^a	Product length (bp)	Reference
		TaqMan probe	(not provided)	FAM-TTGTCTGCTGCT TGACAGATT-TAMRA		
	N protein	Forward primer	HKU-NF	TAATCAGACAAGGAAC TGATTA	110	(Poon et al., 2020)
		Reverse primer	HKU-NR	CGAAGGTGTGACTTCC ATG		
		TaqMan probe	HKU-NP	FAM-GCAAATTGTGC AATTTGCGG-TAMRA		
	N protein	Forward primer	WH-NIC N-F	CGTTTGGTGGACCTC AGAT	57	(Department of Medical Sciences, Ministry of Public Health, T, 2020)
		Reverse primer	WH-NIC N-R	CCCCACTGCGTTCTCC ATT		
		TaqMan probe	WH-NIC N-P	FAM-CAACTGGCAGT AACCABQH1		
	N protein	Forward primer	NIID_2019-nCOV_N_F2	AAATTTTGGGGACCAG GAAC	158	(Shirato et al., 2020)
		Reverse primer	NIID_2019-nCOV_N_R2	TGGCAGCTGTGTAGGT CAAC		
		Reverse primer (revised version)	NIID_2019-nCOV_N_R2ver3	TGGCACCTGTGTAGGT CAAC		
		TaqMan probe	NIID_2019-nCOV_N_P2	FAM-ATGTCGCGCAT TGGCATGGA-BHQ1		
	S protein	Forward primer	RBD-qF1	CAATGGITTAACAGGC ACAGG	121	(Zhou et al., 2020)
		Reverse primer	RBD-qR1	CTCAAGTGTCTGTGGA TCACG		
Nested RT-PCR	ORF1a	1st PCR forward primer	NIID_WH-1_F501	TTCGGATGCTCGAACT GCACC	413	(Shirato et al., 2020)
		1st PCR reverse primer	NIID_WH-1_R913	CTTTACCAGCACGTGC TAGAAGG		
		2nd PCR forward primer	NIID_WH-1_F509	CTCGAACTGCACCTCA TGG		
		2nd PCR reverse primer	NIID_WH-1_R854	CAGAAGTTGTATCGA CATAGC		
	S protein	1st PCR forward primer	WuhanCoV-spk1-f	TGGGCAAAATCAAGA CTCACTTT	547	(Shirato et al., 2020)
		1st PCR reverse primer	WuhanCoV-spk2-r	TGTGGTTCATAAAAAAT TCCTTTGTG		
		2nd PCR forward primer	NIID_WH-1_F24381	TCAAGACTCACTTTCT TCCAC		
		2nd PCR reverse primer	NIID_WH-1_R24873	ATTTGAAACAAAGACA CCTTCAC		

^a Single-letter code: M stands for A or C; R stands for A or G; S stands for C or G; W stands for A or T; and Y stands for C or T. Abbreviations: BBQ, blackberry quencher; BHQ1, black hole quencher 1; FAM, 6-carboxyfluorescein; HEX, hexachloro-6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine.

gene-RT-qPCR assay, which was specific only for SARS-CoV-2, worked well under their RT-qPCR platform (Shirato et al., 2020). The N protein gene is the most widely used gene target for developing RT-qPCR assays (CDC, 2020a; Chu et al., 2020; Corman et al., 2020; Shirato et al., 2020). An N gene-RT-qPCR assay developed by Shirato et al. (2020) was reported to be able to detect as low as ~5 RNA copies per reaction (Shirato et al., 2020), which is comparable to the assay developed by Corman et al. (2020).

Nalla et al. (2020) evaluated the performance of seven RT-qPCR assays targeting RdRp, E, and N genes (CDC, 2020a; Corman et al., 2020), where clinical respiratory and swab samples including SARS-CoV-2 positive samples were tested (Nalla et al., 2020). Based on the results of experiments using dilutions of a SARS-CoV-2-positive sample, the authors found that the N gene- (N2) and E gene-RT-qPCR assays developed by CDC (2020a) and Corman et al. (2020), respectively, showed the highest sensitivity of ~6.3 genomic equivalents per reaction. However, since the limited number of SARS-CoV-2-positive samples collected from a certain region were used in this study, further studies using samples from various locations worldwide are needed to establish a 'gold standard' assay.

Unlike clinical samples, a lower ALOD value is required when SARS-CoV-2 is tested in wastewater samples with low virus concentrations due to dilution and a low prevalence of COVID-19. Unfortunately, data regarding ALOD are not available for many of the existing RT-qPCR

assays, most likely because these methods were designed for application to rapidly screening clinical samples. It is likely that RT-qPCR assays showing an ALOD of <10 copies per reaction could be useful for screening of wastewater samples for SARS-CoV-2 (Corman et al., 2020; Shirato et al., 2020). A SYBR Green-based qPCR targeting spike (S) protein gene has been also developed, although no ALOD data are provided (Zhou et al., 2020). Digital RT-PCR may enable more sensitive and accurate detection/quantification of SARS-CoV-2 RNA in wastewater samples than RT-qPCR as suggested recently for clinical samples (Dong et al., 2020; Suo et al., 2020).

When detecting SARS-CoV-2 RNA in wastewater by RT-qPCR, confirmation of positive RT-qPCR signals by sequencing analysis is highly recommended until the assay specificities have been validated against environmental samples. This is because the currently available RT-qPCR assays were developed for clinical diagnosis, which may be quite different from environmental applications. Some of recent studies on SARS-CoV-2 detection in wastewater reported sequence confirmation of RT-qPCR positive samples by directly sequencing qPCR products or re-amplification with regular PCR followed by sequencing (Ahmed et al., 2020; Nemudryi et al., 2020; F. Wu et al., 2020b).

Two nested RT-PCR assays targeting open reading frame 1a (ORF1a) and S protein genes are also available (Shirato et al., 2020), which could be used to elucidate the genetic diversity of SARS-CoV-2 circulating in human populations. As a thermal cycler is essential for PCR, novel assays

which do not require any thermal cyclers, such as loop-mediated isothermal amplification (LAMP) method, are expected to be developed, which will enable detection of SARS-CoV-2 RNA more easily and rapidly, especially in situations where sufficient laboratory equipment is not available. Additional efforts may be made to assess viral infectivity in wastewater using an engineered cell line with high susceptibility to SARS-CoV-2 (Matsuyama et al., 2020) and/or to detect infectious viral particles selectively by utilizing viability qPCR, such as ethidium bromide monoazide (EMA) or propidium monoazide (PMA) treatment followed by RT-qPCR, or integrated cell culture (ICC)-RT-PCR/qPCR (Farkas et al., 2020).

A critical issue in the application of molecular-based methods, including RT-qPCR, to wastewater samples is PCR inhibition during the detection process. It has been recommended that a process control (s) should be included in the analysis to monitor the levels of loss of targets and/or inhibition from the sample concentration to the detection steps (Haramoto et al., 2018). Three types of process controls are proposed, depending on the points of their inoculation: (i) whole process controls, to be inoculated into a water sample before virus concentration; (ii) molecular process controls, to be inoculated into a viral concentrate before nucleic acid extraction; and (iii) RT-qPCR controls, to be inoculated before RT-qPCR. At least one of these process controls is recommended to be included to avoid false-negative results and for concentration methods with low virus recovery efficiencies. Based on the results, samples may need to be reanalyzed (Haramoto et al., 2018). For a reliable process control, it is appropriate to select a virus which is genetically closely related to and/or has similar physical characteristics as the target virus and is expected not to be present in the tested water. Low-pathogenic animal CoVs such as MHV represent ideal process controls for SARS-CoV-2. Nonetheless, the selection of already established process controls for enteric viruses, such as murine norovirus and mengovirus, which are both single-stranded RNA viruses, may be acceptable at this stage.

6. Survival and inactivation of CoVs and enveloped surrogate viruses in water and wastewater matrices

The magnitude of human health risks varies depending on the inactivation of pathogens, including SARS-CoV-2 in water environments. Understanding the inactivation of SARS-CoV-2 and its RNA will ultimately improve control measures and wastewater treatment requirements, but little has been documented on the persistence of CoVs in water and wastewater matrices. Currently available data on survival of coronaviruses and an enveloped phage in water and wastewater are summarized in Table 4. Wang et al. (2005) investigated the persistence of SARS-CoV, *Escherichia coli* and ϕ_2 phage in hospital wastewater, domestic sewage, tap water, phosphate buffered saline, feces, urine, water, and wastewater with high various concentrations (5, 10, 20 and 40 mg/L) of chlorine and chlorine dioxide. They also investigated the effect of contact time on inactivation of SARS-CoV in wastewater with low (10 mg/L chlorine and chlorine dioxide) and high (20 mg/L of chlorine and 40 mg/L of chlorine dioxide) concentrations. Results indicated that coronavirus persisted longer (inoculated titer of 10^5 TCID₅₀; detectable with RT-PCR for 14 days) at 4 °C compared to 20 °C (3 days) in hospital wastewater, domestic sewage, and dechlorinated tap water. At 20 °C, SARS-CoV persisted in three fecal samples for 3 days and two urine samples for 17 days (inoculated titer of 10^5 TCID₅₀). SARS-CoV was more vulnerable to disinfectants compared to *E. coli* and ϕ_2 phage. Free chlorine was more effective in inactivating SARS-CoV than chlorine dioxide. Free residue chlorine of >0.5 mg/L or chlorine dioxide of 2.19 mg/L in wastewater were sufficient for the complete removal of SARS-CoV (Xin Wei Wang et al., 2005b).

Gundy et al. (2009) determined the survival of human CoV 229E and enteric feline CoV (ATCC-990) in water and wastewater using plaque assay or median culture infectious dose (TCID₅₀) technique. The times for 99 and 99.9% inactivation (T_{99} and $T_{99.9}$, respectively) were

determined for filtered and unfiltered tap water at 23 °C, filtered tap water at 4 °C, filtered and unfiltered primary effluent at 23 °C and secondary effluent (activated sludge) at 23 °C. The survival of both human and feline CoVs showed similar patterns and was highly dependent on water temperature, level of organic matter, and biological activity. The T_{99} for tap water for both human and feline CoV were faster at 23 °C (7–9 days) than 4 °C (>87 days). The inactivation rates of both CoVs were faster in filtered tap water compared to unfiltered tap water at 23 °C, suggesting increased protection and survival in the presence of organic matter and suspended solids. CoVs were inactivated rapidly in wastewater, with T_{99} values of <3 days (Gundy et al., 2009).

Casanova et al. (2009) determined the persistence of two surrogate CoVs, transmissible gastroenteritis virus (TGEV), and MHV in reagent grade water, lake water, and pasteurized settled sewage in North Carolina, USA using quantal assays for cytopathic effect (CPE). In general, both the surrogate viruses persisted for significantly shorter durations at 25 °C compared to 4 °C for all water types. For reagent grade water, TGEV and MHV persisted for shorter durations (T_{99} = 22 and 16 days, respectively) at 25 °C than at 4 °C (>220 days for both viruses). For lake water, TGEV and MHV T_{99} values were 13 and 10 days, respectively, over a 14-day experiment. However, at 4 °C, one log₁₀ reduction was observed at day 14 for TGEV, while no reduction was observed for MHV up to day 14 at 4 °C. Both viruses persisted shorter in pasteurized settled sewage samples, and T_{99} reduction times were nine days for TGEV, and seven days for MHV. At 4 °C, T_{99} values of TGEV and MHV were 49 and 70 days, respectively, suggesting surrogate CoVs can remain infectious for long periods in water and pasteurized settled sewage at a lower temperature (Casanova et al., 2009).

A technical brief from WHO suggested that there is no evidence about the survival of SARS-CoV-2 in wastewater or drinking water. It is likely that enveloped CoVs are less stable in the environment and are more susceptible to chlorine, pH, and temperature than most of nonenveloped enteric viruses (WHO, 2020d). Therefore, conventional wastewater treatment processes should inactivate SARS-CoV-2, and multiple barriers used in drinking water treatment plants should suffice to remove SARS-CoV-2 to levels of non-detect and low risks (< 10^{-4} annual risk). However, limited data published to date suggest that surrogate CoV remain infectious in water environments for days to weeks, depending on temperature and other physico-chemical factors (Pratelli, 2008). Therefore, it is crucial to determine the persistence of SARS-CoV-2 in sewage and environmental waters using molecular and cell culture assays. If biosafety is a concern/limitation, then enveloped surrogate viruses such as low-pathogenic human CoV (e.g., 229E or OC43), feline CoV, MHV, or *Pseudomonas* phage ϕ_6 can be used.

Ye et al. (2016) compared the persistence and partitioning behavior of two model enveloped viruses, MHV and *Pseudomonas* phage ϕ_6 in raw and pasteurized wastewater samples using cell culture and plaque assays. MHV and ϕ_6 were seeded into unpasteurized and pasteurized wastewater and incubated at 10 and 25 °C to mimic typical winter and summer temperatures of wastewater. The T_{90} values of MHV and ϕ_6 in unpasteurized wastewater at 25 °C were 13 and 7 h, respectively. In contrast, the T_{90} values of MHV and ϕ_6 were slower in unpasteurized wastewater at 10 °C with T_{90} values of 36 and 28 h, respectively. Both viruses persisted relatively longer in pasteurized wastewater than unpasteurized wastewater. Based on the results, the authors concluded that although MHV and ϕ_6 were inactivated rapidly in wastewater, their persistence could still be of concern for wastewater treatment facilities, stormwater overflows, and wastewater intrusion in drinking water (Ye et al., 2016). Their results on comparative viral persistence in pasteurized and unpasteurized wastewater implied that enhancement of competition and predation contributed by indigenous microbial communities in wastewater could be a potential medium-term strategy to fight against the ongoing and future viral disease outbreaks.

Similarly, Aquino de Carvalho et al. (2017) evaluated the persistence of ϕ_6 in a variety of matrices, including water and wastewater. The T_{90}

Table 4
Survival of human and animal coronaviruses and an enveloped phage in water and wastewater.

Viruses ^a	Water type	Temperature (°C)	Days persisted	Reduction time			Reference
				T ₉₀	T ₉₉	T _{99.999}	
Human coronavirus	Filtered tap water	23	-	-	6.76 day	-	(Gundy et al., 2009)
	Unfiltered tap water	23	-	-	8.09 day	-	
	Filtered tap water	4	-	-	392 day	-	
	Filtered primary effluent	23	-	-	1.57 day	-	
	Unfiltered primary effluent	23	-	-	2.36 day	-	
Feline coronavirus	Unfiltered secondary effluent	23	-	-	1.85 day	-	(Gundy et al., 2009)
	Filtered tap water	23	-	-	6.76 day	-	
	Unfiltered tap water	23	-	-	8.32 day	-	
	Filtered tap water	4	-	-	87.0 ^b day	-	
	Filtered primary effluent	23	-	-	1.60 day	-	
TGEV	Unfiltered Primary effluent	23	-	-	1.71 day	-	(Casanova et al., 2009)
	Unfiltered secondary effluent	23	-	-	1.62 day	-	
	Reagent grade water	4	-	-	220 day	-	
	Reagent grade water	25	-	-	22.0 day	-	
	Pasteurized settled sewage	4	-	-	7.00 day	-	
MHV	Pasteurized settled sewage	25	-	-	9.00 day	-	(Casanova et al., 2009)
	Reagent grade water	4	-	-	>365 ^b day	-	
	Reagent grade water	25	-	-	17.0 day	-	
	Pasteurized settled sewage	4	-	-	70.0 day	-	
	Pasteurized settled sewage	25	-	-	49.0 day	-	
MHV	Unpasteurized wastewater	10	-	36 h	-	-	(Ye et al., 2016)
	Unpasteurized wastewater	25	-	13 h	-	-	
	Pasteurized wastewater	10	-	149 h	-	-	
	Pasteurized wastewater	25	-	19 h	-	-	
	Pasteurized wastewater	10	-	146 h	-	-	
<i>Pseudomonas</i> phage Φ6	Unpasteurized wastewater	10	-	28 h	-	-	(Casanova and Weaver, 2015)
	Unpasteurized wastewater	25	-	7 h	-	-	
	Pasteurized wastewater	10	-	53 h	-	-	
	Pasteurized wastewater	25	-	146 h	-	-	
	Pasteurized wastewater	10	-	53 h	-	-	
<i>Pseudomonas</i> phage Φ6	Primary influent	22	-	-	-	6 day	(Casanova and Weaver, 2015)
	Primary influent	30	-	1 day	-	-	
	Primary influent	30	-	1 day	-	-	
<i>Pseudomonas</i> phage Φ6	Autoclaved river water	23	-	7.1 day	-	-	(Aquino de Carvalho et al., 2017)
	Nonautoclaved river water	23	-	3.1 day	-	-	
	Dechlorinated tap water	22	-	3.1 day	-	-	
	Autoclaved wastewater influent	22	-	2.5 day	-	-	
	Deionised water	4	-	66.1 day	-	-	
	Deionised water	25	-	1.6 day	-	-	
	Deionised water	37	-	0.34 day	-	-	
SARS-CoV ^c	Deionised water	45	-	0.017 day	-	-	(Xin Wei Wang et al., 2005b)
	Hospital wastewater	20	3	-	-	-	
	Domestic sewage	20	3	-	-	-	
	Dechlorinated tap water	20	3	-	-	-	
	Phosphate buffer saline	20	14	-	-	-	
	Hospital wastewater	4	14	-	-	-	
	Domestic sewage	4	14	-	-	-	
	Dechlorinated tap water	4	14	-	-	-	
	Phosphate buffer saline	4	14	-	-	-	
	Feces	20	3	-	-	-	
	Urine	20	17	-	-	-	

^a TGEV, transmissible gastroenteritis virus; MHV, mouse hepatitis virus; SARS-CoV, severe acute respiratory syndrome coronavirus.

^b Projected value.

^c Determined by RT-PCR.

of Φ6 under these conditions was highly variable, from 24 min to 117 days. Significant factors included temperature, biological activity, and the composition of the test media. Beyond direct study findings, the authors reported that the aqueous stability of enveloped viruses in water matrices was highly variable, and a single surrogate was insufficient to capture the behavior of all enveloped viruses (Aquino de Carvalho et al., 2017).

Given the limited available data on SARS-CoV-2 in water matrices, it may also be informative to consider recent reports of viral persistence on surfaces. van Doremalen et al. (2020) evaluated the surface stability of SARS-CoV-2 compared to SARS-CoV. The half-life of SARS-CoV-2 varied from 0.8 h on copper to 6.8 h on plastic. The authors also identified comparable environmental persistence between the two viruses (van Doremalen et al., 2020). Chin et al. (2020) also reported on the surface persistence and disinfection of SARS-CoV-2. The authors identified a high temperature dependence on the inactivation kinetics, and rapid removal of the virus using bleach, ethanol, benzylalkonium chloride,

povidone-iodine, and chloroxylenol (Chin et al., 2020). Overall, these limited results suggest that previous data on CoVs are likely to be useful for informing the environmental persistence of SARS-CoV-2, and that SARS-CoV-2 is likely rapidly inactivated under increased temperature and by major disinfectants. In fact, a number of the existing disinfectant products have been approved by the United States Environmental Protection Agency (USEPA) for use against SARS-CoV-2 (USEPA, 2020).

7. Respiratory viruses in wastewater and the occupational risk

In the field of environmental virology, the focus on waterborne transmission has been primarily on enteric viruses. However, respiratory viruses including adenoviruses, coxsackieviruses, and indeed CoVs have been known to occur in wastewater (Sinclair et al., 2008; Xin Wei Wang et al., 2005a) and wastewater-polluted waters (Wigginton et al., 2015). Going back to early descriptions, it has been known that these viruses cause diarrheal as well as respiratory diseases

Table 5
Epidemiological studies of health effects for wastewater treatment plant workers.

Study description ^a	No. of individuals evaluated	Results	Reference
WWTP workers in 11 cities in Northern Ohio were evaluated via questionnaire for a 12-month study; controls were college maintenance and refiner workers.	150 WWTP workers vs. 54 controls	The WWTP workers had significantly higher gastroenteritis, abdominal pain, and headaches. No significant differences were reported for respiratory and other symptoms.	(Khuder et al., 1998)
WWTP workers in 67 plants in the Netherlands were evaluated via questionnaire for a 12-month study; no controls; personal endotoxin exposure was assessed (8 h measurements: $n = 460$).	468 WWTP workers	Dose-response relationships were found with endotoxin levels for: "lower respiratory and skin symptoms", "flu-like and systemic symptoms", and "upper respiratory symptoms".	(Smit et al., 2005)
WWTP workers in Iowa were evaluated via questionnaire for a 3-year study; controls were workers at water treatment plant (WTP) workers; endotoxins sampled as an exposure indicator.	93 WWTP workers vs. 54 WTP worker controls	Odds ratios were statistically higher for respiratory, ocular and skin irritation, neurology, and gastrointestinal symptoms in WWTP workers. Tasks related to sludge handling were identified as high-risk.	(Lee et al., 2009)
A 5-year study conducted in Switzerland; controls were gardeners, waterway maintenance, public transport, and forestry workers.	247 WWTP workers; 52 solid waste workers vs. 304 controls	No effects for occupational exposure to bioaerosols were reported.	(Tschopp et al., 2011)

^a WWTP, wastewater treatment plant; WTP, water treatment plant.

(Britton, 1980) but limited study of viral respiratory diseases has been performed in the wastewater context. There are fewer data available on the presence and concentrations of respiratory viruses in wastewater. Fong et al. (2010) found in addition to enteric adenoviruses 40 and 41 (type F) in sewage, combined sewer overflows and rivers receiving these discharges contained respiratory adenoviruses 2 and 3 (types C and B) as well as adenovirus 12 that causes meningoencephalitis with initial replication in the gastrointestinal or respiratory tract (Fong et al., 2010).

There is no doubt that swimming in sewage-contaminated waters is associated with respiratory disease; however, the etiological agent is not frequently identified (Wade et al., 2010). Studies on the Great Lakes suggest this could be due to adenoviruses (Wong et al., 2009). Respiratory disease as an occupational risk for sewage workers has also been studied with mixed results. Four key studies were reviewed as shown in Table 5. In Switzerland, no health impacts were found in garbage collectors and wastewater treatment plant (WWTP) workers (Tschopp et al., 2011). However, in three other investigations, gastrointestinal effects were observed, and two of the three studies noted respiratory health impacts (Khuder et al., 1998; Lee et al., 2009; Smit et al., 2005).

8. Quantitative microbial risk assessment (QMRA) for respiratory viruses and SARS-CoV-2

Environmental engineering and science, and in particular QMRA have major roles to play in reducing the impact of the current COVID-19 outbreak (Haas, 2020; Wigginton and Boehm, 2020). The process of QMRA involves relating an environmental concentration of an infectious agent to an exposure dose and subsequently a probability of developing an infection or illness (Haas et al., 2014). Gaps needed to fill include characterizing persistence, fate, and transport (including airborne transport and deposition, for example), and exposure to be able to define the risk. In addition to basic questions like "what is the risk" (potentially in relation to some baseline) for a particular context, QMRA can be used to address questions for SARS-CoV-2 such as: (i) What ventilation/air exchange rate is recommended for different settings (e.g. workplace, healthcare facility) to prevent transmission consistent with a risk target?; (ii) Is a 6-ft/2 m "social distance" protective enough?; (iii) What should surface disinfection targets be for different settings and what are the best technologies or disinfectants for achieving these targets (e.g. UV light)?; (iv) What wastewater treatment disinfection targets might be needed?

Table 6
Presence of viruses in aerosols at wastewater treatment plants.

Virus	Detection method	Plant description ^c	Country	Wastewater levels ^d	Aerosol levels	Remark	Reference
Coliphages	Plaque assay ^a	Aeration basin - lagoons	USA	3.25×10^3 to 5.53×10^5 PFU/L	<1 to 9 PFU/m ³		(Brenner et al., 1988)
Coliphages	Plaque assay-MPN ^b	Two-stage aeration basins	USA	Not reported	5.0×10^{-3} to 7.6×10^{-2} MPN PFU/m ³		(Fannin et al., 1985)
Somatic coliphages	Plaque assay	7 WWTPs	Finland	Not reported	Up to 380 PFU/m ³		(Heinonen-Tanski et al., 2009)
F-specific coliphages	Plaque assay	7 WWTPs	Finland	Not reported	Up to 70 PFU/m ³		(Heinonen-Tanski et al., 2009)
Adenoviruses	qPCR	79 WWTPs	Switzerland	Not reported	Up to 2.27×10^6 copies/m ³	104/123 (84%) air samples positive	(Masclaux et al., 2014)
Adenoviruses, norovirus GI and GII, FRNA bacteriophages GIII, enteroviruses	qPCR	1 WWTP activated sludge chamber, exhaust duct, and treated air	Japan	Up to 2.5×10^7 copies/L (norovirus GI)	Up to 3.2×10^3 copies/m ³	Air samples positive for adenovirus (4/16), norovirus GI (6/16), FRNA bacteriophages GIII (3/16), and enteroviruses (3/16)	(Matsubara and Katayama, 2019)

^a Three different *E. coli* strains (ATCC 13706, 15,597, and 11,303) were used as hosts.

^b MPN, most probable number.

^c WWTP, wastewater treatment plant.

^d PFU, plaque-forming unit.

SARS-CoV-2 transmission is known to occur via hand-to-face (nasal-pharyngeal: eyes, nose, and mouth) contact with contaminated fomites, and inhalation of aqueous aerosols including coughs. The fecal-oral route or aspiration have been postulated as potential exposure routes, although no cases of transmission via the fecal-oral route have been reported to date (CDC, 2020b; Y. Wu et al., 2020a; Yeo et al., 2020). There are some preliminary data to suggest that the virus is shed longer from the digestive tract than the respiratory tract (Hu et al., 2020).

In general for respiratory viruses, a review by Van Leuken et al. (2016) highlighted the ability of bioaerosols, particularly from farming and wastewater exposures, to carry infectious agents, including viruses, and to present disease risks at considerable distances from the source (Van Leuken et al., 2016). Additionally, numerous exposures are possible at close-range, especially for occupational populations (e.g. wastewater workers and nurses). Many QMRAs have also focused on wastewater biosolids applications, addressing adenovirus, astrovirus, coxsackievirus, echovirus, enterovirus, hepatitis A virus, hepatitis E virus, norovirus, and rotavirus, both during the actual period of application as well as at various exposures from farm to fork (Hamilton et al., 2020). SARS-CoV-2 can be spread and persist in aerosols, hence measurement in wastewater aerosols would be informative for risk assessment (Bushmaker et al., 2020; van Doremalen et al., 2020; Guo et al., 2020).

A key component of bioaerosol QMRA is modeling the dispersion of aerosols and/or transfer of microorganisms from water to air. A key study of stormwater reuse for inhalation-ingestion of adenovirus and norovirus provides an example of considering the aerosol size profile of a particular activity in order to calculate the number of viruses aerosolized and the subsequent deposited dose (Lim et al., 2015). Methods utilized for other microbial risk studies are the use of a water-to-air transfer coefficient or computational fluid dynamics approaches (Hamilton and Haas, 2016). The studies of viral aerosols emerging from wastewater facilities have often focused on coliphage as an indicator for human pathogenic viruses, but most studies have not simultaneously sampled the wastewater and the aerosols produced, or identified how the viruses are distributed in aerosols with respect to the aerosol size profile (Table 6). Fannin et al. (1985) were not able to detect animal viruses in the aerosols (Fannin et al., 1985). Adenoviruses are known to be quite stable in air and high concentrations were found by qPCR, thus viable viruses were not addressed (Masclaux et al., 2014). The phage data suggest a 10,000-fold level of dilution and inactivation (Brenner et al., 1988). There has been no published study to date testing SARS-CoV-2 in aerosols from wastewater facilities, but a recent laboratory-scale study on persistence of coronaviruses in aerosols revealed that SARS-CoV-2 could maintain its infectivity in aerosols

for up to 16 h (Fears et al., 2020), suggesting potential human exposure if wastewater aerosols contain viable SARS-CoV-2. Further investigations are needed to elucidate the presence of SARS-CoV-2 and its viability in wastewater bioaerosols and associated public health risks.

A recent QMRA study for enteric viruses via exposure to wastewater bioaerosols focused on adenoviruses as the hazards (Carducci et al., 2018). The analysis highlighted the following: (i) workers at highest risk were related to exposures at the influent and biological oxidation tanks for >3 min; (ii) adenovirus concentrations drove the risk; (iii) risks of 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} were related to levels of 565, 170, 54 and 6 copies/m³ in the bioaerosol, respectively; (iv) this relates to an estimated level of approximately 10^4 to 10^6 copies/L in the oxidation tank. Similarly, a recent study focusing on rotavirus and norovirus bioaerosol exposures to WWTP workers noted risks that exceeded common public health risk benchmarks of 10^{-4} infections or 10^{-6} disability adjusted life years (DALY) per person per year for airborne concentrations above the aeration tank of 27 and 3,099 viruses/m³-h, for rotavirus and norovirus, respectively (Pasalari et al., 2019). Taken together, these data help to provide a comparison of relative risks and put concentrations of viruses into perspective.

To date, few QMRAs for CoVs have been conducted including one for MERS-CoV in a hospital setting (Adhikari et al., 2019) and another for a residential bathroom exposure (Watanabe et al., 2010). Adhikari et al. (2019) made use of a SARS-CoV dose-response model to examine the impact of a coughing patient and resulting exposures to nurses, healthcare workers, and family visits. Mean daily risks were on the order of $\sim 10^{-4}$ and were highest for healthcare workers and nurses compared to family visitors and patients in the same room. The concentration of viruses in saliva were a driving variable in the Monte Carlo analysis, and respiratory masks were a more effective intervention than increasing the air exchange rate. Watanabe et al. (2010) developed new SARS-CoV dose-response models and reconstructed a dose observed during a 2003 Hong Kong apartment complex outbreak due to exposures to sewage via sewer gas entering through bathroom floor drains and U-traps after a toilet flush under negative pressure by high-capacity bathroom fans. The authors made use of epidemiological attack rate data (ranging from 0.038 to 0.144) by floor of a building where 99 cases of SARS were reported (Li et al., 2005) to arrive at estimates of 16–49 plaque-forming units (PFU) on the first floor, 63–160 PFU for residents on a middle floor, and 42–117 PFU for residents on upper floors, with higher attack rates on higher floors likely due to air flow in the building. Tabulating attack rates for SARS-CoV-2 could provide similarly useful information for estimating doses and/or risks in retrospect; however such an exercise requires information on an

Table 7
QMRA parameters of SARS-CoV-2 and relevant respiratory viruses (SARS-CoV, MERS-CoV, and influenza viruses).

Parameter	SARS-CoV-2	SARS-CoV	MERS-CoV	Influenza virus	Reference
Dose response (see Table 8)	Not available	Available	Available	Available	(Huang et al., 2018; Kitajima et al., 2011; Lunn et al., 2019; QMRA Wiki, 2020a, 2020b; Watanabe et al., 2010)
Excretion in saliva (copies/mL)	9.9×10^2 – 1.2×10^8	7.08×10^3 – 6.38×10^8	7 – 2.02×10^5 (Avg.: 4.17×10^4)	10^1 – 10^7	(Adhikari et al., 2019; Sueki et al., 2016; To et al., 2020a, 2020b; Wang et al., 2004)
Feces (copies/g-feces)	Up to 10^8 copies/swab	5.1×10^1 – 10^7	Up to 10^3	$10^{3.7}$ – 10^6	(Chan et al., 2011; Cheng et al., 2004; Drosten et al., 2013; Hung et al., 2004; Isakbaeva et al., 2004; D. Wang et al., 2020; Wigginton and Boehm, 2020; Wölfel et al., 2020)
Urine (copies/mL)	ND	Up to $10^{4.4}$	Up to 2.69×10^3	Not available	(Drosten et al., 2013; Hung et al., 2004; W. Wang et al., 2020c)
Nasal swabs (copies/mL)	1.4×10^6 – 1.5×10^7	2.47×10^4 – 6.97×10^7	Up to $10^{3.7}$	$10^{2.7}$ – $10^{9.3}$ (varies by strain)	(Drosten et al., 2013; Lee et al., 2009; Ngaosuwanikul et al., 2010; D. Wang et al., 2020b; Wong et al., 2005; Zou et al., 2020)
Attack rate (%)	Up to 80	<1–100 depending on scenario	0.42–15.8	5–30	(Burke et al., 2020; Park et al., 2018; Verity et al., 2020; WHO, 2011, 2019)
Case fatality rate (CFR) (%)	5.3–8.4	Up to 50; most estimates ~9–17%	34.4–69.2	<1 (seasonal flu)–60 (H5N1)	(Jung et al., 2020; Li et al., 2008; Park et al., 2018; C. Wang et al., 2020a; WHO, 2003, 2011; J. Y. Wong et al., 2013b)
Basic reproduction number (R_0)	1.4–6.5	2–5	0.45–8.1	1.7–2.8 (varies by strain)	(Boldog et al., 2020; Cheng and Shan, 2020; Coburn et al., 2009; Jung et al., 2020; Lai et al., 2020; Liu et al., 2020; Park et al., 2018; B. Tang et al., 2020; Wallinga and Teunis, 2004; WHO, 2020e)
Incubation period (days)	2–14	2–10	4.5–7.8	1–4	(CDC, 2004; Lessler et al., 2009; Linton et al., 2020; Park et al., 2018; WHO, 2018)

Table 8
Dose-response parameters available and sporadic dose-response data where there are limited models and gaps.

Virus	Dose units ^a	Host type	Exposure route	No. doses	Model ^b	Parameters ^{c,d}	Health endpoint/ response	N ₅₀	Remarks	Reference
SARS-CoV-2	-	-	Intranasal (Bao et al. 2020a, 2020b, Blanco-Melo et al. 2020), ocular inoculation and intratracheal (Deng et al. 2020), intratracheal and intranasal (Rockx et al. 2020)	-	-	-	-	-	Existing dosing experiments designed to infect all animals ranged from 10 ² TCID ₅₀ (mice)–10 ⁶ TCID ₅₀ (macaques)	(Bao et al., 2020a, 2020b; Blanco-Melo et al., 2020; Deng et al., 2020; Rockx et al., 2020)
SARS-CoV	PFU	Pooled transgenic mice, non-transgenic mice	Intranasal	8	E	$k = 2.45 \times 10^{-3}$	Death ^e	280		(QMRA Wiki, 2020a; Watanabe et al., 2010)
SARS-CoV	PFU	Transgenic mice	Intranasal	4	E	$k = 2.97 \times 10^{-3}$	Death	233		(QMRA Wiki, 2020a; Watanabe et al., 2010)
SARS-CoV	PFU	Mice	Intranasal	4	E	$k = 2.14 \times 10^{-3}$	Death	324		(QMRA Wiki, 2020a; Watanabe et al., 2010)
SARS-CoV	PFU	Rhesus macaques	Intratracheal	2	-	-	Infection	-	Monkeys: 2/2 infected at 10 ⁸ PFU	(Zhou et al., 2005)
MERS-CoV	TCID ₅₀	Mice	Intranasal	6	E	$k = 5.71 \times 10^{-3}$	Shedding/ mortality	≈ 121	Pooled endpoint	(Lunn et al., 2019)
MERS-CoV	PFU	Mice	Intranasal	3	-	-	Infection/ death	-	All animals infected: LD ₅₀ ~1–2×10 ⁴	(Douglas et al., 2018)
MERS-CoV	PFU	Mice	Intranasal	2	-	-	Infection/ death	-	Authors stated “sublethal” 5×10 ³ and “lethal” 5×10 ⁵ doses (no deaths in test animals observed; all sacrificed 4 days post inoculation)	(Leist et al., 2019)
MERS-CoV	TCID ₅₀	Rhesus macaques	Intratracheal	1	-	-	Death	-	0/4 died at 6.5×10 ⁷	(Yao et al., 2014)
MERS-CoV	TCID ₅₀	Rhesus macaques	Intratracheal	1	-	-	Infection	-	4/4 infected at 6.5×10 ⁷	(Yao et al., 2014)

Human coronavirus 229E	TCID ₅₀	Humans	Intranasal	4	E	$k = 5.39 \times 10^{-2}$	Illness (cold)	13		(Watanabe et al., 2010)
Animal coronaviruses (MHV-S, HEV-67N, IBVA-5968)	PFU or CD ₅₀	Mice, rats, chicks	Intranasal	3-6	E	$k = 8.78 \times 10^{-5} - 9.16 \times 10^{-2}$	Death	-8 - 5.95×10^5	Various coronavirus models fit for comparison with SARS	(Watanabe et al., 2010)
Influenza virus (H5N1)	PFU, TCID ₅₀	Mice	Intranasal	6	T	$\alpha = 4.640 \times 10^{-1}$; $J_0 = 3.015 \times 10^2$; $J_1 = 1.000$; $J_2 = 1.793$	Infection	$<10^{1.5} - >10^7$ (depending on strain)		(Kitajima et al., 2011)
Influenza virus (H5N1)	PFU, TCID ₅₀	Mice	Intranasal	7	T	$\alpha = 2.730 \times 10^{-1}$; $J_0 = 9.617 \times 10^4$; $J_1 = 2.7082$; $J_2 = 4.666$	Infection	$<10^{1.5} - >10^7$ (depending on strain)		(Kitajima et al., 2011)
Influenza virus (H5N1)	PFU, TCID ₅₀	Ferrets	Intranasal	2	T	$k_0 = -1.707 \times 10^{-1}$; $k_1 = -1.502 \times 10^{-1}$	Infection	$<10^{1.5} - >10^7$ (depending on strain)		(Kitajima et al., 2011)
Influenza virus (H5N1)	PFU, TCID ₅₀	Ferrets	Intratracheal	2	T	$k_0 = -1.480 \times 10^1$; $k_1 = -7.092$	Infection	$<10^{1.5} - >10^7$ (depending on strain)		(Kitajima et al., 2011)
Influenza virus (H1N1)	TCID ₅₀	Human	Intranasal	4	B	$\alpha = 9.04 \times 10^1$	Infection	1.25×10^6		(QMRWiki, 2020b)
Influenza virus (H1N1)	TCID ₅₀	Human	Intranasal	9	B	$\alpha = 5.81 \times 10^1$	Infection	9.45×10^5		(QMRWiki, 2020b)
Influenza virus (H3N2)	TCID ₅₀	Human	Intranasal	5	B	$\alpha = 4.29 \times 10^1$	Infection	6.66×10^5		(QMRWiki, 2020b)
Influenza virus (H5N1)	EID ₅₀	Mice	Intranasal	6	E	$k = 1.09 \times 10^{-2}$,	Death	6.38×10^1		(QMRWiki, 2020b)
Influenza virus (H1N1, H3N2)	TCID ₅₀	Human	Intranasal	4-5	B	$\alpha = 1.57 \times 10^{-1} - 9.05 \times 10^{-1}$; for fixed parameters ($\alpha = 2.95 \times 10^{-1}$; $N_{50} = 4.42 \times 10^5$) attenuation parameter $\gamma = 1.07 \times 10^{-3}$	Infection	Children $3.3 \times 10^2 - 1.2 \times 10^5$; Adults $2.7 \times 10^4 - 1.2 \times 10^6$	Pooled data analysis from 11 datasets with respect to virus subtype (H1N1 or H3N2), attenuation method (cold-adapted or avian-human gene reassortment), and human age (adults or children)	(Watanabe et al., 2012)
Influenza virus (H3N1, H1N1, influenza A, influenza B)	HI titer	Various	Various	Various	B-HI	$\lambda = 0.002 - 0.245$	Various	Depends on HI titer	Authors developed a relationship between HI titer and protection against influenza virus	(Huang et al., 2018)

^a PFU, plaque forming unit; TCID₅₀, median tissue culture infectious dose; EID₅₀, median egg infectious dose, HI, hemagglutination inhibition.

^b E, exponential model; B, Beta-Poisson model; T, dose response time model; B-HI, modified Beta-Poisson model to include a parameter for hemagglutination inhibition titer.

^c Best fit dose response model parameters are given in table (where a model was not available, available information relating dose to an outcome in an animal or human model is provided); ID₅₀, median infectious dose; LD₅₀, median lethal dose.

^d The N₅₀ represents the median dose associated with a particular health endpoint.

^e Watanabe et al. (2010) considered the animal death endpoint to be representative of a SARS-CoV human illness in the dose response model.

environmental measurement coupled with the total number of persons exposed in addition to the number of individuals infected and/or ill. Given the parameters developed in the existing QMRA models, a summary of parameters for potential use in QMRA models and data gaps are summarized in Table 7.

9. Dose-response of SARS-CoV-2 and relevant respiratory viruses

To conduct a QMRA, a dose-response model is required providing the relationship between exposure and health outcome or endpoint. To date, no quantitative dose-response model is available for SARS-CoV-2. This is partially due to the absence of an appropriate animal model for pathogen dosing (Gralinski and Menachery, 2020). Contributing factors to the lack of appropriate animal model include the inability to cause disease without passing the virus through a mouse host and milder disease in primates compared to humans (Gralinski and Menachery, 2020). The search for an appropriate animal model for various applications (treatments and vaccines) is underway and several models are being explored, placing strains on the supply of transgenic laboratory mice (Boodman, 2020; Callaway, 2020; Warren, 2020).

Existing animal models for SARS-CoV and MERS-CoV include non-human primates (macaques, cynomolgus monkeys, African green monkeys, rhesus macaques, and common marmosets), hamsters, ferrets, and transgenic mice (Gretebeck and Subbarao, 2015). A recent study by Rockx et al. (2020) indicated that SARS-CoV-2 results in a severity of infection that is intermediate between that of SARS-CoV and MERS-CoV based on a direct comparison of the three viruses in a combined intratracheal and intranasal dosing study of female adult cynomolgus macaques with a dose of 10^6 TCID₅₀ for all viruses. Shedding varied depending on the age of the animal, with higher levels detected in nasal swabs of aged animals compared to younger animals (Rockx et al., 2020).

The reported viral doses in current animal model experiments underway for SARS-CoV-2 are 10^4 PFU – $10^{6.5}$ TCID₅₀ in ferrets (Blanco-Melo et al., 2020; Kim et al., 2020; Richard et al., 2020), 10^2 – 10^5 TCID₅₀ in mice (Bao et al., 2020b; S. Xia et al., 2020a), 10^5 PFU in hamsters (Chan et al., 2020), and 10^5 – 10^6 TCID₅₀ in macaques (Bao et al., 2020a; Deng et al., 2020; Munster et al., 2020). These studies generally indicated 100% infection or isolation of viral RNA from animals at the inoculated dose and therefore it is not possible to designate a median infectious dose (ID₅₀) or median lethal dose (LD₅₀). Xia et al. (2020a) reported 100% mortality in 12 newborn mice challenged intranasally with 10^2 TCID₅₀ (J. Xia et al., 2020b). Deng et al. (2020) reported that macaques infected with 10^6 via an ocular (2/3) or intratracheal (1/3) route of exposure had a positive viral load in nose and throat swabs from 1 to 7 days post inoculation, supporting reports of ocular transmission reported for a healthcare worker infected with SARS-CoV-2 while working with a patient without eye protection (Lu et al., 2020; S. Xia et al., 2020a). Previously infected rhesus macaques challenged intratracheally with SARS-CoV-2 at 10^6 TCID₅₀ did not display reinfection characteristics when challenged again with the same dose, indicating some immunity conferred from an initial infection (Bao et al., 2020a).

Sufficient data are not available for modeling or pooling dose groups from multiple studies at this time that meets the criteria of (i) >3 unique dose groups and (ii) at least three unique responses. A lack of a dose-response model for SARS-CoV-2 is a critical gap for conducting QMRA for this pathogen. Emerging areas for dose-response testing include dosing of organoids to represent aspects of specific pathogenesis processes such as liver damage (Zhao et al., 2020); however, these approaches have not been reconciled with existing quantitative dose-response modeling calculations as they do not fully encapsulate the ability to represent host immune processes (Haas, 2015).

Existing dose-response models are available for SARS-CoV (QMRA Wiki, 2020a; Watanabe et al., 2010), MERS-CoV (Lunn et al., 2019), and various influenza virus strains (Huang et al., 2018) including

H5N1 (Kitajima et al., 2011; QMRA Wiki, 2020b), H1N1 (QMRA Wiki, 2020b; Watanabe et al., 2012), and H3N2 (QMRA Wiki, 2020b; Watanabe et al., 2012) subtypes, but not SARS-CoV-2 (Table 8). Available health endpoints (infection or death) varied for these models with infection endpoints available for influenza virus but not MERS-CoV or SARS-CoV, and death endpoints available for influenza virus and SARS-CoV. Modelled LD₅₀ for SARS-CoV ranged from 233 to 324 PFU, compared to estimates ranging from <32 – 10^7 PFU for H5N1 highly pathogenic avian influenza virus (Kitajima et al., 2011; QMRA Wiki, 2020b). Modelled ID₅₀ for influenza virus ranged from 6.66×10^5 to 1.25×10^6 TCID₅₀ (QMRA Wiki, 2020b). The MERS-CoV median infectious/lethal dose calculated from Lunn et al. (2019) by solving the exponential model using $-\ln(0.5/k)$ (authors specified a pooled analysis with both endpoints) would be approximately 121 PFU.

Previously, “sublethal”, “moderate”, and “lethal” concentrations of MERS-CoV of 5×10^3 , 5×10^4 , and 5×10^5 PFU, respectively, have been used for a mouse animal model (Leist et al., 2019), but concentrations of 6.5×10^7 TCID₅₀ or 10^8 PFU were not lethal in macaques (Yao et al., 2014; Zhou et al., 2005). The lowest dose used in SARS-CoV-2 experiments designed to infect all animals was 10^2 TCID₅₀, indicating the ID₅₀ might be lower than that value, although this cannot be determined with certainty at this time. Chen et al. (2020a, 2020b) indicated that in a placebo (unvaccinated) group of SARS-CoV-dosed macaques, 3/4 dosed animals at 10^5 TCID₅₀ displayed respiratory distress or alveolar damage after virus inoculation and 3/4, 4/4, and 2/4 had quantifiable viral RNA in pharyngeal swab samples at day 2, 5, and 7, respectively (Y. Chen et al., 2020a). These modeling efforts and sparse animal model experimental data that could not be modelled generally indicated that SARS-CoV and SARS-CoV-2 are more infectious than influenza virus, with SARS-CoV-2 potentially more infectious than SARS-CoV. MERS-CoV had uncertain infectivity but overlapped with ranges for SARS-CoV and SARS-CoV-2. For a death endpoint, LD₅₀ values were higher for influenza virus compared to SARS-CoV (indicating SARS-CoV is more deadly), and MERS-CoV estimates were again overlapping the influenza virus and SARS-CoV values. This is roughly comparable with the severity of infection ranking provided by Rockx et al. (2020) of SARS-CoV > SARS-CoV-2 > MERS-CoV.

10. Knowledge gaps and research needs

At present, significant knowledge gaps exist on the potential role of wastewater in the transmission of SARS-CoV-2. Survival of SARS-CoV-2 in environmental media, including wastewater and water, remains mostly unknown. Recent data indicate that the stability of SARS-CoV-2 is similar to that of SARS-CoV in aerosols and on surfaces (van Doremalen et al., 2020). Using a similar approach, the stability of SARS-CoV-2 in various water matrices should be investigated. The persistence of SARS-CoV-2 needs to be determined in wastewater and environmental water for tropical, sub-tropical and temperate climatic zones as the persistence may be highly variable in different temperatures, as demonstrated in a recent study (Hart and Halden, 2020). Moreover, the persistence of SARS-CoV-2 in wastewater and receiving waters and inactivation mechanisms, such as predation, UV, sunlight, and disinfection should be investigated. Data on SARS-CoV-2 removal and/or inactivation by wastewater and water treatment processes, such as activated sludge, membrane filtration, coagulation-sedimentation, and disinfection (chlorine, chloramine, UV, ozone, etc.) is scarce. If it is difficult to determine log₁₀ reduction values of SARS-CoV-2 itself due to availability of the virus and/or biosafety restrictions, model enveloped virus such as human CoVs, MHV, or *Pseudomonas* phage $\Phi 6$ can be used for laboratory- or pilot-scale experiments.

Currently RT-qPCR assays developed for clinical specimen testing are being used for SARS-CoV-2 RNA detection in environmental water samples. Recent environmental studies reported that different assays might produce conflicting results (Ahmed et al., 2020; Medema et al., 2020). Moreover, the false-negative rates (due to improperly designed

primers/probe or virus mutation in the targeted genome region) of these assays need to be assessed by multiple laboratories. For environmental application, the sensitivities of these RT-qPCR methods need to be evaluated. The major limitation of qPCR is that it does not provide information on viability. When viability needs to be assessed, cell culture infectivity assay, EMA/PMA-RT-qPCR, and ICC-RT-qPCR may provide useful information. A standardized protocol to recover and detect SARS-CoV-2 from environmental water samples, including concentration method, PCR assay, and process controls, should be established.

Wastewater surveillance is critical as WBE may provide valuable information on the prevalence of infections in the community. Continuous and systematic monitoring of wastewater may provide early warning signs and will potentially identify undiagnosed or successive disease at the population level, thus alerting public health officials on the ongoing or future viral disease outbreaks. Nationwide and international wastewater surveillance campaigns should be carried out to better understand temporal and spatial dynamics of disease prevalence, molecular epidemiology and evolution of the virus, and efficacy of public health interventions.

QMRA has a potential role to play in reducing the impact of the ongoing COVID-19 outbreak. However, currently available QMRA parameters for SARS-CoV-2 are limited, although previous studies on relevant respiratory viruses (SARS-CoV, MERS-CoV, and influenza viruses) help to assess the likely risks of SARS-CoV-2. Our understanding on the potential role of wastewater in SARS-CoV-2 transmission is largely limited by knowledge gaps in its occurrence and survival in wastewater and environmental waters and removal by wastewater treatment processes. There is an urgent need for collecting these pieces of information to understand and mitigate the human health risks associated with exposure to wastewater and environmental waters potentially contaminated with SARS-CoV-2.

Declaration of competing interest

The authors declare no conflict of interest.

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