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Review

Wastewater surveillance to infer COVID-19 transmission: A systematic review

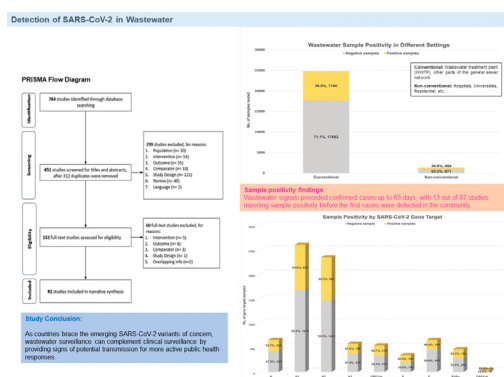
Shimoni Shah¹, Sylvia Xiao Wei Gwee¹, Jamie Qiao Xin Ng, Nicholas Lau, Jiayun Koh, Junxiong Pang^{*}

Saw Swee Hock School of Public Health, National University of Singapore and National University Health System, Singapore 117549, Singapore
Centre for Infectious Disease Epidemiology and Research, National University of Singapore, Singapore 117549, Singapore

HIGHLIGHTS

- Overall wastewater positivity was 29.2% (sewage system: 28.9%, others: 34.8%).
- While N gene was the most common, S gene had maximum sample positivity (80.5%).
- Positive samples detected before first case in 13 studies (2–63 days before).
- Positive signals typically anticipate cases by 10 days minimally (24 studies).

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 17 May 2021

Received in revised form 27 August 2021

Accepted 27 August 2021

Available online 8 September 2021

Editor: Jay Gan

Keywords:

COVID-19

Wastewater

Epidemiological factors

Systematic review

ABSTRACT

Successful detection of SARS-CoV-2 in wastewater suggests the potential utility of wastewater-based epidemiology (WBE) for COVID-19 community surveillance. This systematic review aims to assess the performance of wastewater surveillance as early warning system of COVID-19 community transmission. A systematic search was conducted in PubMed, Medline, Embase and the WBE Consortium Registry according to PRISMA guidelines for relevant articles published until 31st July 2021. Relevant data were extracted and summarized. Quality of each paper was assessed using an assessment tool adapted from Bilotta et al.'s tool for environmental science. Of 763 studies identified, 92 studies distributed across 34 countries were shortlisted for qualitative synthesis. A total of 26,197 samples were collected between January 2020 and May 2021 from various locations serving population ranging from 321 to 11,400,000 inhabitants. Overall sample positivity was moderate at 29.2% in all examined settings with the spike (S) gene having maximum rate of positive detections and nucleocapsid (N) gene being the most targeted. Wastewater signals preceded confirmed cases by up to 63 days, with 13 studies reporting sample positivity before the first cases were detected in the community. At least 50 studies reported an association of viral load with community cases. While wastewater surveillance cannot replace large-scale diagnostic testing, it can complement clinical surveillance by providing early signs of potential transmission for more active public health responses. However, more studies using standardized and validated methods are required along with risk analysis and modelling to understand the dynamics of viral outbreaks.

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^{*} Corresponding author at: Saw Swee Hock School of Public Health, National University of Singapore and National University Health System, Singapore 117549, Singapore.

E-mail addresses: ephssu@nus.edu.sg (S. Shah), ephsgxw@nus.edu.sg (S.X.W. Gwee), e0439226@u.nus.edu (J.Q.X. Ng), niclauwh@nus.edu.sg (N. Lau), ephkj@nus.edu.sg (J. Koh), ephjiv@nus.edu.sg (J. Pang).

¹ Authors contributed equally.

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1. Introduction

Wastewater surveillance, commonly used to monitor the epidemiology of poliovirus and noroviruses, is a potential approach for community surveillance of COVID-19 (Kitajima et al., 2020). Established evidence on fecal shedding of SARS-CoV-2 by patients and successful virus genomic detection in wastewater studies suggest the applicability of this surveillance technique (Wang et al., 2020). Monitoring of sewage for detecting pathogens has been ongoing for over 40 years especially for poliovirus (Sinclair et al., 2008). Past studies have established that small outbreaks and epidemics of enterovirus and adenovirus disease within a community can be predicted by monitoring a community's sewage (Sinclair et al., 2008). Virus during a Coxsackievirus B5 outbreak was detected 10 days before clinical cases were detected while the Public Health Laboratories of Israel have been conducting an environmental surveillance of sewage to assess the spread of wild type poliovirus, on a monthly basis since 1989 (Nelson et al., 1967; Manor et al., 1999). This helped in the detection of the most recent outbreak of wild poliovirus type 1 in 2013–14 in Rahat, Israel (Brouwer et al., 2018). Sensitivity of sewage surveillance has also been validated in Mumbai, India wherein the wild type poliovirus was detected three months before any clinical cases were observed (Deshpande et al., 2003). Viruses causing gastroenteritis can also be detected in wastewater with only a few infected people. Hellmer et al., concluded that detection of pathogenic viruses in sewage provided early warnings of Hepatitis A virus and Norovirus outbreaks even before the causative pathogens were recognized in health care (Hellmér et al., 2014; La Rosa et al., 2014). Wastewater surveillance can also help predict the prevalence of certain types of disease in the community better than clinical data obtained from hospitalizations, like non-polio EV infections wherein EV-A and EV-C tend to produce subclinical infections that do not require hospitalization (Hellmér et al., 2014; Bisseux et al., 2020). These studies highlighted that sustained wastewater surveillance could be used to assess the introduction of a new infectious agent in the community.

The ongoing COVID-19 pandemic is caused by newly diagnosed SARS-CoV-2 virus, which has been detected in feces and urine of infected patients (Agrawal et al., 2021a). One of the public health challenges in this pandemic has been to implement high coverage and timely COVID-19 testing on populations, and the mainstay has been to conduct laboratory-based diagnostic testing on individuals, whether be it with Polymerase Chain Reaction (PCR) or serological tests. The

use of wastewater surveillance as an early warning system to monitor the appearance and resurgence of COVID-19 is commonly suggested along with its use is in tailoring containment and mitigation measures and determining target populations for testing (Aguiar-Oliveira et al., 2020). Wastewater surveillance has a potential advantage of being able to predict the overall status of a given catchment area and being able to include asymptomatic individuals with much less effort compared to clinical surveillance. Given the median duration of virus persistence in feces exceeds that in respiratory samples (Wang et al., 2020), the probability of viral RNA detection in wastewater may be greater than clinical testing. Wastewater surveillance can complement the surveillance pyramid by providing mass monitoring via a low-cost, efficient and non-invasive approach. It can also shed light on prevalence rates 'hidden' by asymptomatic infections, poor health-seeking behaviour as well as in settings with low diagnostic capacity (Lodder and de Roda Husman, 2020).

There is still limited systematic synthesis of the progress made in applying wastewater surveillance to monitor COVID-19 trends exclusively, but rather reviews on the different methodologies used by various research groups and application of wastewater surveillance for SARS-CoV-2 along with other waterborne pathogens (Zahedi et al., 2021; Bivins et al., 2020). While we came across 22 reviews in our search, only one review by Li et al. was a systematic review conducted until Jan 2021 (Hamouda et al., 2021). This systematic review can complement Li et al.'s work and assess the performance of wastewater surveillance in conducting qualitative or quantitative risk assessment of COVID-19 community cases until July 2021, to help understand the potential of wastewater surveillance in driving public health decisions.

2. Material and methods

2.1. Search strategy

This systematic review was conducted in line with Cochrane's Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A systematic search was conducted in Pubmed, Medline and Embase for published literature. Additionally, grey literature on wastewater surveillance of COVID-19 were taken from the Wastewater-based epidemiology Consortium Registry which listed resources from COVID-19 dashboards/government websites. (<https://www.covid19wbec.org/covidpools19>). Keywords such as "wastewater",

“COVID-19”, “SARS-CoV-2”, “fecal/urine” and “surveillance/detection” were used in the search to identify peer-reviewed articles on wastewater surveillance published between 1st January 2020 and 4th November 2020. An updated search for only published literature was conducted from 4th November 2020 until 31st July 2021. The publications were imported and managed in EndnoteX9 during the screening process. Publications identified were screened for their title & abstracts, and subsequently their full text according to eligibility criteria drawn out with the PICOS tool. The inclusion criteria are as follows:

- Population: Any population-based community in a geographical region or groups within public/private facilities (including hospitals and cruise ships) that have a sewage system
- Intervention: Wastewater epidemiology with any RT-PCR based COVID assay applied to wastewater (fecal &/or urine) samples, regardless of sampling and laboratory methodology. Excluding studies that do not exclusively look at wastewater surveillance sampling
- Comparator: Epidemiological case counts or trends within the defined community.
- Outcome: Any form of comparison (including graphical) or statistical reporting (e.g. Correlation or association/sensitivity & specificity) of wastewater surveillance results with epidemiological trends.
- Study Design: Cross-sectional or prospective, longitudinal (observational) studies, including technical/government reports and unpublished (non-peer-reviewed i.e. preprints) data, excluding newspaper reports.

Studies that conducted any form of wastewater surveillance and sampling were included if they fulfilled all of the aforementioned criteria. The following study types: newspaper reports, reviews, and all non-English language sources were excluded. All screening was done in duplicate by five authors (SS, JK, JN, NL and SXWG). Discrepancies, if any, were resolved by a third author and JP.

2.2. Data extraction

Data was extracted according to five main themes - study details, sample collection process, sample processing and testing, and various outcomes - by five authors (SS, JK, JN, NL, SXWG) independently in an excel sheet template. The following data fields were condensed and presented in this manuscript: data collection period, country, region, population served, sampling site, sample type, sample collection approach, PCR assays used, kit names, gene targets, viral load recovered, number of positive samples, quantitative/qualitative association, temporal trends. To determine sample positivity, the overall number of samples was extracted as the denominator as much as possible. Amount of SARS-CoV-2 recovered was extracted in terms of gene copies as well as Ct values. For studies that presented sample positivity in terms of sites or days, each site or day was considered a positive sample. Outcome measures including samples positive and viral load are also stratified by gene target subject to data availability. Detailed information on the aforementioned fields, sample processing, sequencing and modelling can be found in the supplementary material (Appendix A: Supplementary Tables 1–3). This review defined grab sampling as sample collected at a single time point, and composite sampling as a sample merged from sampling over multiple time points, regardless of usage of autosampler or manual sampler. Settings other than WWTP and sewage systems are considered as settings outside of the conventional sewage system in this review.

2.3. Quality assessment

Given the novelty of wastewater surveillance as an epidemiological tool, there was no precedent in risk assessment for this study's reference. In order to assess the methodological quality of included studies, this review piloted a risk assessment tool adapted from Bilotta et al.'s

work on quality assessment tools for environmental science and CDC guidelines on wastewater surveillance (Bilotta et al., 2014; CDC, 2021). Themes from Bilotta et al.'s work were adopted to form the framework of this assessment: 1) study design (selection and performance bias), 2) measurement of outcome (assessment bias), and 3) bias linked to clarity and publication bias. The tool comprises of nine signaling questions, modified according to CDC guidelines on sample collection, processing and testing. There are four questions in the study design domain, and three questions in the measurement of outcome domain and two questions for the clarity domain. Studies were assigned as of “high” or “low” risk of bias in response to each signaling question. Due to the lack of technical expertise to assign numerical scores in accordance to the importance of each aspect in the methodology, the review authors did not establish an approach to derive the overall quality of each study nor deemed fit to confer a study a high risk of bias based on a single question. Quality of the studies was assessed on a domain basis, with any domain rendered at high risk of bias if at least one question in the domain is answered “high”.

Data extraction and risk assessment for each study was conducted by a single author, and further verified by another. Any disagreement was resolved by a third author and JP.

2.4. Role of the funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

3. Results

3.1. Literature search results & selected study characteristics

A total of 763 studies were identified from the database search. Following removal of 312 duplicates, 299 studies, including two non-English articles were excluded during the primary screening of title and abstract. Of the 152 studies selected for full-text review, 92 studies were shortlisted for qualitative synthesis (Fig. 1).

An overview of study characteristics is presented in Table 1. All 92 included studies reported testing on wastewater samples for SARS-CoV-2 – 87 were research studies on wastewater surveillance (Acosta et al., 2021a; Agrawal et al., 2021b; Ahmed et al., 2021a; Bar-Or et al., 2021a; Bertrand et al., 2021a; Betancourt et al., 2021a; Bhattarai et al., 2021a; Black et al., 2021a; Cao and Francis, 2021a; Chakraborty et al., 2021a; Chavarria-Miró et al., 2021a; Colosi et al., 2021a; D'Aoust et al., 2021a; Ciesielski et al., 2021a; Fernandez-Cassi et al., 2021a; Fongaro et al., 2021a; Gerrity et al., 2021a; Gibas et al., 2021a; Giraud-Billoud et al., 2021a; Graham et al., 2021a; Hillary et al., 2021a; Hokajärvi et al., 2021a; Hong et al., 2021a; Johnson et al., 2021a; Karthikeyan et al., 2021a; Kitamura et al., 2021a; Koureas et al., 2021a; Kumar et al., 2021a; Kumar et al., 2021b; Li et al., 2021a; Melvin et al., 2021a; Mondal et al., 2021a; Mota et al., 2021a; Pillay et al., 2021a; Prado et al., 2021a; Rafiee et al., 2021a; Róka et al., 2021b; Rusiñol et al., 2021a; Saguti et al., 2021a; Saththasivam et al., 2021a; Scott et al., 2021a; Sharma et al., 2021a; Spurbek et al., 2021a; Tanhaei et al., 2021a; Tomasino et al., 2021a; Wannigama et al., 2021a; Weidhaas et al., 2021a; Wilder et al., 2021a; Wilton et al., 2021a; Wong et al., 2021a; Wu et al., 2021a; Wurtz et al., 2021a; Wurtz et al., 2020a; Xiao et al., 2021a; Xu et al., 2021a; Yaniv et al., 2021a; Ahmed et al., 2020a; Ahmed et al., 2020b; Albastaki et al., 2021a; D'Aoust et al., 2021b; Fongaro et al., 2021b; Gonçalves et al., 2021a; Gonzalez et al., 2020a; Guerrero-Latorre et al., 2020a; Hasan et al., 2021a; Hata et al., 2021a; Jørgensen et al., 2020a; Kumar et al., 2020a; Kuryntseva et al., 2020a; La Rosa et al., 2020a; La Rosa et al., 2021a; Martin et al., 2020a; Medema et al., 2020a; Nemudryi et al., 2020b; Peccia et al., 2020a; Prado et al., 2020a; Randazzo et al., 2020a; Randazzo et al., 2020b; Rimoldi et al., 2020a; Sharif et al., 2020a; Sherchan et al., 2020a; Trottier

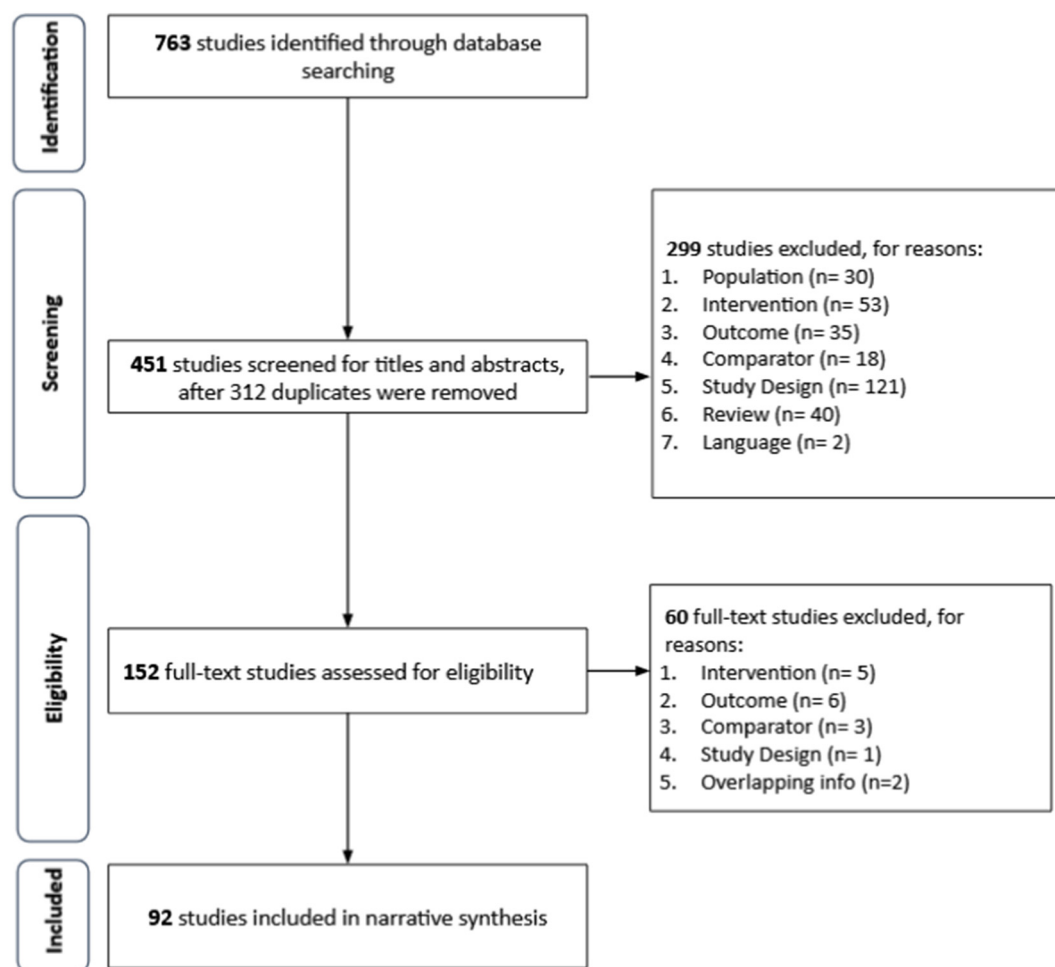


Fig. 1. PRISMA flow diagram.

et al., 2020a; Vallejo et al., 2020a; Westhaus et al., 2021a; Wu et al., 2020a; Arora et al., 2020a; Haramoto et al., 2020a) while five were reports on ongoing wastewater surveillance programs (*Gambier Wastewater SARS-CoV-2 Virus Report 2021*, n.d.; Government NSW, 2020a; Government NSW, 2020b; Government NSW, 2020c; Government NSW, 2020d) undertaken by New South Wales, Australia and Kenyon college, USA. Details on the five reports can be found in the supplementary file and tables, and would not be further discussed in this review. Six of the 87 research studies were pre-prints that have yet to be peer reviewed. The 87 research studies were geographically distributed across at least 34 countries – United States (23) (Betancourt et al., 2021a; Bhattarai et al., 2021a; Cao and Francis, 2021a; Ciesielski et al., 2021a; Colosi et al., 2021a; Gerrity et al., 2021a; Gibas et al., 2021a; Graham et al., 2021a; Karthikeyan et al., 2021a; Li et al., 2021a; Melvin et al., 2021a; Mondal et al., 2021a; Scott et al., 2021a; Spurbeck et al., 2021a; Weidhaas et al., 2021a; Wilder et al., 2021a; Wu et al., 2021a; Xiao et al., 2021a; Gonzalez et al., 2020a; Nemudryi et al., 2020b; Peccia et al., 2020a; Sherchan et al., 2020a; Wu et al., 2020a), India (6 studies) (Chakraborty et al., 2021a; Kumar et al., 2021a; Kumar et al., 2021b; Sharma et al., 2021a; Kumar et al., 2020a; Arora et al., 2020a), Brazil (5 studies) (Fongaro et al., 2021a; Mota et al., 2021a; Prado et al., 2021a; Fongaro et al., 2021b; Prado et al., 2020a), Spain (5 studies) (Chavarria-Miró et al., 2021a; Rusiñol et al., 2021a; Randazzo et al., 2020a; Randazzo et al., 2020b; Vallejo et al., 2020a), Australia (4 studies) (Ahmed et al., 2021a; Black et al., 2021a; Ahmed et al., 2020a; Ahmed et al., 2020b), France (4 studies) (Bertrand et al., 2021a; Wurtz et al., 2021a; Wurtzer et al., 2020a; Trottier et al., 2020a), Canada (3 studies)

(Acosta et al., 2021a; D'Aoust et al., 2021a; D'Aoust et al., 2021b), Italy (3 studies) (La Rosa et al., 2020a; La Rosa et al., 2021a; Rimoldi et al., 2020a), Japan (3 studies) (Kitamura et al., 2021a; Hata et al., 2021a; Haramoto et al., 2020a), United Kingdom (3 studies) (Hillary et al., 2021a; Wilton et al., 2021a; Martin et al., 2020a), Germany (2 studies) (Agrawal et al., 2021b; Westhaus et al., 2021a), Iran (2 studies) (Rafiee et al., 2021a; Tanhaei et al., 2021a), Israel (2 studies) (Bar-Or et al., 2021a; Yaniv et al., 2021a), South Africa (2 studies) (Johnson et al., 2021a; Pillay et al., 2021a; Albastaki et al., 2021a; Hasan et al., 2021a), United Arab Emirates (2 studies), and Argentina, Ecuador (Guerrero-Latorre et al., 2020a), Finland (Hokajärvi et al., 2021a), Greece (Koureas et al., 2021a), Hong Kong (Xu et al., 2021a), Hungary (Róka et al., 2021b), Netherlands (Medema et al., 2020a), Pakistan (Sharif et al., 2020a), Portugal (Tomasino et al., 2021a), Qatar (Saththasivam et al., 2021a), Russia (Kuryntseva et al., 2020a), Saudi Arabia (Hong et al., 2021a), Singapore (Wong et al., 2021a), Slovenia (Gonçalves et al., 2021a), Sweden (Saguti et al., 2021a), Switzerland (Fernandez-Cassi et al., 2021a), and Thailand (Wannigama et al., 2021a) (one study each) and a mixed-country study involving Belgium, Denmark and France (Jørgensen et al., 2020a). Majority of studies were conducted in the Americas and Europe.

Samples were collected between January 2020 and May 2021 from various locations. Settings in which sewage were sampled from include: Waste Water Treatment Plant (WWTP) (69 studies), parts of the sewage system (21 studies), hospitals (11 studies), university (6 studies), river/lakes (5 studies), residential (2 studies), aircraft (2 studies) and canal, cruise, airport and shopping malls/office/food market/entertainment venues (1 study each) (Table 1). Only 61 studies exclusively

Table 1
Summary of study details, sample collection and testing methodology of included studies.

Study details				Sample collection			Testing			
Author	Data collection period	Country	City/region	Total population served	Sampling Site	Sample type	Sample collection approach	PCR assays used	PCR kit name	Gene target
Wu, F., et al., (2020b)	January–mid-May 2020	USA	Middlesex, Norfolk, Suffolk (counties in Massachusetts) & Boston (city in Massachusetts)	2,254,000–2,289,000	WWTP, sewage system	Raw sewage	Composite & Grab	RT-PCR, RT-qPCR	ProtoScript II Reverse Transcriptase (RT), TaqMan® Fast Advanced Master Mix, TaqMan™ Fast Virus 1-Step Master Mix	N1, N2
Westhaus, S., et al. (2021b)	8 April 2020	Germany	North Rhine-Westphalia (State)	3,725,633	WWTP	Raw sewage, effluent (post-tertiary treated & after ozonation/filtration)	Composite	RT-qPCR	Luna Universal Probe One-Step RT-qPCR Kit, LightCycler® Multiplex RNA Virus Master	E, M, N, RdRp
Vallejo, J. A., et al. (2020b)	15 April–4 June 2020	Spain	A Coruña, Oleiros, Culleredo, Cambre & Arteixo (municipalities in A Coruña)	>369,098	WWTP, hospital sewer (treating COVID-19 patients)	Wastewater	Composite	RT-qPCR	qCOVID-19	N
Trottier, J., et al. (2020b)	7 May–20 July 2020	France	Lattes (commune in Montpellier)	470,000	WWTP	Effluent	Composite	RT-qPCR	TaqPath One-Step RT-qPCR, CG Master Mix (ThermoFisher Scientific)	N1, N3
Sherchan, S. P., et al. (2020b)	13 January–29 April 2020	USA	Louisiana	290,321	WWTP	Raw wastewater, secondary treated, final effluent	Composite & Grab	RT-qPCR	Perfecta qPCR ToughMix	N1, N2
Sharif, S., et al. (2020b)	20 March–28 April 2020	Pakistan	38 districts	3,800,000–11,400,000	Pumping station, Open drain	Raw wastewater	Grab	RT-qPCR, RT-PCR	Real-Time Fluorescent RT-PCR Kit, 2019-nCoV Nucleic Acid Diagnostic Kit (PCR Fluorescence Probing), 2019 Novel Coronavirus RNA (PCR Fluorescence Probing)	ORF 1ab, N, E
Rimoldi, S.G., et al. (2020b)	14 & 22 April 2020	Italy	Milan & Monza e Brianza (city & province in Lombardy)	2,780,000	WWTP, River, Canal	Raw/treated wastewater, Riverwater	Grab	RT-qPCR	2019-nCoV real-time RT-PCR kit panel	N, ORF1ab, E
Randazzo, W., et al. (2020c)	12 March–14 April 2020	Spain	Murcia, Cartagena, Molina de Segura, Lorca, Cieza, Totana (cities in Murcia)	750,132	WWTP	Influent, secondary, tertiary treated effluent	Grab	RT-qPCR	One Step PrimeScript™ RT-PCR Kit (Perfect Real Time)	N1, N2, N3
Randazzo, W., et al. (2020d)	12 February–14 April 2020	Spain	Valencia	~1,200,000	WWTP	Raw/treated sewage water	Grab	RT-qPCR	PrimeScript™ One Step RT-PCR Kit	N1, N2
Prado, T., et al. (2020b)	15 April 2020	Brazil	Niterói	NR	WWTP, hospital sewer, sewage system	Raw sewage	Composite	RT-qPCR	NR	N2
Peccia, J., et al. (2020b)	19 March–1 June 2020	USA	New Haven (city in Connecticut)	~200,000	WWTP	Primary sewage sludge	NR	RT-qPCR	Bio-Rad iTaq Universal Probes One-Step Kit	N1, N2
Nemudryi, A., et al. (2020a)	Late March–June 2020	USA	Bozeman (city in Montana)	49,831	WWTP	Raw wastewater	Composite	RT-qPCR	2019-nCoV CDC EUA Kit	N1, N2
Medema, G., et al. (2020b)	5 February–25 March 2020	Netherlands	Amsterdam, Den Haag, Utrecht, Apeldoorn, Amersfoort, Tilburg and Schiphol Airport	2,802,800	WWTP, airport	Sewage (influent)	Composite	RT-qPCR	Taqman Fast Virus 1-Step Master Mix	N1, N2, N3, E

(continued on next page)

Table 1 (continued)

Study details			Sample collection			Testing		Gene target		
Author	Data collection period	Country	City/region	Total population served	Sampling Site	Sample type	Sample collection approach		PCR assays used	PCR kit name
Martin, J., et al. (2020b)	14 January–12 May 2020	UK	South East England	4,000,000	WWTP	Raw sewage (influent)	Composite	RT-qPCR, nRT-PCR	qScript XLT qPCR Toughmix system, Invitrogen SuperScript III One-Step RT-PCR System with Platinum Taq High-Fidelity DNA Polymerase	RdRP, E, nsp2-PLPro, ORF8b-N
La Rosa, G., et al. (2021b)	9 October 2019–28 February 2020	Italy	Milan (City in Lombardy), Turin (City in Piedmont) and Bologna	4,998,600	WWTP	Raw sewage	Composite	nRT-PCR, RT-qPCR	PCR reaction Kit Platinum SuperFi Green PCR Master Mix	RdRp, ORF1ab
La Rosa, G., et al. (2020b)	3 February–2 April 2020	Italy	Milan, Rome	3,000,000	WWTP	Raw sewage	Composite	nRT-PCR, RT-qPCR	Kit Platinum™ SuperFi™ Green PCR Master Mix; SuperScript III Reverse Transcriptase, Dream Taq polymerase and buffer, UltraSense one-step qRT-PCR System	ORF1ab, S, RdRP
Kuryntseva, P., et al. (2020b)	30 March & 30 July 2020	Russia	Kazan	1790	Sewage access points (city, Kazan Federal University campus)	Raw sewage	Composite	RT-qPCR	OM-Screen-2019-nCoV-RT	Orf1ab
Kumar, M., et al. (2020b)	8 & 27 May 2020	India	Ahmedabad (city in Gujarat)	NR	WWTP (hospital included in catchment)	Raw sewage, final effluents (after UASB & aeration pond)	Composite	RT-PCR	TaqPath Covid-19 RT-PCR Kit	ORF1ab, N, S
Jorgensen, A., et al. (2020b)	February–June 2020	Denmark, France, Belgium	NR	NR	WWTP (Denmark, France), hospitals (Denmark, Belgium)	Raw sewage	Composite	RT-PCR	VIRSeek Screen kit, VIRSeek Ident kit	E, RdRP
Hata, A., et al. (2021b)	5 March–23 April 2020	Japan	Ishikawa and Toyama	465,243	WWTP	Influent wastewater	Grab	RT-qPCR	qTOWER3	N, N2, N3
Hasan, S. W., et al. (2021b)	May–June 2020	UAE	NR	NR	WWTP, sewer access points, pumping stations	Influent and treated effluents, raw wastewater	Composite	RT-qPCR	GENESIG COVID-19 kits	RdRP
Haramoto E., et al. (2020b)	17 March–7 May 2020	Japan	Yamanashi Prefecture	NR	WWTP, river	Influent, secondary-treated wastewater activated sludge process before chlorination, river water	Grab	RT-qPCR, nRT-PCR	NR	N, N1, N2, ORF1a, S
Guerrero-Latorre, L., et al. (2020b)	5 June 2020	Ecuador	Quito	~3,000,000	River	River water impacted by urban wastewater	NR	RT-qPCR	TaqMan™ Fast Virus 1-Step Master Mix	N1, N2
Gonzalez, R., et al. (2020b)	9 March–2 August 2020	USA	Virginia	1,700,000	WWTP	Raw wastewater	Composite & Grab	RT-ddPCR	One-Step RT-ddPCR Advanced Kit for Probes	N1, N2, N3
Fongaro, G., et al. (2021c)	30 October 2019–4 March 2020	Brazil	Florianopolis (city in Santa Catalina)	~5000	Sewage system	Raw sewage	NR	RT-qPCR	OneStep qPCR Quantinova kit, Seegene Allplex™ 2019-nCoV commercial kit	N1, S, RdRp
D'Aoust, P.M., et al. (2021c)	1 April–30 June 2020	Canada	Ottawa (City in Ontario) and Gatineau (City in Quebec)	~1,300,000	WWTP	Post-grit chamber influent solids (PGS) & primary clarified sludge (PCS)	Composite & Grab	RT-qPCR, RT-ddPCR	Reliance One-Step Multiplex RT-qPCR Supermix, One-Step RT-ddPCR Advanced Kit for Probes	N1, N2, E
Arora, S., et al. (2020b)	3 May–14 June 2020	India	Jaipur (City in Rajasthan)	NR	WWTP (jail included in catchment), hospitals	Raw sewage, treated sample, tertiary treated sample	Grab	RT-qPCR	Allplex™ 2019-nCoV Assay kit, TaqPath™ COVID-19 Combo Kit	E, RdRp, N, ORF1ab, S
Albastaki, A., et al. (2021b)	22 April–7 July 2020	UAE	Dubai	NR	WWTP, pumping stations, aircrafts	Raw sewage, treated wastewater, raw sewage from airplane	Grab	RT-qPCR	TaqPath™ Covid-19 RT-PCR Kit	ORF1ab, N, S

Ahmed, W., et al. (2020d)	23 April–10 May 2020	Australia	NA	> 321	Cruise ship, aircrafts	Influent, effluent of the membrane bioreactor of the cruise ship, aircraft wastewater samples	Grab	RT-qPCR, RT-ddPCR	iTaq™ Universal Probes One-Step ReactionMix	N1, N2, N, E
Ahmed, W., et al. (2020e)	24 February–1 April 2020	Australia	Brisbane (City in Queensland)	736,172	WWTP, pumping station	Raw sewage	Composite	RT-qPCR	iTaq™ Universal Probes One-Step Reaction Mix	N
Gonçalves, J., et al. (2021b)	1 June–15 June 2020	Slovenia	Ljubljana	NR	Hospital pumping station	Raw wastewater	Composite	RT-qPCR	Roche LightCycler® Multiplex RNA Virus Master, LightMix® Modular SAKS-CoV primers and probes mix	E, RdRp
Acosta, N., et al. (2021b)	5 Aug–17 Dec 2020	Canada	Calgary, Alberta	2129	Manholes (3 tertiary-care hospitals)	Raw waste water	Composite	RT-qPCR	TaqMan™ Fast Virus 1-Step Master Mix (Applied Biosystems)	N1, N2, E
Agrawal, S., et al. (2021c)	April–August 2020	Germany	Frankfurt metropolitan area	~1,200,000	WWTP	Raw sewage	Composite	RT-qPCR	TaqPath COVID-19 RT-PCR Kit	N, S, ORFlab
Ahmed, W., et al. (2021b)	24 Feb–1 May 2020	Australia	Brisbane	934,000	WWTP	Raw wastewater	Composite & Grab	RT-qPCR	iTaq™ Universal Probes One-Step Reaction Mix (Bio-Rad Laboratories, Richmond, CA).	N1, N2, E
Bar-Or, I., et al. (2021b)	August 2020 to February 2021	Israel	El Hamra, Tzfat, Haifa, Natanya, Shafdan, Ashdod, Jerusalem, Rahat, Beer Sheva	4,493,300	WWTP	Raw wastewater	Composite	RT-qPCR	SensFast reaction mix (Bioline)	E
Bertrand, I., et al. (2021b)	2 April–28 May 2020	France	French Grand Est region.	250,000	WWTP	Influent wastewater	NR	RT-PCR, RT-ddPCR	RNA UltraSens™ One-Step Quantitative RT-PCR system	RdRp, E
Betancourt, W., et al. (2021c)	24 August–20 November 2020	USA	the University of Arizona, Tucson	NR	University (sewer manholes)	Raw sewage	Grab	RT-PCR	RT-PCR assays manufactured at Integrated DNA Technologies (IDT, Coralville, IA)	N1, N2
Bhattarai, B., et al. (2021b)	18 May–21 July 2020	USA	Utah	1,449,860	Water reclamation facilities (WRF)	Raw influent, primary sludge, return activated sludge, digested sludge	Composite & Grab	RT-qPCR	Quantstudio™ 3 Real-Time PCR machine (ThermoFisher Scientific, USA).	N1
Black, J., et al. (2021b)	25 Aug–27 Oct 2020	Australia	Metropolitan & regional Melbourne	4,336,602	WWTP & main sewer pipes	Raw sewage	Composite & Grab	RT-qPCR	PerkinElmer® SARS-CoV-2 Nucleic Acid Detection Kit (RUO)	N, ORFlab
Cao and Francis (2021b)	8 April 2020–Current	USA	Indiana	~30,000	WWTP	Influent	Composite	NR	NR	NR
Chakraborty, P., et al. (2021b)	Chennai: 5–11 Sept 2020 Hospital WW: Aug–Sept 2020	India	Chennai	9,675,613	Sewage treatment plant, sewage pumping station, hospital wastewater	Influent, effluent, sludge	Composite and grab	RT-qPCR	IDT 2019-nCoV CDC-EUA kit	N1, N2
Chavaria-Miró, G., et al. (2021b)	13 April–7 July 2020	Spain	Barcelona (Metropolitan area)	~2,700,000	WWTP	Raw sewage	Composite	RT-qPCR	NR	RdRp, IP2, IP4, E, N1, N2
Ciesielski, M., et al. (2021b)	9 March–6 Sept 2020	USA	18 cities in Virginia	1,427,336	WWTP	Influent	Grab & composite	RT-qPCR, RT-ddPCR	Reliance One-Step Multiplex RT-qPCR Supermix; Bio-Rad's One-Step RT-ddPCR Advanced Kit	N2
Colosi, L., et al. (2021b)	7 July–2 September 2020	USA	Virginia	> 14,505	WWTP, hospitals, dormitories	Raw sewage, primary solids	Grab & composite	RT-qPCR	NR	N1, N2
D'Aoust, P., et al. (2021d)	20 June–4 Aug 2020	Canada	Ottawa	~1000,000	water resource recovery facility (WRRF)	Primary clarified sludge	Composite	RT-qPCR	One-Step Multiplex RT-qPCR Supermix	N1, N2
Fernandez-Cassi, X., et al. (2021b)	26 February–30 April 2020	Switzerland	Lugano, Lausanne, Zurich	815,000	WWTP	Influent	Composite	RT-qPCR	RNA UltraSense™ One-Step	N1, N2
Fongaro, G., et al. (2021d)	Aug–20	Brazil	Minas Gerais State region (presidio, worker accommodation, rural community)	1750	Sink storage box, river water	Raw sewage, river water upstream and downstream	NR	RT-qPCR	NR	N1, N2

(continued on next page)

Table 1 (continued)

Study details				Sample collection			Testing		Gene target	
Author	Data collection period	Country	City/region	Total population served	Sampling Site	Sample type	Sample collection approach	PCR assays used		
Gerrity, D., et al. (2021b)	Early March–late May 2020	USA	Southern Nevada	1,060,000	WWTP, untreated surface water, finished drinking water	Influent and primary effluent	Composite and grab	RT-qPCR	iScript™Select cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA) or the Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific)	N1, N2, E, ORFla
Gibas, C., et al. (2021b)	28 September–23 November 2020	USA	Charlotte, North Carolina	>2550	University (plumbing cleanouts & manhole access points)	Raw sewage	Composite	RT-qPCR	iTaq universal probes reaction mix (Bio-Rad laboratories), iScript reverse transcriptase (Bio-Rad laboratories), 2019-nCoV CDC RUO Kit (Integrated DNA Technologies IDT, #10006713)	N1
Giraud-Billoud, M., et al. (2021b)	22 July 2020–January 2021	Argentina	Mendoza	~1,191,649	WWTP	Influent samples	Manual	RT-qPCR	2019 nCoV CDC EUA Kit (IDT#10006606, L#0000512209)	N1, N2
Graham, K., et al. (2021b)	16 March–12 July 2020	USA	Santa Clara County, California	~1,700,000	WWTP	Influent, primary solids	Grab & composite	RT-qPCR, ddRT-PCR	TopTaq Master Mix Kit, ddPCR SARS CoV-2 BioRad kit triplex assay	N1, N2
Hillary, L., et al. (2021b)	March–July 2020	UK	Gwynedd, Cardiff, Liverpool, Manchester, the Wirral, Wrexham	3,032,000	WWTP	Influent, treated effluent	Grab & composite	RT-qPCR	RNA Ultrasense Reaction Mix, RNA Ultrasense Enzyme Mix	N1, E
Hokajärvi, A., et al. (2021b)	19–20 April and 24–25 May 2020	Finland	Helsinki	860,000	WWTP	Influent	Composite	RT-qPCR	TaqMan Fast Virus 1-step Master Mix and a QuantStudio 6 Flex real-time PCR system	E, N2
Hong, P., et al. (2021b)	15 April–9 July 2020	Saudi Arabia	Jeddah	42–236	Hospital (WWTP within hospital)	Raw sewage, partially treated wastewater	Grab	RT-qPCR	NR	N1, N2, N3
Johnson, R., et al. (2021b)	18-Jun-20	South Africa	Cape Town and Stellenbosch municipality	2,375,000	WWTP	Raw influent	Composite	RT-qPCR	iTaq Universal Probes One-Step Reaction Mix	N1, N2
Karthikeyan, S., et al. (2021b)	20 July 2020–21 October 2020	USA	San Diego	>2,300,000	WWTP	Influent	Composite	RT-qPCR	TaqPath™ 1-Step RT-qPCR Master Mix	N1, N2, E
Kitamura, K., et al. (2021b)	9 June–19 August 2020	Japan	Metropolitan region	NR	Manhole, WWTP	Influent	Grab	RT-qPCR	QIAamp Viral RNA kit (Qiagen)	N1, N2
Koureas, M., et al. (2021b)	29 October 2020–14 April 2021	Greece	Larissa and Volos	305,000	WWTP	Influent	Composite	RT-PCR	TaqPath™ COVID-19 CE-IVD RT-PCR Kit (Applied Biosystems™)	ORF1ab, S, N
Kumar, M., et al. (2021c)	7 Aug–30 Sept 2020	India	Gandhinagar	NR	WWTP (4 wards and an academic institution)	Influent	Grab	RT-PCR	TaqPath™ Covid-19 RT-PCR Kit	ORF1ab, N, S
Kumar, M., et al. (2021d)	3 September–26 November 2020	India	Ahmedabad	NR	WWTP, pumping station, lakes, river	Influent	Grab	RT-PCR	TaqPath™ 1 Step Multiplex Master Mix	N, ORF 1ab, S
Li, B., et al. (2021b)	27 Aug–4 Oct 2020	USA	Honolulu (Sand Island and Honouliuli)	697,680	WWTP	Influent	Composite	RT-qPCR	ABI 7300 qPCR System (Applied Biosystem; Beverly, MA, USA)	E, N1, N2
Melvin, R., et al. (2021b)	May–August 2020	USA	Minnesota	2,362,610	WWTP	Influent	Composite	RT-qPCR	Go-Taq Probe 1-Step RT-qPCR system 2× master mixes	N1, N2
Mondal, S., et al. (2021b)	Mid-October 2020–early Jan 2021	USA	Wisconsin (Dane county)	NR	WWTP	Primary influent	24 h composite	RT-qPCR	SARS-CoV-2 RT-qPCR Detection Kit for Wastewater (Promega Corp.)	N1, N2, E
Mota, C., et al. (2021b)	11 May–7 August 2020	Brazil	Belo Horizonte	~2,000,000	WWTP, sewer network	Influent	Composite	RT-qPCR	iTaq™ Universal probes One Step reaction mix & reverse transcriptase	N1
Pillay, L., et al. (2021b)	7 July–6 October 2020	South Africa	KwaZulu-Natal	NR	WWTP	Raw sewage	Grab	One-step RT-ddPCR	One-Step RT-ddPCR Advanced Kit for Probes from Biorad (USA)	N2
Prado, T., et al. (2021b)	15 April–25 August 2020	Brazil	Niterói, Rio de Janeiro	513,584	WWTP, sewer network	Raw sewage	Composite	RT-qPCR	SuperScript™ III Platinum™ One-Step qRT-PCR Kit (Invitrogen)	N2

Rafiee, M., et al. (2021b)	12–20 November 2020, 6–14 May 2021	Iran	Tehran	8,694,000	Sewer access points (manholes)	Raw sewage	Grab, composite & Moore swabs	RT-qPCR	Novel coronavirus nucleic acid diagnostic real time PCR kit (Sansure Biotech, China)	N, ORFlab
Róka, E., et al. (2021a)	June–November 2020	Hungary	Budapest	1,800,000	WWTP	Raw sewage	Composite & grab	RT-qPCR	LightCycler Multiplex RNA Virus Master kit (Hoffmann-La Roche)	N1
Rusiñol, M., et al. (2021b)	Mid-March–early November 2020	Spain	Catalonia	2,011,300	WWTP	Influent	Composite	RT-qPCR	TaqMan™ Environmental Master Mix 2.0	N1, N2
Saguti, F., et al. (2021b)	10 February–5 July 2020	Sweden	Gothenburg	755,940	WWTP	Influent, treated effluent	Composite & grab	RT-qPCR	Reaction Mix (Invitrogen), 20 U RNaseOUT™ (Invitrogen), 1 µL SuperScript® III/platinum® Taq Mix (Invitrogen)	RdRP
Saththasivam, J., et al. (2021b)	21 June–30 August 2020	Qatar	greater Doha	2,503,457	WWTP	Influent	Composite	RT-qPCR	SARS-CoV-2 (2019-nCoV) CDC qPCR Probe Assay Research Use Only (RUO) kit	N1, N2
Scott, L., et al. (2021b)	19 August–1 December 2020	USA	New Orleans (Louisiana)	14,062	University (manholes, sewer cleanouts, and municipal manholes)	Raw sewage	Grab	RT-qPCR	qScript one-step RT-qPCR ToughMix (QuantaBio)	N1, N2
Sharma, D., et al. (2021b)	Pre-Covid & 11–22 May 2020	India	Mumbai	4,704,883	Pumping station, open drain	Raw sewage	Grab	RT-PCR	LabGun COVID-19 real-time RT-PCR kit (LabGenomics)	E, RdRP
Spurbeck, R., et al. (2021b)	14, 22 & 29 July 2020	USA	Toledo (Ohio)	NR	Hospitals, nursing home, residential neighbourhood (manholes)	Raw sewage	Composite & grab	RT-qPCR	TaqMan Fast Viral One-Step Master Mix (ThermoFisher)	N1
Tanhaei, M., et al. (2021b)	30 June–31 July 2020	Iran	Tehran	NR	WWTP	Raw influent, treated effluent	Grab	RT-qPCR	Sansure Biotech Inc. (Changsha, China), Shanghai Zhijiang Biotechnology Co. (Shanghai, China) Kits	ORFlab, N
Tomasino, M., et al. (2021b)	14 May 2020–10 March 2021	Portugal	Porto	370,000	WWTP	Raw sewage	Composite	RT-qPCR	1) BGI's Real-Time Fluorescent RT-PCR kit 2) Water SARS-CoV-2 RT-PCR ready-to use kit (IDEXX Laboratories)	May-Sep: NR Sep-Mar: N1, N2, N3, E
Wannigama, D., et al. (2021b)	7 July–27 December 2020	Thailand	Bangkok & 26 nearby suburbs	15,934,500	Bangkok: shopping centers, condominium complex, office complex, and WWTP, Entertainment venues Suburbs: shopping centers, housing complex, office complex, and food markets	Primary wastewater samples	NR	RT-qPCR	Bio-Rad iTaq Universal Probes One-Step Kit	
Weidhaas, J., et al. (2021b)	1 April–28 May 2020	USA	Utah	1,260,000	WWTP	Influent, interceptor sample from sub-sewershed, effluent	Composite & grab	RT-qPCR	TaqPath™1-step RT-qPCR (ThermoFisher), qScript XL1T Onestep RT-qPCR (QuantaBio)	N1, N2
Wilder, M., et al. (2021b)	28 April–24 June 2020	USA	New York (upstate)	1000,898	Sewage Network Access Points	Influent	Composite	RT-qPCR	Reliance One-Step Multiplex RT-qPCR Super-mix (Bio-Rad® California, USA)	RdRp
Wilton, T., et al. (2021b)	14 January 2020–26 January 2021	UK	London	4,000,000	WWTP	Inlet samples	Composite	nRT-PCR	NR	RdRP, ORF8b, S
Wong, J., et al. (2021b)	4–20 July 2020	Singapore	Singapore	NR	Manhole of apartment building WWTP or catchments	Raw sewage	Composite	RT-qPCR	Luna Universal Probe One-Step RT-qPCR Kit (NEB, USA)	ORFlab
Wu, F., et al. (2021b)	18 February–2 June 2020	USA	Nationwide (40 States)	42,500,000	WWTP or catchments	Raw wastewater	NR	RT-qPCR	TaqMan® Fast Advanced Master Mix, TaqMan™ Fast Virus 1-Step Master Mix	N1, N2

(continued on next page)

Table 1 (continued)

Study details				Sample collection		Testing		
Author	Data collection period	Country	City/region	Total population served	Sampling Site	Sample type	Sample collection approach	Gene target
Wurtz, N., et al. (2021b)	1 July–15 December 2020	France	Marseille	973,746	Sewer networks	Wastewater	NR	NR
Wurtz, S., et al. (2020b)	5 March–23 April 2020	France	Paris	3,000,000	WWTP	Influent	Composite	NR
Xiao, A., et al. (2021b)	4 March 2020–13 May 2021	USA	Boston (Massachusetts)	~2,300,000	WWTP	Raw sewage	Composite	NR
Xu, X., et al. (2021b)	8 June–29 September 2020	Hong Kong	Hong Kong	4,555,001	Manholes of isolation ward building (hospital), public housing estate, WWTP, sewer network	Raw sewage	Composite	NR
Yaniv, K., et al. (2021b)	17–19, 25 May 2020	Israel	Ashkelon	34,100	Manholes, WWTP	Raw sewage	Composite	NR
NSW Government (2020a)	July–October 2020	Australia	New South Wales	147,908–6,430,642	WWTP	Raw sewage	NR	NR
NSW Government (2020b)	4–10 October 2020	Australia	New South Wales	6,472,811	WWTP, quarantine hotels	Raw sewage	NR	NR
NSW Government (2020c)	11–17 October 2020	Australia	New South Wales	6,526,714	WWTP, quarantine hotels	Raw sewage	NR	NR
NSW Government (2020d)	18–24 October 2020	Australia	New South Wales	6,475,464	WWTP, quarantine hotels	Raw sewage	NR	NR
Kenyon College (Gambier Wastewater SARS-CoV-2 Virus Report, n.d.)	Ongoing	USA	Ohio	700	NR	NR	Composite	NR

sampled a single type of setting: WWTP (47 studies), hospital and university (three studies each), river and residential (one study each), parts of the sewage system (six studies). The population served by each WWTP or sampling site ranged from 321 to 11,400,000 inhabitants, totaling at least 188,907,989 inhabitants (Table 1).

Raw sewage, the most commonly collected sample type, was collected singly or in combination with other sample types in 80 studies (Table 1). Nineteen studies collected wastewater effluent treated to secondary, tertiary, and post-tertiary levels and seven studies collected primary sludge samples. Three studies were unclear in reporting the sample type collected (Wurtz et al., 2021a; Medema et al., 2020a; Vallejo et al., 2020a). A total of 8078 studies clearly reported their sampling approach – grab samples only (19 studies), composite samples only (43 studies), both grab and composite sampling (18 studies). One study also conducted Moore sampling (Rafiee et al., 2021a). All but one study conducted polymerase-chain testing to ascertain sample positivity – quantitative real-time PCR (RT-qPCR) (74 studies), qualitative PCR (RT-PCR) (10 studies) and digital droplet PCR (RT-ddPCR) (seven studies). Six studies also carried out nested PCR. A total of 49 studies focused on only one genetic target for positive signals (N gene – 4332 studies, RdRp – 3 studies, Orf1ab – 2 studies and E – 1 study), while all others looked at more than one gene target. The N gene (inclusive of N/N1/N2/N3) was the most commonly targeted, being used in 70 of the included studies. Other gene targets used include envelope (E), ORF1ab, Spike (S), RNA-dependent RNA polymerase (RdRp). While only a couple of studies used primers/probe detecting the membrane (M) gene, nsp2-PLPro and ORF8ab-N (Wilton et al., 2021a; Martin et al., 2020a) (Table 1).

3.2. Positivity in wastewater samples

A total of 7644 positive samples were detected from at least 26,197 samples (29.2%) collected by 66 studies (Table 2). Twenty-two papers were unclear in reporting the outcome of sampling (Vallejo et al., 2020a; Wu et al., 2020a). Notably, all papers that detailed testing outcome reported sample positivity. Majority of samples reported were attributed to Peccia et al., who made 3632 positive detections in 17,661 samples (20.6%) of primary sludge (Peccia et al., 2020a). Separately, the sewage surveillance program by the New South Wales government saw the detection of positive signals at least 33 times out of 472 sampling events over three months. Raw sewage influent constituted majority of positive sample, followed by secondary and tertiary treated-effluent, effluent after ozonation and filtration, post-grit chamber influent solids, primary sludge, and river samples in no particular order (Table 2).

Positive samples were detected in various settings – WWTP, parts of the sewage system, hospitals, rivers, aircrafts and airport. In settings within the conventional sewage system, 7180 samples were positive (28.9%) of 24,862 samples taken. In settings outside of the conventional sewage system (WTP, sewage access points, drains, pumping stations), samples were positive on 464 out of 1335 occasions (34.8%). Nine of 10 papers that sampled sewage systems serving hospitals reported clear outcomes showing a total of 185/284 (65.1%) positive samples. Three papers that examined samples from aviation settings, specifically two from aircrafts and one from airport, found 34 positive samples from 212 samples (Ahmed et al., 2020b; Albastaki et al., 2021a; Medema et al., 2020a). In the sole paper that sampled cruise ships, Ahmed et al., reported eight positive detections in 11 samples constituting both influent and effluent of the cruise sewage system (Ahmed et al., 2020b). Studies investigating travel-related settings (airport, aircraft & cruise) reported 42/223 (18.8%) samples positive collectively (Ahmed et al., 2020b; Albastaki et al., 2021a; Medema et al., 2020a). Another two of three papers that sampled rivers made seven positive detections in 12 (58.3%) collected samples (Guerrero-Latorre et al., 2020a; Rimoldi et al., 2020a; Haramoto et al., 2020a). Six of seven university-based studies reported clear outcomes with 222 positive samples (27.7%) of

802 samples collected. Two studies involving samples from residential areas reported 7 out of 11 (63.6%) samples positive (Tomasino et al., 2021a; Yaniv et al., 2021a). Separately, a single study collected wastewaters from nursing home (1 of 3 samples positive; 33.3%) (Tanhaei et al., 2021a). Viral load measured in the positive samples ranged from 1 to 4.8×10^9 gene copies per liter of wastewater (Table 2).

In terms of sample positivity, regions within the spike (S) gene had the highest percentage of positive samples: S (153/190; 80.5%), ORF1ab (215/327; 65.8%), RdRp (180/284; 63.4%), N2 (880/1462; 60.2%), N1 (920/1678; 54.8%), N (226/429; 52.7%), N3 (199/378; 52.7%), E (188/465; 40.4%). Although high positivity was recorded when ORF8-N (17/19 samples; 89.5%) was targeted, this should be interpreted with caution as the data was only obtained from two studies (Wilton et al., 2021a; Martin et al., 2020a). N genes remain the most commonly used gene targets in samples taken from settings outside of the conventional sewage system. Maximum sample positivity outside of the conventional sewage system was seen in ORF1ab (12/20; 60%), followed by S (8/14; 57.1%), N (48/99; 48.5%), RdRp (10/21; 47.6%), N2 (185/397; 46.6%), N3 (26/61; 42.6%), N1 (254/707; 35.9%), E (26/148; 17.6%). For river samples, N1 and N2 had positivity rates of 50% each given by 3/6 positive samples, while N and ORF1ab had positivity rates of 44.4% given by 4/9 positive detections each and six samples that were tested with E gene assay returned null detection. In the hospital setting, N1 gene was the most commonly used (138/228; 47.4%), followed by N2 (105/224; 46.9%), E (18/117; 15.4%), N3 (23/57; 40.4%), RdRp (10/21; 47.6%), N (9/19; 47.4%), ORF1ab and S (1/1; 100% each). In the university setting, the highest sample positivity was observed in N gene (8/10; 80%), followed by ORF1ab and S at 70% each (7/10 samples positive), N2 gene (58/110; 52.7%), and N1 gene (98/442; 22.2%). In residential setting, N gene was 50% positive (4/8 samples), and N1 gene was 100% positive (3/3 samples). The N2 gene was used in the only study involving nursing home, which reported 1 out of 3 positive samples (33.3%). Samples taken from travel-related setting were only tested with N and E genes, giving sample positivity as follows – N3 (3/4; 75%), N1 (11/25; 44%), E (8/25; 32%), N (6/21; 28.6%) and N2 (6/25; 24%) (Table 2).

3.3. Association with COVID-19 cases in the community

All but four studies reported potential association between wastewater signals and community COVID-19 cases. Twenty-three studies reported an association between positive detection in wastewater samples and number of community cases. These studies that focused on qualitative detection of SARS-CoV-2 in wastewater consistently reported the following three trends: 1) positive detection in areas with recent history of COVID-19 patients, 2) positive detection in wastewater samples coinciding with presence or increase in community cases, or 3) decreasing number of positive wastewater samples coinciding with decline in community cases. Another 53 studies reported associations between trend in viral load and community cases, with number of community cases directly proportional to the viral load. Six studies reported both qualitative and quantitative associations between WWTP positivity and case trends, while a single study by Bar-Or et al. (2021a) reported the presence of B.1.1.7 variant concurrently with clinical detection. Notably, thirteen studies reported detection of positive signals in wastewater before community cases surfaced in the population. The most prominent finding was that by Fongaro et al., who reported stable viral load in November 2019 through early March 2020, nearly 60 days before the first confirmed case in South America, and more than 90 days before the first confirmed case in Brazil (Fongaro et al., 2021b). La Rosa et al., reported similar findings of SARS-CoV-2 in wastewater samples dated between 18 December 2019 and 29 January 2020, two months before the first autochthonous Italian case was reported on 21 February 2020 (La Rosa et al., 2021a). Likewise, Chavarria-Miro et al. detected SARS-CoV-2 in sewage 41 days before the first COVID-19 cases was declared on 25 February 2020 (Chavarria-Miró et al., 2021a). In

Table 2
Summary of outcomes of included studies.

Author	Viral load		Sample positivity		Association		Temporal trends	
	Gene copies	Ct values	Overall	Gene specific	Case trend (Y/N)	Correlation, estimated prevalence of infection or comparison with case numbers	Trend	Duration
Wu F., et al. (2020b)	N1: NR N2: NR Overall: >15 copies/mL	NR	NR	NR	Y	No positive detection before March, correspond to absence of case reported. Low viral titer matched low community cases (2 cases in early March), exponential increase in titers matched peak in ILI (late March), decreased titers matched decline in new clinical cases (Mid-April)	Higher correlations when comparing new clinical cases back-dated by 4–10 days; maximum agreement is a 4-day time lag (consistent with 4–5 days incubation period)	4–10 days before
Westhaus S., et al. (2021b)	Overall: 2.7–37 gene equivalents/mL	M: 32–35 RdRP: 33–37	13/13 (Influent, effluent, post-tertiary treatment, effluent after ozonation & filtration)	NR	Y	SARS-CoV-2 load correlated to cumulative and acute prevalence at the WWTP; no conclusive correlation between incidence and SARS-CoV-2 concentration	NA	NA
Vallejo, J. A., et al. (2020b)	Overall: <1000–~500,000 gene copies/L	NR	NR	NR	Y	- Viral load in WWTP Bens consistent with number of estimated COVID-19 cases in the metropolitan area of A Coruña, both reached lowest numbers by beginning of June; mean viral load at WWTP Bens decreased with asymptotic fashion, while estimated cases decreased approx. 6 folds in the same period - Strong linear correlation between active cases and logarithm of daily mean viral load at Bens. - Higher viral load at CHUAC (hospital) compared to WWTP Bens in mid-May	NA	NA
Trottier, J., et al. (2020b)	NR	N1: 36.4 N3: 37.6	7/7	NR	Y	Low detection of viral RNA in the second half of May but abnormally high COVID-19 cases (might be due to readjustment), in early June, there was increase in viral RNA detected as daily cases remained relatively low. In mid-late June, there was sharp increase in amount of detected viral RNA matched by slowly increasing COVID-19 daily case. By early July, high viral titers was consistently detected, accompanied by sharp increase in COVID-19 daily case. COVID-19 daily cases peaked in late July	Number of new COVID-19 patients surged roughly 2–3 weeks after the increase of SARS-CoV-2 RNA levels in wastewater	2–3 weeks before
Sherchan, S. P., et al. (2020b)	N1: 7.5×10^3 copies/L N2: 3.1×10^3 – 4.3×10^3 copies/L Overall: 4.3×10^3 ; 3.1×10^3 – 7.5×10^3 copies/L	NR	2/15 (Influent)	N1: 1/15 N2: 2/15	Y	No detection in wastewater before first COVID-19 case (March 9), samples positive in April when COVID-19 cases surfaced.	NA	NA
Sharif, S., et al. (2020b)	NR	32–38 (only known for 6 samples)	21/78	(Only known for 20 samples) ORF1ab: 6/20 N: 6/20 E: 6/20	Y	Detection of SARS-CoV-2 RNA in wastewater samples collected from areas with recent history of COVID-19 patients	NA	NA
Rimoldi, S.G., et al. (2020b)	NR	NR	WWTP: 4/12 (raw) River: 4/6	WWTP N: 2/12 ORF1ab: 4/12	Y	Lower viral load on 22 April than 14 April, corresponds to the overall declining case trend whereby cases on 14 April higher	NA	NA

Randazzo, W., et al. (2020c)	N1: 5.1 ± 0.3 log ₁₀ gc/L N2: 5.5 ± 0.2 log ₁₀ gc/L N3: 5.5 ± 0.3 log ₁₀ gc/L	N1: 34.68–37.28 N2: 35.1–38.39 N3: 35.2–38.14 Values not inclusive of SD	37/73 (raw, secondary treated)	N1: 12/24 N2: 13/24	Y	Amplification signals detected in wastewaters when cases were diagnosed within the municipality.	SARS-CoV-2 detected weeks before the first confirmed case. Positive signals in wastewater found 12–16 days before COVID-19 cases were diagnosed clinically.	12–16 days before
Randazzo, W., et al. (2020d)	N1: 5.55; 5.31–5.75 log ₁₀ gc/L N2: 5.73; 5.22–5.99 log ₁₀ gc/L Overall: 5.56; 5.22–5.99 log ₁₀ gc/L	N1: 34.79; 33.87–36.02 N2: 36.95; 34.17–37.84 Overall: 35.12; 33.87–37.84	13/24 (raw)	N1: 12/24 N2: 13/24	Y	First positive sample on 24 February corresponds to incipient community cases (first local case reported on 25 Feb); local community transmission likely undergoing when the first cases were declared in Valencia. By 9 & 11 March, consistent detection in samples correspond to 50 and 76 cases in Valencia (not that high yet)	First positive sample detected 1 day before community case; wastewater viral RNA anticipated the ascent in cases (increased and plateaued faster than declared cases)	at least 1 day before
Prado, T., et al. (2020b)	NR	N2: 36.3–39.8	WWTP/SS: 5/10 (raw) Hospital: 0/2	WWTP/SS: N2: 5/10 Hospital: N2: 0/2	Y	Majority of positive samples (4/5) correctly reflected neighbourhood with higher cases (n = 70), sole detection at Camboinhas suggests outbreak expansion. Given the uncertainties in sludge data and epidemiological data, the study did not attempt to correlate absolute numbers of sludge SARS-CoV-2 RNA concentrations and COVID-19 cases.	NA	NA
Peccia, J., et al. (2020b)	1.7 × 10 ³ mL ⁻¹ to 4.6 × 10 ⁵ mL ⁻¹ of primary sludge	Average Ct N1: 34.6 N2: 34.5	3632/17661 (primary sludge)	NR	N	Sludge results led tests by date of specimen collection by 0–2 days, hospital admissions by 1–4 days, reported positive tests by 6–8 days.	0–2 days - before positive test by date of specimen collection 1–4 days - before hospital admissions 6–8 days - before positive tests by report date	
Nemudryi, A., et al. (2020a)	N1: 43.5–5400.5 copies/L (mean) N2: 22.2–6116.2 copies/L (mean)	NR	13/17	N1: 13/17 N2: 13/17	Y	After 1 month (late April – early May) of undetectable levels of SARS-CoV-2, the wastewater began testing positive again in late May, which coincided with an increase in COVID-19 cases in the community	In mid-March, SARS-CoV-2 RNA concentration in the wastewater lagged behind symptom onset data by 8 days, preceded laboratory test results for individuals by 2 days. In May, wastewater detection trailed symptom onset by 5 days and foreshadowed the increase in positive tests by 4 days.	5–8 days after symptom onset 2–4 days before clinical PCR test results
Medema, G., et al. (2020b)	N1: 195; 2.6–790 gc/mL N2: 425; 12–2200 gc/mL N3: 430; 6.6–1800 gc/mL Overall N: 310; 2.6–2200 gc/mL E - NA	N: NR E - 32.95; 29.9–39.2	WWTP: 17/25 (influent) Airport: 3/4 (influent)	WWTP N1: 15/25 N2: 16/25 N3: 16/25 E: 16/25 Airport N1: 3/4 N2: 2/4 N3: 3/4 E: 2/4	Y	No samples were positive 3 weeks before the first cases were detected in Netherland (Feb 27). One week into the epidemic (March 4–5), samples were positive in Utrecht, Den Haag, Schiphol and Amersfoort although no cases were detected in Amersfoort on March 5 but first case surfaced 6 days later. As the epidemic spread (march 18), all samples (March 15 and 16) were positive and correlated with increase in cases at different rates in each city (Tilburg > Utrecht > Amsterdam). An increase in the SARS-CoV-2 RNA concentration was observed as the number of	Virus circulation in Amersfoort 6 days before the first cases were noted. N and E assays started to produce signals in sewage samples when the observed COVID-19 prevalence was around or <1 case in 100,000 people.	6 days before the first case

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Table 2 (continued)

Author	Viral load		Ct values		Sample positivity		Association		Temporal trends	
	Gene copies				Overall	Gene specific	Case trend (Y/N)	Correlation, estimated prevalence of infection or comparison with case numbers	Trend	Duration
Martin, J., et al. (2020b)	RdRp: <3.50–5.91 Log ₁₀ gc/L E: 4.63–5.84 Log ₁₀ gc/L	NR			4/5 (raw)	RdRP: 4/5 E: 2/5 nsp2-PLPro: 3/5 ORF8b-N: 4/5	Y	reported COVID-19 cases increased. Low levels of viral RNA detected was detected in February sample; 11 days after first two COVID-19 cases were confirmed in York, northern England, and 3 days before the first case was reported in the population. The viral RNA levels found in sewage in March were higher than cases detected; most likely due to limitations in testing capacity. Large reduction of viral RNA concentration in April–May samples correlated with observed reduction in confirmed cases as lockdown was introduced from 23rd March.	Low levels of viral RNA detected 3 days before the first case was reported in the population	3 days before the first case
La Rosa, G., et al. (2021b)	1200; 290–56,000 c.g./L	NR			15/40 (raw)	NR	Y	Samples were positive before the first autochthonous Italian case was reported (February 21). First positive sewage samples from Milan and Turin (18 Dec 2019) and Bologna (29 Jan 2020) correlated with clinical cases detected in January and February samples, except February sample from Bologna. While infections were still limited in Milan and the first autochthonous case was reported earlier on 21 February, positive samples were detected on 24 and 28 February. Positive samples in Rome were detected on 31 March and 2 April correlating with considerable spread of the epidemic.	SARS-CoV-2 was already circulating in northern Italy at the end of 2019, more over in different geographic regions simultaneously	23–63 days before first case
La Rosa, G., et al. (2020b)	NR	NR			6/12 (raw)	ORF1ab: 6/12 Spike: 2/12 RdRP: 0	Y	February sample from Bologna. While infections were still limited in Milan and the first autochthonous case was reported earlier on 21 February, positive samples were detected on 24 and 28 February. Positive samples in Rome were detected on 31 March and 2 April correlating with considerable spread of the epidemic.	NR	NR
Kuntyntseva, P., et al. (2020b)	NR	NR			City: 3/20 (sewage) School: 1/2 (sewage)	NR	Y	Positive samples correlated to an average of 1.2% of sickness rate in the residences that use the sewage inspection chamber. While level of viral particles in March were not detectable, there were positive samples (4/11) detected in July which correlated to the growth in registered COVID-19 cases in the city. Higher detection (ten times increase) of positive samples on 27 May as opposed to 8 May which correlated with the infection numbers in the area (4912 and 10,674 cases on 8 and 27 May respectively). High virus concentrations were detected between mid-late March which correlated with the COVID-19 peak in Denmark. Samples from Solrød were positive on 24 Feb, three days prior to the reporting of “patient zero” from another city in Denmark. Decrease in the virus load correlated with the introduction of preventive measures by the local authorities.	Kazan Federal University Campus (3 buildings); Presence of viral particles in wastewater but none reported feeling sick or having a fever on the day of investigation. 1 case registered within 5 days after investigation, 3 more cases within 7 days.	5 days before first case
Kumar, M., et al. (2020b)	5.6 × 10–3.5 × 10 ² copies/L	ORF1ab: 32.65–35.52 N: 34.18–35.39 S: 34.83–39.56			2/4 (raw)	ORF1ab: 2/4 N: 2/4 S: 2/4	Y	COVID-19 cases in the city. Higher detection (ten times increase) of positive samples on 27 May as opposed to 8 May which correlated with the infection numbers in the area (4912 and 10,674 cases on 8 and 27 May respectively). High virus concentrations were detected between mid-late March which correlated with the COVID-19 peak in Denmark. Samples from Solrød were positive on 24 Feb, three days prior to the reporting of “patient zero” from another city in Denmark. Decrease in the virus load correlated with the introduction of preventive measures by the local authorities.	NR	NR
Jorgensen, A., et al. (2020b)	NR	32 (hospital sample)			WWTP: 18/51 Hospital: 6/9	NR	Y	High virus concentrations were detected between mid-late March which correlated with the COVID-19 peak in Denmark. Samples from Solrød were positive on 24 Feb, three days prior to the reporting of “patient zero” from another city in Denmark. Decrease in the virus load correlated with the introduction of preventive measures by the local authorities.	Virus was detected in a sample 3 days before patient zero was identified in the country.	3 days before first case

Hata, A., et al. (2021b)	N: 1×10^0 – 1.3×10^4 copies/L N2: 1×10^0 – 1.8×10^4 copies/L N3: 1×10^0 – 3.5×10^4 copies/L Overall: 1×10^0 – 3.5×10^4 copies/L	N: NIL N2: NIL N3: 31.6; 29.9–36.2 Overall: 31.6; 29.9–36.2	20/21 (influent)	N2: 12/21 N3: 16/21 N: 4/21	Y	The detection frequency seemed to be higher when the number of total confirmed SARS-CoV-2 cases in 100,000 peoples became above 10 in each prefecture. The SARS-CoV-2 detection frequency was 15% (3 positives out of 20 samples) before the number became >10, whereas it reached 57% (4 positives out of 7 samples) after the number became >10. When the viral load was high in May, there were more positive wastewater samples detected. More community prevention measures in June and July, lesser cases (decreased viral load) than in May.	NR	NR	NR
Hasan, S. W., et al. (2021b)	1.49×10^3 ; 1.06×10^2 – 2.91×10^4 gc/L	NR	33/45 (influent)	NR	Y		NR	NR	NR
Haramoto E., et al. (2020b)	2.4×10^3 copies/L	39.96	WWTP: 1/10 (secondary treated) River: 0/3	WWTP N: 1/10 N1: 0/10 N2: 0/10 ORF1a: 0/10 S: 0/10 River N: 0/3 N1: 0/3 N2: 0/3 ORF1a: 0/3 S: 0/3	Y	Correspondence between RNA detection and highest peak in daily cases in Yamanshi Prefecture; Cumulative COVID-19 cases low (4.4 cumulative cases/100,000 inhabitants) but SARS-CoV-2 RNA detected when weekly reported community cases were high.	NR	NR	NR
Guerrero-Latorre, L., et al. (2020b)	N1: 2.91×10^6 ; 2.84×10^5 – 3.19×10^6 gc/L N2: 8.55×10^5 ; 2.07×10^5 – 2.23×10^6 gc/L Overall: 1.54×10^6 ; 2.07×10^5 – 3.19×10^6 gc/L 10^1 – 10^4 copies/100 mL	NR	3/3 (river)	River N1: 3/3 N2: 3/3	Y	There were high viral loads from the samples taken in M1 (south-center city) where higher active cases were registered.	NR	NR	NR
Gonzalez, R., et al. (2020b)		NR	150/198 (raw)	N1: 107/198 N2: 125/198 N3: 113/198	Y	Positive detection in at least one WWTP on all sample dates, even during the first week of sampling when there were only two cases in the region. Increased detection in Williamsburg and Nansemond (mid-March & mid-April) due to 2 different outbreaks in those areas.	NR	NR	NR
Fongaro, G., et al. (2021c)	5.74; 5.49–6.68 log ₁₀ gc/L (SD excluded)	NR	4/6 (raw)	NR	Y	Stable viral load in November 2019–March 2020. On 4 March 2020, an increase of approximately 1 log ₁₀ of viral gene copies were detected in wastewater when the first COVID-19 case diagnosed in region.	60 days before first case (continent) >90 days before first case (Brazil)	60 days before first case (continent) >90 days before first case (Brazil)	60 days before first case (continent) >90 days before first case (Brazil)
D'Aoust, P.M., et al. (2021c)	1.7×10^3 – 3.8×10^5 copies/L	NR	11/NR (PCS, PGS)	NR	Y	Positive detection in PCS from Ottawa and Gatineau between 1 April and 30 June 2020; decreasing and continued low COVID-19 prevalence in two cities (56.7 → 4.8 and 57.3 → 10.2 confirmed cases/100 K inhabitants in Ottawa and Gatineau, respectively). No correlation between sample detection and any of the three epidemiological metrics in Ottawa while significant correlations between sample detection and number of active cases in Gatineau. Strong, significant and positive correlation between PMMoV normalized	NR	NR	NR

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Table 2 (continued)

Author	Viral load	Sample positivity		Association		Temporal trends		
		Ct values	Overall	Gene specific	Case trend (Y/N)	Correlation, estimated prevalence of infection or comparison with case numbers	Trend	
Arora, S., et al. (2020b)	NR	33.63; 16.8–37.52	WWTP: 5/18 (influent) Hospital: 1/7 (influent)	WWTP: RdRp: 4/18 E: 6/18 N: 5/18 ORF1ab: 0/1 S: 0/1 Hospital: RdRp: 0/6 E: 2/6 N: 2/7 ORF1ab: 1/1 S: 1/1	Y	N1/2 signals and 7-day rolling average percent positive epidemiological unit in Ottawa. Areas served by WWTPs with positive detections reported a continuous increase in confirmed positive patients soon after the first sampling. A significant increase in positive tested cases within 6–14 days of first sampling (4 May 2020) was correlated to a surge in cases at site 5 (Central jail) within six days of sampling. At site 6, the 1st sample (15 May 2020) was negative owing to low number of positive cases in area. Positive samples in the subsequent weeks correlated with the sudden surge in COVID-19 cases up to fivefold within next six days of detection. Decrease in SARS-CoV-2 concentration in municipal wastewater samples over study duration, corresponding to decrease in cases in the city.	4–15 May 2020: samples positive 10–14 days preceding a large jump in positive cases in the respective area 16 May–12 June 2020: gap between positive sewage detection and positive cases decreased from 14 days to 6 days	6–14 days before case surge
Albastaki, A., et al. (2021b)	NR	32–34, 33–36 (aircraft)	Municipal - 847/2470 (raw) Aircraft - 27/198	NR	Y	Decrease in SARS-CoV-2 concentration in municipal wastewater samples over study duration, corresponding to decrease in cases in the city.	NR	NR
Ahmed, W., et al. (2020d)	N: 49.95; 0.66–156 copies/100 mL N1: 163; 43.9–596 copies/100 mL N2: 13; 1.33–60.9 copies/100 mL E: 138; 53.6–272 copies/100 mL RT-ddPCR N1: 103–387 (83.5–387 copies/100 mL)	RT-qPCR N: 36.6; 35.1–42.1 N1: 34; 32.1–36.8 N2: 38.2; 35.8–41.3 E: 37.7; 36.3–39.0 RT-ddPCR N1: NR N: 37.5–39	Aircraft - 4/10 Cruiseship - 8/11 (influent, effluent)	Aircraft N1: 0/10 N2: 0/10 N: 1/10 E: 3/10 Cruiseship N1: 8/11 N2: 4/11 N: 5/11 E: 3/11	Y	SARS-CoV-2 detected in airplanes but no infected passengers identified within 14 days. SARS-CoV-2 detected in cruise ship corresponded to 24 cases onboard.	NR	NR
Ahmed, W., et al. (2020e)	1.9–12 copies/100 mL	N: 37.5–39	2/9	N: 2/9	N	Estimated a median number of 1090 infections on 27 March; positive sample; 297 cases in the PHNs in Brisbane and median number of 171 infections on 1 April; positive sample; 404 cases in the PHNs in Brisbane	NA	NA
Gonçalves J., et al. (2021b)	NR	E: 33.61–38.45 RdRp: 29.65–38.46 Average Cq in triplicates	10/15	E: 7/15 RdRp: 10/15	Y	SARS-CoV-2 RNA was detected in hospital wastewater when only one patient was hospitalized. The sample from the 5th of June was negative for RdRp, which may have been caused by the heavy rainfall recorded on that day, which may have diluted the sample collected, or by a variable shedding rate of the patients. SARS-CoV-2 RNA signal in wastewater increased over time in both the amount detectable and the proportion of samples that were positive, consistent with increasing cases and hospitalizations, coinciding with Calgary's COVID-19 'second wave'.	RdRp gene - 1 day after the first hospitalization of one patient (4th of June). E gene - 5 days after the first patient was hospitalized (8th of June).	1–5 days after hospitalization
Acosta, N., et al. (2021b)	N1: 0.12–23,861.79 copies/5 µL (avg 921.27 copies/5 µL) N2: 0.096–26,881 (avg 1014) E: 8.65–20,323.42 (avg 3261.92)	Overall: Avg (min, max) N1: 33.4 (26.1, 39.8) N2: 36.9 (27.544.9) E: 30.7 (25.9,	102/165	N1: 102/165 N2: 79/165 E: 9/96	Y	Significant differences in median SARS-CoV-2 N-1,2 between outbreak-free periods vs outbreak periods when measured as copies/mL and normalized for PMMoV was observed at each hospitals	5–14 days before first hospital acquired case	

39.2)	N1 median (IQR) H1: 32.5 (30.6–36.4) H2: 31.8–35.9 H3A: 32.8. (30.7–35.6) H3B: 34.6 (33.8–36.5) H3C: 36.4 (34.7–37.1) N2 median (IQR) H1: 35.3 (33.5–37.7) H2: 35.9 (34.5–37.8) H3A: 36.2 (33.2–41.2) H3B: 39.6 (38.1–41.7) H3C: 41.1 (39.7–41.7) E median (IQR): H1: 35.5 (31.3–37.3) H3A: 30.6 (28.5–30.7) H3C: 34.9						
Agrawal, S., et al. (2021c)	1.29×10^{12} – 1.63×10^{15} copies/day	44/44	NR	Y	Continuous increase again in COVID-19 cases in Frankfurt since August, with an average of 28.6 incidences, compared to 28.7 incidences in April. In August, a resurgence in the SARS-CoV-2 RNA load was observed, reaching 3×10^{13} copies/day, which represented similar levels compared to April with approx. 2×10^{14} copies/day. A corresponding increase in total viral load across all samples was observed when incidence increased. In June and July, the incidences were less than ten COVID-cases per 100,000 persons per week and the load ranged between 1.29×10^{12} and 1.91×10^{13} copies/d. Increase in SARS-CoV-2 RNA load clearly preceded the reported cases with the first step-increase in the middle of July 2020.	Increase in the SARS-CoV-2 RNA load clearly preceded the reported cases with the first step-increase in the middle of July 2020.	NR
Ahmed, W., et al. (2021b)	N1: 135–11,992 gc/100 mL E: 113 & 222 gc/100 mL	N1: 32.8–41.46 N2: 0 E: 36.6 & 37.6	21/63	N1: 19/63 N2: 0/63 E: 2/63	Y	Wastewater samples were generally positive during the period with highest caseload data. Most SARS-CoV-2 RNA detections were in WWTP C wastewater, and were one month before and during the main wave of the pandemic. The decay of the 7-day average detections in mid-April align with the decrease of active cases; As might be expected, the 7-day	Detections were made in a Southern Brisbane WWTP in late February 2020, up to three weeks before the first clinical case was reported there 2–3 weeks before the first case

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Table 2 (continued)

Author	Viral load		Sample positivity		Association		Temporal trends	
	Gene copies	Ct values	Overall	Gene specific	Case trend (Y/N)	Correlation, estimated prevalence of infection or comparison with case numbers	Trend	Duration
Bar-Or, I., et al. (2021b)	NR	27–35	58/58	E: 58/58	Y	average wastewater detections tracked similarly to the 7-day average GC/100 mL; however, the GC/100 mL did not appear to agree well with daily case numbers. Mutation analyses of SARS-CoV-2 in wastewater samples successfully identified the penetration of the B.1.1.7 variant into Israel in December 2020 in the Shafdan and Tzfat regions, and its spread into additional regions by February 2021, corresponding with clinical sampling results (first identified B.1.1.7 case in Israel in December 2020). Identification of B.1.1.7 variant in Tzfat in December 2020, a region in which non-sufficient clinical sampling was available, exemplifies that surveillance by wastewater is a robust approach that can cover large areas by few samples.	Penetration of the B.1.1.7 variant into Israel in December 2020 in the Shafdan and Tzfat regions, first identified case in Israel via clinical sampling and sequencing in December 2020.	NR
Bertrand, I., et al. (2021b)	$\geq 3.0 \times 10^3$ gc/L	NR	12-Oct	RdRp: 9/10 E: 10/10	Y	A parallel decrease in cases in patients and genome concentration in wastewater was observed, confirming the link between the circulation of the virus in the human population and its presence in wastewater.	NR	NR
Betancourt, W., et al. (2021c)	From 1/13 dorms N1: 1.00×10^4 – 3.84×10^5 gc/L N2: 1.61×10^5 – 1.06×10^6 gc/L	NR	99/319	NR	Y	On August 25, wastewater from dorm A tested positive. On August 26, antigen testing identified one positive individual (Person A) despite demonstrating no symptoms. Person B with symptoms reported. Person C inconclusive by antigen test. PCR testing on 26 August revealed all three positive. 76% (sensitivity), 79.8% (PPV), 88.6% (NPV). Overall, while positive COVID-19 cases increased significantly in most sewersheds, the gene copy number for influent and sludge samples remained relatively stable over the sampling period. Of the seven WRFs, only ECWRF exhibited a positive correlation between the influent gene copies per liter and the daily number of COVID-19 cases.	On August 26, wastewater from dorm A tested positive. On August 26, antigen testing identified one positive individual (Person A) despite demonstrating no symptoms. Person C inconclusive by antigen test on August 25, but tested positive on August 29. Persons A, B, C were positive by PCR testing (tested on August 26, results out several days later). A significant correlation between SARS-CoV-2 gene copy numbers in influent and daily reported cases of COVID-19 was seen in ECWRF, after accounting for a one-week time lag. Digested sludge gene copy numbers significantly predicted the weekly number of cases with a two-week lag for all three WRFs that possessed anaerobic digesters.	≤ 4 days before first case in building
Bhattarai, B., et al. (2021b)	RAS: $6.89\text{E}+02$ ($3.33\text{E}+02$)– $1.59\text{E}+04$ ($1.03\text{E}+04$) gc/L Influent: $2.38\text{E}+04$ ($1.83\text{E}+04$)– $4.60\text{E}+04$ ($6.97\text{E}+04$) gc/L	NR	NR	NR	Y	Probability of detection varied widely with the number of known infected people. It fell with decreasing case numbers, but was still around 10% for the presence of a single known case at any point in the illness course. Although unlikely, it provides some evidence that a single infected person in a catchment on a given day could trigger the detection of virus at the sampling site on the same day.	The odds of detection in sewage were between 5 and 20 times higher where known cases were present, with less effect of distance than time since onset. Each known SARS-CoV-2-infected person was determined on each day from two days before onset to 55 days after onset	1 week before (sludge) 2 weeks before (digested sludge)
Black, J., et al. (2021b)	NR	NR	71/346	NR	Y			2 days before - 55 days after onset

Cao and Francis (2021b)	Mean (SD): 318.37 (463.98) $\times 10^6$ gc/L Median (IQR): 137.9 (458.72) $\times 10^6$ gc/L	NR	NR	NR	Y	It is clear that an increase in SARS-CoV-2 concentration has been consistently associated with an increased number of COVID-19 infections (weekly). (Model) An increase in the SARS-CoV-2 concentration in the current week implies a steadily increase in the COVID-19 cases in the following three weeks, peaking in the third week.	An increase in the SARS-CoV-2 concentration in the current week implies a steadily increase in the COVID-19 cases in the following three weeks, peaking in the third week.	3 weeks before case surge
Chakraborty, P., et al. (2021b)	N1: STP/SPS: 1.41×10^4 – 1.99×10^5 gc/L HWW: $>1.19 \times 10^4$ gc/L	STP and SPS overall: N1: 29.2–36.87 N2: 30.03–36.08 HWW: N1: 28.68–38.24 N2: 25.85–32.07	12/17 (incl 0 effluent)	NR	Y	Higher viral loading in HWW samples during partial lock-down (1.10×10^5 – 1.62×10^5 gc/L) over post-lockdown (1.20×10^4 – 1.15×10^5 gc/L) can be related with the lower number of active COVID-19 patients admitted in SRM hospital during that period. The wastewater surveillance showed the presence of higher number of infected people in communities with high population density.	NR	NR
Chavarria-Miró, G., et al. (2021b)	NR	NR	NR	NR	Y	In WWTP 2, the analysis of archival samples revealed the increasing occurrence of SARS-CoV-2 genomes in samples from 15 January to 4 March employing the IP2, IP4, and E targets. Of note, SARS-CoV-2 was detected in sewage 41 days (15 January) ahead of the declaration of the first COVID-19 case (25 February), clearly evidencing the validity of wastewater surveillance to anticipate cases in the population. Progressive decline in genome copy numbers in both WWTPs paralleled the diminution in the estimated cumulative number of shedders, based on the actual number of reported symptomatic cases and figures for 7-day, 14-day, and 21-day excretion periods before the sampling date; evidences the effect of lockdown measures on the spread of the infection.	Of note, SARS-CoV-2 was detected in sewage 41 days (15 January) ahead of the declaration of the first COVID-19 case (25 February).	41 days before first case
Ciesielski, M., et al. (2021b)	RT-ddPCR: 0– $3.2E+05$ copies/L RT-qPCR: 0– $1.16E+06$ copies/L	N2: RT-ddPCR: 0– $3.2E+05$ copies/L RT-qPCR: 0– $1.16E+06$ copies/L	NR	N2: 39/60	N	NR	NR	NR
Colosi, L., et al. (2021b)	NR	Wastewater (influent) Hospital N1: 30.6–41.9 N2: 32.5–41.2 WWTP N1: 30.3–34.2 N2: 31.2–35.2	Wastewater (Influent) Hospital: 11/11 WWTP: 2/3 School dorms: 15/29 Primary solids WWTP: 3/3	NR	Y	Wastewater results are highly consistent with known presence or absence of COVID-19 cases detected via clinical testing. Across all hospital and dorm samples collected after completion of the side-by-side method comparison, outcomes were as follows: 25 true positives, 0 false positives, 9 true negatives,	Interval between successive clinical and wastewater-based testing was never more than 8 days. Positive wastewater results were obtained 8 days after the first positive clinical results and 5 days after the second set of clinical positive results.	5–8 days after sample positivity

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Table 2 (continued)

Author	Viral load		Sample positivity		Association		Temporal trends	
	Gene copies	Ct values	Overall	Gene specific	Case trend (Y/N)	Correlation, estimated prevalence of infection or comparison with case numbers	Trend	Duration
D'Aoust, P., et al. (2021d)	School dormitory N1: 32.6–38.1 N2: 35.1–41.5 Primary solids WWTP N1: 30.8–36.2 N2: 32.4–39.2 NR		Overall: 31/46			and 1 apparent false negative. Corresponding sensitivity was 96.2% and specificity was 100% (94.74% and 100%, respectively, if duplicate samples are excluded). There was a very weak negative correlation between CT values and known hospital case counts by date for both the N1 and N2 amplicons.		
	N1 and N2: below 1.00×10^{-4} copies/copies PMMoV An absolute increase of 2.03×10^{-4} and 3.01×10^{-4} copies/copies PMMoV is reported during this period of increase for N1 and N2 gene regions		NR	NR	Y	Positive correlations were observed between the normalized viral RNA signal (N1 and N2) and both the number of new daily positive COVID-19 cases and clinical testing percent positivity. A weak positive correlation was also seen between N1 and N2 SARS-CoV-2 PMMoV-normalized RNA signal and COVID-19-caused hospitalizations	A times-step correlation analysis suggests that increases in SARS-CoV-2 signal in wastewater precedes increases in new daily positive COVID-19 cases and clinical testing percent positivity by two days. Furthermore, it also suggests that increases in SARS-CoV-2 signal in wastewater precedes increases in the number of hospitalized cases by four days	2 days before case resurgence 4 days before hospitalization increased rates
Fernandez-Cassi, X., et al. (2021b)	NR	NR	NR	NR	Y	RNA (N1) load generally corresponded to confirmed cases in the regions over sampling period. Wastewater-derived incidence (model) outperformed case numbers with respect to the timing and shape of the peak incidence, whereas confirmed case numbers were a better indicator for incidence decline.	NR	NR
Fongaro, G., et al. (2021d)	Sewage: average of 1.3×10^4 – 4.3×10^4 gc/mL Downstream river water: average 1.1×10^2 – 1.7×10^3 gc/mL Upstream river water: ND N1: 3.6×10^4 gc/L N2: 1.2×10^5 gc/L Overall: 10^4 – 10^6 gc/L	NR	NR	NR	N		NR	NR
Gerrity, D., et al. (2021b)		N1: 33.8 ± 1.4 N2: 35.1 ± 1.6 E. Sarbeco: 35.2 ± 1.1 orf1a: 33.3 ± 1.8	NR	N1: 30/56 N2: 26/56 E. Sarbeco: 9/56 orf1a: 4/56	Y	Consistent wastewater detections of SARS-CoV-2 occurred once the confirmed daily case load and cumulative cases exceeded 0.001% relative incidence and 0.01% relative prevalence, respectively. The strongest observed wastewater signal, as determined by the number of positive assays and replicate reactions, occurred when the confirmed daily case load and cumulative cases reached 0.01% relative incidence and 0.1% relative prevalence, respectively, which corresponds with a model concentration of nearly 105 gc/L.	NR	NR
Gibas, C., et al. (2021b)	394–2,990,271 copies/L	28.38–45.21	40/332	N1: 40/332	Y	The number of new daily positive cases in Mecklenburg County as a whole and the number of buildings detected as positive on each sampling event are correlated over this time frame.	NR	NR
Giraud-Billoud, M., et al. (2021b)	N1: 3.97×10^2 – 1.05×10^5 gc/100 mL (avg 34.4) N2: 0.5×10^2 – 1.45×10^5 gc/100 mL (avg 1.49 * 10^4)	N1: 30.7–38.3 (avg 34.4) N2: 30.8–40 (avg 34.6)	39/46	N1: 32/46 N2: 37/46	Y	Wastewater samples were not detectable in July when the weekly cases were 356. The samples were positive for SARS-CoV-2 RNA genetic markers on August 3rd and 11th before the biweekly doubling of	N2 was the first marker to become positive on July 30, 2020. N1 and N2 showed the highest concentration when the cases were at its peak. Wastewater samples were detectable before the	NR

Graham, K., et al. (2021b)	NR	NR	NR	N1: 79/96 N2: 68/96	Y	weekly COVID-19 cases. Between September and the end of October, when the cases were high, wastewater samples were positive for N1 and N2 consecutively. WW samples from EP remained detectable between late November and December, when only 140 and 290 weekly COVID-19 cases were reported. Positive association between N1 and N2 in solids and the number of new COVID-19 infections. The concentrations of N1 and N2 tracked the reporting of new cases in the sewershed (based on specimen collection date) when accounting for autocorrelation and technical errors associated with the wastewater measurements. Down sampling wastewater data collection and analysis frequency to twice per week yields significant associations between case counts and wastewater concentrations, not fortnightly or once a week.	exponential growth phase of weekly COVID-19 cases. As cases diminished from November 9 to November 29, wastewater samples remained positive. In addition, the N2 marker showed a second peak in the week of November 2–8, 2020.	NR
	NR	NR	NR	NR	Y	Drop in wastewater SARS-CoV-2 RNA concentration, new positive clinical tests and COVID-19 related deaths following the imposition of the UK-wide lockdown in late March 2020. WWTPs in Manchester, Liverpool and the Wirral showed strong correlations between SARS-CoV-2 RNA concentration and daily positive tests. Negative correlations were also observed between viral concentrations in all sites and time following the implementation of national lockdown, except Cardiff, indicating these measures lowered the prevalence of the virus in local populations.	Our downsampling analysis suggests that sampling solids twice per week would be frequent enough to identify the global trends in the clinical case data.	2–4 days before testing
Hillary, L., et al. (2021b)	<1.2 × 10 ³ –1.5 × 10 ⁴ gc/100 mL	NR	NR	NR	Y	The presence of SARS-CoV-2 in wastewater influent samples collected during April and May 2020 were in agreement with confirmed COVID-19 cases in the municipalities of the Viikimäki WWTP sewerage network area preceding the sampling.	If only considering daily clinical testing data, the SARS-CoV-2 wastewater RNA concentration leads testing data by 2–4 days but this can be extended by approximately 1 day by using a rolling sum of positive clinical test cases over a series of days leading up to the clinical testing date being considered. The overall effect of varying these parameters is minimal since correlation coefficients stay between 0.8 and 0.9 over a range of permutations.	NR
Hokajärvi, A., et al. (2021b)	E: mean 2.8–3.2 log10 copies/100 mL N2: mean 3.6–4 log10 copies/100 mL	NR	2-Feb	E: 2/2 N2: 2/2	Y	Despite presence of infected patients since the first sampling date, SARS-CoV-2 was not detected in untreated wastewaters sampled on 15, 16, 22 and April 24, 2020. A relatively consistent detection of the SARS-CoV-2 N1 gene was only observed after April 27, 2020.		NR
Hong, P., et al. (2021b)	Overall: 81.1 ± 11.0–1327.4 ± 176.6 gc/L Untreated wastewater N1: 173.7 ± 32.2 gc/L N2: 772.1 ± 172.5 gc/L N3: 1327.4 ± 176.6 gc/L Partially treated wastewater N1: 81.1 ± 11.0 gc/L N2: 1115.8 ± 173.1 gc/L N3: 411.2 gc/L	NR	43/57	N1: 31/57 N2: 26/57 N3: 23/57	Y	N1 and N2 gene exhibited a weak positive correlation, but there was no apparent correlation between abundance of N3 genes and the number of hospitalized patients. An estimated range of 253–409 positive cases out of 10,000 persons is required prior to detecting SARS-CoV-2 RNA in		NR

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Table 2 (continued)

Author	Viral load		Sample positivity		Association		Temporal trends	
	Gene copies	Ct values	Overall	Gene specific	Case trend (Y/N)	Correlation, estimated prevalence of infection or comparison with case numbers	Trend	Duration
Johnson, R., et al. (2021b)	Overall: 4.6×10^3 – 454×10^3 copies/mL N1: $\sim 5 \times 10^3$ – 400×10^3 copies/mL N2: 4.6×10^3 – 454×10^3 copies/mL	Overall: 29–32	5-May	N1: 5/5 N2: 5/5	Y	wastewater. The number of confirmed COVID-19 cases on 18 June 2020 in the Khayelitsha subdistrict (one of the areas served by the Zandvliet WWTP) was higher ($n = 80$) than in the Stellenbosch municipality ($n = 43$) which corresponded with the SARS-CoV-2 data from Zandvliet and Stellenbosch WWTP.	NR	NR
Karthikeyan, S., et al. (2021b)	487,224.07–4,303,251.63 gc/L	NR	94/94	NR	Y	Peaks in the wastewater data were frequently followed by peaks in the clinically confirmed cases at a later date; suggests a correlation between wastewater and the number of new cases with the caveat of a time delay, where the wastewater data predicts future trends in the new number of cases. SARS-CoV-2 viral gene copies correlated with the daily hospital caseload; suggesting wastewater data could at least be used to identify the peak.	Forecasts successfully captured general trends on the number of new cases (shown here up to 3 weeks in advance), ultimately reinforcing that wastewater analysis can be expository of previously undetected SARS-CoV-2 infections in the population.	3 weeks before
Kitamura, K., et al. (2021b)	Overall: 1.1×10^2 – 1.3×10^4 gene copies/L	NR	18/32	NIID_N2: 13/32 CDC_N1N2: 17/32	Y	Spearman's correlation analysis revealed a significant correlation between the number of COVID-19 cases and viral RNA concentration in WWTP B. No SARS-CoV-2 RNA was detected within the first two weeks of the sample collection period (June 9–17) at any of the three collection sites. The first detection was made within the third week, on June 23, corresponded to a sample from Manhole A. Viral RNAs were continuously detected in samples from Manhole A and WWTP B after the third and sixth weeks, respectively. Notably, WWTP B is downstream of Manhole A and is located in a high COVID-19 prevalence area. Conversely, WWTP C is located in a low COVID 19 prevalence area, and samples from this WWTP rarely showed the presence of SARS-CoV-2 RNA.	NR	NR
Koureas, M., et al. (2021b)	12–8000 copies/mL	NR	NR	NR	Y	Wastewater based predictions seem to capture short term changes in disease incidence and resembled the epidemic curve in both municipalities. Considerable deviations between actual and predicted cases were observed in certain periods (e.g., January), while very high accuracy was observed during the first epidemic wave in both municipalities (from October until December).	Our results indicate that estimating cumulative cases choosing a 7-day window is feasible.	NR
Kumar, M., et al. (2021c)	Overall S: average ~1223 copies/L	Average values: S: 32.66	Overall: 39/43 Wards: 32/33	Overall: N: 41/43	Y	A comparison of monthly variation demonstrated higher SARS-CoV-2 genome	Examining the potential of WBE for COVID-19 surveillance as a potential tool	1–2 weeks before

	(21–4159 copies/L) N: average ~1022 copies/L (22–6768 copies/L) ORF 1 ab: average ~485 copies/L (34–2441 copies/L) Wards S: 42–4159 copies/L N: 22–6768 copies/L ORF 1 ab: 34–2441 copies/L Academic institute S: 21–2976 copies/L N: 101–2357 copies/L ORF 1 ab: 43–1476 copies/L Overall: 0.13 × 10 ² –1049.2 × 10 ² copies/L N: 0.15 × 10 ² –522.7 × 10 ² copies/L ORF 1 ab: 0.13 × 10 ² –1049.2 × 10 ² copies/L S: 0.15 × 10 ² –374.5 × 10 ² copies/L	N: 33.03 ORF 1 ab: 33.95	Academic institute: 7/10	ORF: 39/43 S: 39/43 Ward: N: 33/33 ORF: 32/33 S: 32/33 Academic institute: N: 8/10 ORF: 7/10 S: 7/10	concentration in September (~925 copies/L) than August (~898 copies/L) in line with the ~2.2-fold rise in the number of confirmed cases during the study period	showed that the percentage change in genome concentration level on a particular date was in conjunction with the confirmed cases registered 1–2 weeks later on a temporal scale by the regulatory authority based on clinical tests	
Kumar, M., et al. (2021d)		Average N: 32.50 ORF 1 ab: 32.36 S: 33.85	111/116	N: 109/116 ORF 1 ab: 109/116 S: 109/116	Monthly variation depicted a significant decline in gene concentration in October compared to September 2020, followed by a sharp increment in November 2020. The PCR products and effective gene concentration for all three genes were maximum in wastewater samples of November, followed by September and October, in line with a ~1.5-fold rise in the number of confirmed cases during the study period (3rd September 2020 and 26th November 2020). The linear regression between changes in SARS-CoV-2 effective gene concentration and the number of confirmed cases showed a positive but statistically insignificant correlation. Corresponding to the decrease in new clinical cases resulting from the lockdown, the measured SARS-CoV-2 RNA concentration in the wastewater samples also exhibited an overall downward trend and significant concentration fluctuations in both WWTPs.	Percentage change in effective gene concentration level on a particular date was in conjunction with the confirmed cases registered 1–2 weeks later; relationship between percentage changes in effective gene concentration and confirmed cases that can be used as a pre-alarming tool, which gives a lead of ~2 weeks for the upcoming scenario.	1–2 weeks before
Li, B., et al. (2021b)	Liquid fractions N1: 10 ³ 3.0–10 ⁵ 5.1 GC/L N2: 10 ⁴ 1.2–10 ⁴ 4.5 GC/L E: 10 ² 0.0–10 ⁴ 4.5 GC/L Solid fractions N1: 10 ⁴ 4.1–10 ⁵ 5.5 GC/g N2: 10 ⁴ 1.5–10 ⁶ 6.0 GC/g E: 10 ⁴ 1.4–10 ⁶ 6.2 GC/g	NR	NR	NR	detected significant daily fluctuation of the wastewater SARS-CoV-2 RNA abundance, even within the same weeks, indicating the fine-scale temporal dynamics of SARS-CoV-2 RNA in wastewater needs to be taken into consideration in designing and implementing wastewater-based surveillance		NR
Melvin, R., et al. (2021b)	NR	NR	NR	NR	The clinical data for the state showed that SARS-CoV-2 virus was present in late Spring and then began to decrease until around mid-July, but then it steadily increased through August. Similar trends were observed for indexed wastewater SARS-CoV-2 RNA in each region across Minnesota, with the exceptions of the South East and South West regions. Interestingly, indexed wastewater SARS-CoV-2 RNA data pooled for the entire state had a similar pattern to clinical cases of COVID-19 around the same period, except it appeared to be slightly offset. Levels in wastewater samples (indexed SARS-CoV-2) appeared to increase by mid-June, preceding the apparent increase in new clinical cases. This trend was recapitulated in most of the other regions of Minnesota, except for the	Statewide, lag analysis showed that wastewater predicted confirmed new cases by 15 d for N1 and 17 d for N2. The predictive window when Melvin's Indices for N1 and N2 were averaged was 17 d. Lag analysis had less predictive power when regions were considered.	15–17 days before

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Table 2 (continued)

Author	Viral load		Sample positivity		Association		Temporal trends	
	Gene copies	Ct values	Overall	Gene specific	Case trend (Y/N)	Correlation, estimated prevalence of infection or comparison with case numbers	Trend	Duration
Mondal, S., et al. (2021b)	NR	NR	NR	N1: 36/36 N2: 36/36 E: 36/36	Y	South West and South East regions. Pearson correlation coefficient comparing N1 to new cases was 0.38, that comparing N2 to new cases was 0.38, and that comparing the mean of N1 and N2 to new cases was 0.39.	Concurrent peak with 7 day moving average	7 days
	N1: 56–214,430 gc/L	NR	202/202	N1: 202/202	Y	The peak of SARS-CoV-2 genetic signal observed in the wastewater is concurrent with the peak of positive SARS-CoV-2 reported in mid-November of 2020. The four-week cumulative number of individuals seeking health care with COVID-19 symptoms and the load of SARS-CoV-2 RNA in sewage followed similar trends, with sharp increases in weeks 24 to 26, followed by surges and later decreases in weeks 27 to 32. Local restrictions had a strong effect on hospital bed occupation, as well as suspected COVID-19 cases and SARS-CoV-2 RNA loads in sewage.	NR	NR
Pillay, L., et al. (2021b)	N2: average $0-7.32 \times 10^5$ copies/100 mL	NR	39/42	N2: 39/42	Y	The increase in clinical cases during the period of 9th–12th August 2020, corroborated with an increase in the SARS-CoV-2 loads in wastewater. This trend continued to rise and reaching its peak on 18th August 2020.	NR	NR
Prado, T., et al. (2021b)	$3.07-7.12 \log_{10} \text{ gc}/100 \text{ mL}$	NR	188/223	N2: 188/223	Y	High titers of SARS-CoV-2 RNA in sewage samples coincided with the period when the city registered the highest numbers of COVID-19 cases and related deaths (end of April to mid-June).	NR	NR
Rafiee, M., et al. (2021b)	NR	N: 29.59–39 ORF1ab: 31.06–40	34/34	N: 34/34 ORF1ab: 31/34	Y	As expected, the temporal variations on November 2020 and May 2021 did not reach statistical significance ($p = 0.074$). These results are consistent with the so-called second and fourth epidemic waves in Iran (Iran Ministry of Health and Medical Education, 2020).	NR	NR
Róka, E., et al. (2021a)	N1: 8×10^4 – 1.93×10^5 gc/day (up to 7.14×10^5 GC/L) Hospital building: 3.27×10^5 GC/L	NR	NR	NR	Y	First positive signals of SARS-CoV-2 in sewage were detected 2 weeks before case numbers started to rise. From week 34, new cases doubled every week, and the viral titres also increased exponentially. Viral load correlated to the number of new cases on the week of sampling and the active cases on the next week. Highest correlation was seen at +3 and +11 days for new cases and active cases, respectively. No clear association was seen when the number of cases	Viral load correlated to the number of new cases on the week of sampling and the active cases on the next week. Highest correlation was seen at +3 and +11 days for new cases and active cases, respectively.	2 weeks before case surge

Rusiñol, M., et al. (2021b)	N1: $2.18 \times 10^{-3.92} \times 10^6$ gc/L N2: $9.24-2.82 \times 10^6$ gc/L Overall: $9.24-3.92 \times 10^6$ gc/L	NR	N1: 118/184 N2: 102/184 Overall: 128/184	NR	Y	plateaued. Correlation to hospitalisations and deaths were low. The highest concentration of SARS-CoV-2 in WW matched the infection peak for the first wave of the pandemic in Barcelona. While the second wave of the pandemic (July-onwards) showed higher recorded cases but the concentration of N1 in WW was lower than that recorded in the first wave, suggesting under-diagnosis in the first wave. The efficacy of the lockdown measures implemented nationwide were reflected by WW monitoring, with viral load decreasing with daily new cases and prevalence estimates. Besides the incidence level, concentrations of SARS-CoV-2 (N1 and N2) were consistently higher in larger facilities compared with smallest plants. During the first weeks of the second wave, no SARS-CoV-2 RNA was detected in the smallest WW facilities, it was only when the infection rate relative to population was higher than ever, SARS-CoV-2 was detected and quantified in their WW samples. In all 10 WWTPs, WW loads are congruent with estimations of active cases within the watershed. The lowest incidence of infection which resulted in a quantifiable concentration of SARS-CoV-2 in WW for medium sized WWTPs was 0.11 cases/1000 inh. while for small WWTPs this was 0.82 cases/1000 inh. The four peaks of SARS-CoV-2 during the sampling period coincided with the peaks of SARS-CoV-2 in treated effluent wastewater with a 4-log10 reduction compared to the influent wastewater. The SARS-CoV-2 peaks in wastewater preceded the peaks of number of hospitalized patients with COVID-19 at Sahlgrenska University Hospital by three to four weeks. Daily reported SARS-CoV-2 positive cases decreased by over 66% during the study time period which was mirrored by declining CRNA trends observed in all the WWTPs. A momentary surge in the CRNA was observed during the first two weeks of August 2020 which coincided with the rise in the daily reported cases.	Concentrations of SARS-CoV-2 (N1 assay) in WW showed good correlations with the number of cases that would be diagnosed from 0 to 7 days after sampling.	≤1 week before
Saguti, F., et al. (2021b)	Influent RdRp: $<3-16 \log_{10}$ gc/L Treated effluent RdRp: $0.14-1.87 \log_{10}$ gc/L	NR	18/35	RdRp: 18/35	Y	Correlation between the increase in concentrations of viral genomes in wastewater and the number of newly hospitalized patients with COVID-19 with the former preceding the latter by three to four weeks.	3-4 weeks before hospitalisations	
Saththasivam, J., et al. (2021b)	N1: 7889-542,056 copies/L N2: 14,980-485,050 copies/L	N1: 27.5-32.4 N2: 27.9-33.3 Overall: 27.7-33.3	43/43	N1: 43/43 N2: 43/43	Y	Overall, the estimated infected population dropped approximately 17-fold the numbers of daily positive cases in Qatar fell by 66.4% between the third week of June and the end of August 2020. Results did not indicate that SARS-CoV-2 had appeared earlier in the wastewater than the confirmed virological laboratory testing.	0 days before	
Scott, L., et al. (2021b)	N1: $2.25 \times 10^{-5.27-10^3}$ gc/100 mL (average 1.75×10^3 copies/100 mL) N2: $8.16 \times 10^{-3.91} \times 10^4$ gc/100 mL (average 6.76×10^3 copies/100 mL) Overall: $2.25 \times 10^{-3.91} \times 10^4$ gc/100 mL	NR	60/110	N1: 58/110 N2: 58/110	Y	Temporal trends of SARS-CoV-2 in wastewater, particularly, after human fecal marker normalization, mirrored trends in COVID-19 case data.	NR	

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Table 2 (continued)

Author	Viral load		Sample positivity		Association		Temporal trends	
	Gene copies	Ct values	Overall	Gene specific	Case trend (Y/N)	Correlation, estimated prevalence of infection or comparison with case numbers	Trend	Duration
Sharma, D., et al. (2021b)	NR	E: 31.92–43.54 RdRp: 30.46–42.19	20-Dec	E: 12/12 RdRp: 12/12	Y	None of the sewage samples (from any site) collected before March 20, 2020 showed amplification of E and RdRp genes of SARS-CoV-2 RNA. All sewage samples collected in May 2020 (except one sample from Kurla) were positive for SARS-CoV-2, just as cases exceeded 1000 cases in 5 of 6 regions sampled.	NR	NR
Spurbeck, R., et al. (2021b)	N1 Overall: 1–110.6 gc/mL Hospital 1 N1: 1–2.3 gc/mL Hospital 2 N1: 9–110.6 gc/mL Nursing home N1: 0.6 gc/mL Community N1: 2.9–39.1 gc/mL N: 7.18E + 01 to 1.09E + 03 GC/mL	NR	12-Sep	N1: 9/12 Hospital: 5/6 Nursing Home: 1/3 Residential: 3/3	Y	For Hospital V and Nursing Home N, and a weak positive correlation was observed between the number of infected and viral load in the wastewater. Data from Hospital P and Community C were not available for correlation.	NR	NR
Tanhaei, M., et al. (2021b)		ORF1ab: 29.62–34.45 N: 27.60–30.53	11-Sep	ORF1ab - 9/9 N - 9/9	Y	Comparison of the average level of SARS-CoV-2 genomes in the last wastewater samples with the earlier wastewater samples confirmed that the increase of viral genome in effluent perfectly monitored the increase in the number of new cases confirmed in the country on the onset of the second peak of COVID-19.	NR	NR
Tomasino, M., et al. (2021b)	2.05–1104.9 ng/μL	Overall: 28.3–43.9	16/68	NR	Y	From week 21 (2020/09/30) onwards, we started detecting SARS-CoV-2 RNA in both WWTPs and on week 23 (2020/10/14) we detected SARS-CoV-2 RNA for the first time in all samples collected for this study. The improved detection in mid-October was concomitant with an increase in the number of COVID-19 cases in the region. Good fit between the SARS-CoV-2 gene copy numbers in the analyzed wastewater and the COVID-19 incidence peaks in the region were observed, especially with the peak observed in mid-November. The highest SARS-CoV-2 copy numbers observed for both WWTPs in the liquid and solid phases were coincide with the mid-November peak, with the exception of a few higher values observed during the	NR	NR

Wannigama, D., et al. (2021b)	Overall: 22.86–967.926 copies/mL	NR	NR	Y	mid-January peak in the liquid phase of Sobreiras WWTP. Significant positive relationships between the SARS-CoV-2 copy numbers and the weekly moving average of COVID-19 cases in both phases of both WWTPs. In December, the number of SARS-CoV-2 RNA copies from the city center and suburbs in wastewater increased, reflecting the increased number of positive SARS-CoV-2 patients (previously 0 in Bangkok and nearby suburbs); higher copy number of the viral genome could correlate with an elevation in infected people in the community. Communities with higher confirmed COVID-19 caseloads tended to have higher SARS-CoV-2 MVGC/cap/d in wastewater while the SARS-CoV-2 wastewater loads in ECWRF decreased over the nine-week observation period as the COVID-19 case rates dropped. A sharp increase in viral loads was seen from 2 WWTPs just prior to increases in their weekly COVID-19 case rates. While the overall estimated number of SARS-CoV-2 shedders in each sewershed was found to be linearly correlated with the cumulative diagnosed COVID-19 cases, the daily estimated number of SARS-CoV-2 shedding individuals did not correlate with daily COVID-19 cases	NA
	0.6 (1.7) gc/mL–1038 (1294) $\times 10^3$ gc/mL	NR	109/242	Y	When a one-week lag was applied to the weekly COVID-19 case rates, the ECWRF virus RNA in wastewater did correlate with the COVID-19 case rates, suggesting that the increase in case counts may occur concurrently or precede the increase in SARS-CoV-2 RNA in wastewater, while the decline in SARS-CoV-2 RNA in wastewater may lag the decline in case counts.	NR
Wilder, M., et al. (2021b)	Average: 2.16×10^4 (2.11 $\times 10^4$) gc/L Maximum: 1.02×10^5 (7.96 $\times 10^3$) gc/L	NR	111/169	Y	Samples with quantifiable levels of SARS-CoV-2 RNA were associated with higher levels of positive test results the week following sampling. Over the seven days following wastewater sample collection, both the average number of new positive tests per 10,000 persons and the testing positivity rate (among people contributing to a sewershed in 7 days after sampling) were significantly higher in quantifiable samples than in samples classified as BLOD or DINQ for SARS-CoV-2. Significant relationship between the SARS-CoV-2 (normalized by crAssphage DNA) and the number of positive tests per 10,000 population.	7 days before cases/10,000 population
Wilton, T., et al. (2021b)	NR	NR	13/14	Y	SARS-CoV-2 RNA in sewage samples from London throughout the COVID-19 pandemic, with changes in viral RNA levels shown to be in good agreement with those of confirmed COVID-19 cases reflecting national restrictions.	NA
Wong, J., et al. (2021b)	600–246,700 copies/L	NR	NR	Y	The index case (Case 1) returned from the healthcare facility on 8 July 2020 after clinical recovery. Therefore, wastewater signals detected on 9 July 2020 could be either due to viral shedding from the newly	3–4 days before case was symptomatic

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Table 2 (continued)

Author	Viral load		Ct values		Sample positivity		Association		Temporal trends	
	Gene copies				Overall	Gene specific	Case trend (Y/N)	Correlation, estimated prevalence of infection or comparison with case numbers	Trend	Duration
Wu, F., et al. (2021b)	2.47–16,878.71 copies/mL	NR	NR	830/1751	NR	Y	identified case (Case 10) or from the returned index case. SARS-CoV-2 levels declined after the newly identified case was conveyed to a healthcare facility (1100–39,400 to 600–1700 RNA copies/L sewage from 10 to 13 July 2020). SARS-CoV-2 levels increased again during 15–16 July 2020 (3900–28,300 RNA copies/L sewage) and was attributed to two recovered cases who returned on 14 July 2020. SARS-CoV-2 levels were not detected from the evening of 19 July 2020, corroborating the viral shedding pattern of recovered cases and the cessation of active virus transmission in the building.	A weak positive correlation was found between the incidence of daily new cases at the county level, and the wastewater viral titers at the catchment. A linear relationship was observed between the total viral load and catchment size-normalized daily new cases.	Mean viral titers increased from early March and became relatively stable between late March to late April, followed by a small downward trend until June 2, mirroring the trends of clinical new cases and deaths at the national level, and precedes clinical data. Wastewater viral titers also reflected, and seemed to precede, the rise and fall of hospitalization and intensive care unit admissions.	NR
Wurtz, N., et al. (2020b)	<300–9000 copies/mL	NR	NR	NR	NR	Y		When looking at the evolution between the number of SARS-CoV-2 copies in wastewater and the number of SARS-CoV-2 positive cases, from 1 July to 1 September, the amount of virus in the sewer increases as does the number of positive cases. Then, while the number of positive patients stagnates, the amount of virus in the sewer drops from 1 to 23 September. In this phase, a discrepancy between the number of SARS-CoV-2 positive cases and the amount of virus in the wastewater was observed. From September 24, a perfect correspondence was observed between the number of positive cases and the amount of virus observed in the sewers, with a peak observed on October 22. Then, a decrease in the amount of virus was observed in wastewater, in agreement with the decrease in the number of SARS-CoV-2 positive cases. The cross correlation testing of the curves shows a maximal correlation with a lag = 0. The shape of the concentration curve was reminiscent of the disease dynamics at the regional level. Observed delay between epidemiological curves in humans compared to viral RNA quantification in	NR	NR
Wurtz, S., et al. (2020b)	Max: 2.5×10^6 genome units/L	NR	NR	NR	NR	Y			Viral concentration similar to the number of daily positive cases, with an 8-day temporal shift.	8 days before

Xiao, A., et al. (2021b)	NR	NR	NR	Y	WW. Wastewater viral titers generally mirrored trends in disease incidence: an exponential rise from March to mid-April, a decline through July, a slow increase over the summer, followed by a sharper increase in the fall and second peak in the winter. Trends in wastewater data differed from clinical data after some key events; a short peak in wastewater viral titers at the start of August, which was only slightly reflected in the clinical data; after colleges and universities welcomed students back in late August/early September, we observed another peak in wastewater viral titers, but not in clinical cases; after the start of Phase 3 Step 2 reopening, wastewater viral titers increased steeply, while clinical cases had a shallower slope. WW data more sensitive than clinical data.	The relationship and time lag between wastewater viral titers and positive clinical tests changed over the course of the pandemic. Wastewater was more effective as a leading indicator in the first wave of the pandemic, and this early warning effect diminished drastically in the second wave, perhaps as clinical testing availability increased. Delay between wastewater data and clinical reporting shrank from average of 6.2 days to no significant difference between the wastewater and clinical trend after August 15, 2020.	6.2 days before in early stage of pandemic (low testing capacity)
Xu, X., et al. (2021b)	NR	NR	23/107	Y	Hospital N: 7/12 Individual buildings N: 4/8 WWTP/sewer network N: 12/87 Total N: 23/107	SARS CoV-2 was detected in sewage samples 2 days before COVID-19 was first diagnosed in two individual buildings, community testing for these buildings only began on 27 July, after the positive sewage tests were discovered.	2 days before first case in building
Yaniv, K., et al. (2021b)	N1: 3.32×10^6 – 3.71×10^7 copies/mL 33.18–36.66	N1: 19-Sep	NR	Y	Although the association between sewage measurements of SARS CoV-2 and clinically reported COVID-19 cases has been observed in sewage treatment facilities, a similar relationship was not observed in this study. In May, no relationship was observed between wastewater signals and reported COVID-19 cases. The low morbidity in the city reported during May, particularly at the end of the month, might have been an underestimation of COVID-19 cases due to limited testing and untested asymptomatic cases. Despite no new COVID-19 positive cases reported in Ashkelon, there were traces of the SARS-CoV-2 RNA in sewage originating from the different sampling manholes in the city. A positive SARS-CoV-2 RNA signal was detected in the city sewage system about one week prior to July COVID-19 initial outbreak of the second wave of in the city. A resurgence in COVID-19 cases became evident in the city's population in June–July 2020 a month following the wastewater sampling. Detections from North Richmond and West Camden not associated with	A positive SARS-CoV-2 RNA signal was detected in the city sewage system about one week prior to July COVID-19 initial outbreak of the second wave of in the city.	7 days before second outbreak
NSW Government (2020a)	NR	NR	17/312	N	NR	NA	NA

(continued on next page)

Table 2 (continued)

Author	Viral load		Ct values		Sample positivity		Association		Temporal trends	
	Gene copies				Overall	Gene specific	Case trend (Y/N)	Correlation, estimated prevalence of infection or comparison with case numbers	Trend	Duration
NSW Government (2020b)	NR		NR		4/52	NR	N	previously reported cases (only one case in West Camden WWTP area since 3 October); suggest recently recovered or undiagnosed cases	NR	NA
NSW Government (2020c)	NR		NR		6/55	NR	N	Sites with positive detections had previously reported cases, i.e. positive detection at West Camden WWTP; recent cases identified due to increased testing	NR	NA
NSW Government (2020d)	NR		NR		6/53	NR	Y	Sites with positive detections had previously reported cases, i.e. positive detection at West Camden WWTP; recent cases identified due to increased testing	NR	NA
Kenyon College (Cambier Wastewater SARS-CoV-2 Virus Report, n.d.)	NR		NR		NR	NR	NR	All detections apart from Bathurst were associated with reported cases	Positive sample not linked to reported cases in Bathurst	2 weeks before
								NR	Wastewater signal often precede individual clinical tests by 2 weeks; likely that wastewater reveals earlier cases missed by clinical qPCR (false negative rate = 30%)	

Australia, Ahmed et al. Detected SARS-CoV-2 up to up to three weeks before the first clinical case in Brisbane (Ahmed et al., 2021a). Interestingly, Martin et al., and Jorgensen et al., both detected viral RNA in samples dated three days before the first case surfaced in the sampled population in Southeastern England and a city in Denmark respectively (Jorgensen et al., 2020a; Martin et al., 2020a). Randazzo et al., reported detection of positive cases in two studies conducted in Spain, 12–16 days before in cities in Murcia and at least one day before in Valencia (Randazzo et al., 2020a; Randazzo et al., 2020b). Two studies detected SARS-CoV-2 in apartment buildings 2–3 days before the detection of a positive case in the buildings (Wong et al., 2021a; Xu et al., 2021a). Two campus-dormitories studies detected positive signals 4–5 days before a case was registered (Betancourt et al., 2021a; Kuryntseva et al., 2020a). Lastly, Medema et al., reported their earliest positive detection on 24 February 2020 when community case was incipient in Valencia, Spain, further affirming community transmission in affected regions before clinical cases were reported (Medema et al., 2020a).

Thirty-nine studies reported temporal trends between wastewater signals and community cases, with wastewater signals found to precede confirmed cases in the community by up to 63 days. Positive signals typically anticipated confirmed cases by 10 days – 25 studies reported a minimum duration of up to 10 days, nine studies reported a minimum precedence of 14 days, and three studies detected SARS-CoV-2 minimally 23 days before a clinical case surfaced, and up to a maximum of 63 days. Only one study conducted by Saththasivam et al., concluded that SARS-CoV-2 in wastewaters did not precede clinical cases (Saththasivam et al., 2021a). At the minimum, studies reported that wastewater signals anticipated cases up to five days in advance. Peccia et al., Nemudryi et al. and Saguti et al., further investigated temporal association between wastewater signals and other markers of community cases, including positive tests by date of specimen collection, hospital admission and days after symptom onset (Saguti et al., 2021a; Nemudryi et al., 2020b; Peccia et al., 2020a). Wastewater signals led positive tests by date of specimen collection by a mere 0–2 days as compared to 1–4 days for hospital admissions. Notably, there were three occurrences of wastewater signals lagging cases/hospitalisations – when cases were defined by symptom onset and in a low prevalence setting respectively (Colosi et al., 2021a; Gonçalves et al., 2021a; Nemudryi et al., 2020b) (Table 2).

3.4. Sequencing & modelling studies

Twenty-five out of 87 studies conducted sequencing analysis of their SARS-CoV-2 isolates, fifteen of which reported the resemblance of the strains with those listed in GenBank database. A study conducted in Brisbane between 24 Feb–1 April 2020 reported the presence of genome that originated in Israel (Ahmed et al., 2020a), another study conducted in Japan between March and April 2020 reported genome that was partially identical to the Wuhan strain (Hata et al., 2021a). A study conducted in North Rhine-Westphalia, Germany found a strain in April 2020 that was closely related to the California strain, which was also detected in a study conducted in Bozeman, Montana, USA between late March and June 2020 (Nemudryi et al., 2020b; Westhaus et al., 2021a). Local strains were identified in four studies, namely, two studies conducted in Italy between 3 Feb–2 April 2020 and mid-April, a study conducted in the metropolitan region of Japan between 9 June–19 Aug, 2020 and a study conducted in Dane county, Wisconsin between mid-October 2020–early Jan 2021 (Kitamura et al., 2021a; Mondal et al., 2021a; La Rosa et al., 2020a; Rimoldi et al., 2020a). Notably, Bar-Or et al. and Wilton et al. detected the B.1.1.7 variant in wastewater in Israel from Aug 2020–Feb 2021 and London from Jan 2020 to Jan 2021 (Bar-Or et al., 2021a; Wilton et al., 2021a). Ten studies reported genome coverage of their isolates (85–100%). Among the 17 studies that conducted predictive modelling analysis of COVID-19 cases, three studies adopted the Monte Carlo simulation approach to estimate infection prevalence according to viral load (Prado et al., 2021a; Ahmed et al., 2020a; Hata et al., 2021a), while majority of the studies conducted

regression modelling to estimate the number of infected individuals based on viral load. Separately, Wu et al. simulated wastewater titers and detection probability using a Poisson model (Wu et al., 2020a). The results were varied with the probability for detection being reported between 88% and 100% (Supplementary Table 4).

3.5. Risk assessment

Majority of the studies (30 out of 87) had a high risk of bias linked to clarity and publication. A total of 26 out of 31 studies (29.3%) had a high risk of bias for the study design domain and 22 (25.3%) studies had a high risk of bias for assessment of outcome domain. Notably two studies, only four studies, Agrawal et al., Jorgensen et al., Prado et al. and Wurtz et al., showed high bias in all three domains (Agrawal et al., 2021b; Wurtz et al., 2021a; Jorgensen et al., 2020a; Prado et al., 2020a), while 33 (38%) studies had low bias for all three domains.

Within the study design domain, majority of the studies (20 out of 87) showed high bias for clarity of sampling, while only three studies had a high bias for the method of virus extraction (Wurtz et al., 2021a; Yaniv et al., 2021a; Jorgensen et al., 2020a). Presence of viral quantification process showed maximum risk of bias in the assessment of outcome domain while least bias was seen for association with human cases/community transmission with only six studies (Sharif et al., 2020a) showing high risk of bias. Bias linked to clarity on sampling site had eight studies with high bias, while nineteen studies showed high bias for transparency in results (Supplementary Table 5).

4. Discussion

This review sought to provide a systematic synthesis of wastewater surveillance studies for SARS-CoV-2. Studies in this review covered wide geographical areas serving up to 42,500,000 inhabitants as well as targeted areas like university, residential area, cruise, airlines and hospitals. Although the performance of wastewater surveillance in settings outside conventional sewage system was better (34.8% vs. 28.9%) than conventional sewage system, number of samples in the latter were far more than the former (24,862 vs. 1335). On the note of ethical considerations, wastewater surveillance seems better suited for larger catchment areas as focusing on targeted and high risk areas allows population to be easily identified (Zahedi et al., 2021). In case of wastewater surveillance for pharmaceuticals, it is generally accepted that populations over >10,000 can be considered to give anonymity and posing no risk to smaller groups of people (Sims and Kasprzyk-Hordern, 2020). However, targeting confined sub-populations within high-risk facilities may provide clearer indication of infection source as opposed to widespread geographical testing. Key high-risk facilities such as schools, dormitories, nursing homes that entail frequent prolonged close interactions between individuals have been shown to promote COVID-19 outbreaks (Daughton, 2020; Koh, 2020; Thompson et al., 2020). In this study, SARS-CoV-2 was detected in the targeted surveillance of hospitals (65.1%), university settings (27.7%), residential buildings/regions (63.6%) and nursing home (33.3%). Notably, in their study on hospital wastewater, Gonçalves et al. reported positive signals in wastewater in the presence of only one hospitalized COVID-19 case (Gonçalves et al., 2021a). Persistent positive signals were detected in the hospital's wastewater throughout the study period, which saw only a maximum of four hospitalized COVID-19 patients at its peak (Gonçalves et al., 2021a), suggesting high sensitivity of facility based sewage sampling. Similar findings were reported by Betancourt et al. and Xu et al., which detected the presence of SARS-CoV-2 even before the first case surfaced in the facilities (Betancourt et al., 2021a; Xu et al., 2021a). Targeted surveillance of living facilities can also sieve out sub-populations underserved by healthcare due to unavailability of healthcare services or avoidance in seeking healthcare (Daughton, 2020). Given that sewage can be diluted by rain or industrial discharge running into the sewer network (CNA, 2021), infection prevalence

deduced from WWTP samples may be underestimated without proper accountability of these factors during analysis. Nevertheless, it would still be challenging to interpret the wastewater samples tested positive as potential COVID-19 transmission when there are known reported recovered cases who have returned back into these targeted non-healthcare facilities (Wang et al., 2020; Wong et al., 2021a).

Inadequate sewage infrastructure in developing countries can hinder the application of wastewater surveillance, further stunting surveillance efforts in the very countries that ought to reap the most benefit from this cost-effective surveillance approach. Several constraints in sanitation infrastructure of developing countries challenge the representativeness of wastewater surveillance, such as high proportions of households not being connected to the sewage network, underperformance of WWTP, dysfunctional operational facilities, and mismanagement of sewage. Surveillance approaches that typically begin with sampling influent of WWTP is not representative of actual disease prevalence in developing countries (Panchal et al., 2021; Pandey et al., 2021; Street et al., 2020). Despite several studies based in developing countries, these limitations were insufficiently addressed. Only a single paper included in this review focused on SARS-CoV-2 surveillance in low sanitation settings, detecting three positive samples out of three samples collected from rivers receiving untreated sewage in Ecuador. Approaches should be contextualized to consider both areas served and not-served by sewage networks to present an accurate picture of estimated prevalence (Calabria de Araujo et al., 2020). More investigation of wastewater surveillance approach in developing/low-income settings is warranted.

Positive samples were detected as early as two months before the first case was clinically identified (Hata and Honda, 2020). Virus shedding in feces is suggested to occur five days prior to symptom onset and last up to 17.2 days (Cevik et al., 2021; Jones et al., 2020). COVID-19 has a short serial interval of 3–8 days, given that SARS-CoV-2 reportedly has an incubation period of 5.6 days, it suggests that transmission typically occurred before or near symptom onset (Quesada et al., 2021; Cheng et al., 2020; Park et al., 2020; Pung et al., 2020). In this review, Kuryntseva et al. detected SARS-CoV-2 in wastewater discharged from Kazan Federal University Buildings when nobody reported illness, five days before the first COVID-19 case was registered (Kuryntseva et al., 2020a) while dormitories in the USA and residential buildings in Hong Kong reported positive samples 2–4 days before the first case surfaced. Four cases were detected within seven days after investigation, demonstrating fecal shedding of virus in pre-symptomatic individuals. Studies included in this review typically reported anticipation of COVID-19 cases 16 days prior to clinical detection (≤ 10 days; 24 studies, ≥ 14 days; 8 studies), which provides adequate time for containment measures to be put in place to prevent an imminent cluster/outbreak given the short serial interval of COVID-19. However, there were studies that did not report any positive detection before cases were clinically reported in the community (Medema et al., 2020a; Sherchan et al., 2020a; Wu et al., 2020a), or detection was only observed when there were higher incidence rate reported (Hata et al., 2021a; Nemudryi et al., 2020b; Prado et al., 2020a; Haramoto et al., 2020a). This phenomenon may be due to the fact that only about 50% of the infected cases would shed viruses in the excreta (Gupta et al., 2020). Reports of SARS-CoV-2 signals in wastewater at the beginning of the COVID-19 pandemic should be interpreted with caution given the initial delay in establishing targeted assays and laboratory surveillance for a novel virus.

All papers reported SARS-CoV-2 detection with viral load ranging from 1 to 4.8×10^9 gene copies per liter of wastewater, overall sample positivity was relatively low at 29.2%. Detection of SARS-CoV-2 signals in wastewater depends on viral load present and sensitivity of detection assays. Hata et al. reported that SARS-CoV-2 was detectable in WWT if one in 100,000 persons sheds 10^9 copies/g-feces in 200 g of feces, and the success of wastewater surveillance is dependent on the viral load in feces and the detection limit of the method of analysis, estimated as 2 copies/mL in wastewater using the RT-qPCR assay (Hata and Honda, 2020). Evidence from two meta-analyses demonstrated pooled virus

detection in stool of 40.5% and 48.1% of patients respectively (Cheung et al., 2020; Parasa et al., 2020). Higher detection rates were also reported in females, those with gastrointestinal symptoms and more severe disease (Wong et al., 2020). Such differential viral shedding profiles in feces further challenge the representation of disease prevalence deduced by wastewater surveillance. On this note, targeting endogenous biomarkers instead of utilizing clinical diagnostic testing platforms can potentially mitigate the aforementioned limitations (Daughton, 2020). The accuracy of disease prevalence captured can be potentially improved with the identification of infection markers that are more likely to be universally excreted in either urine or feces. An example of its potential is provided by a preprint published on medRxiv, which reported heightened sensitivity by assaying viral proteins using Multiplex Paired-antibody Amplified Detection (Neault et al., 2020). Since significant inflammation is typically induced in severe patients, the identification of appropriate endogenous markers warrant deeper biomarker evaluation (Panchal et al., 2021), especially for universal indicators applicable to mild cases. Apart from cost reduction from lowered reliance on SARS-CoV-2 immuno- and molecular assays (Daughton, 2020), false negativity caused by low viral load falling below assay detection and analytical limits can be mitigated. Other plausible benefits include earlier alerts supplemented by infection indicators and simplified sampling and processing workflow from using biomarkers present in urine (Daughton, 2020).

Process efficiency is a major challenge as little is known of the recovery efficiency of SARS-CoV-2 (Ahmed et al., 2020c). Beyond proving SARS-CoV-2 detection in wastewater, several studies were driven by the need to propose an adequate workflow to optimise recovery efficiency, comparing the use of different concentration methods adapted from surveillance of other pathogenic viruses, primers and probes for detection (Arora et al., 2020a; Haramoto et al., 2020a). Nineteen studies included in this review trialed more than one virus concentration protocol to evaluate performance differences. The most commonly used approaches were ultrafiltration (34 studies) and polyethylene glycol (PEG) precipitation (31 studies). This was further corroborated by Hjelmsø et al., who concluded PEG as a powerful extraction method in their comparison of viral concentration and RNA isolation methods (Hjelmsø et al., 2017). Some also reported detection of SARS-CoV-2 in different sample types, prompting questions on the optimal wastewater type to sample. While suspended solids – primary sewage sludge, post-grit chamber influent solids and primary clarified sludge – were less frequently collected (7 studies) as compared to raw sewage into WWTP (80 studies), Bhattarai et al. concluded that sludge samples had greater predictability than influent samples, especially for monitoring purposes that exhibits a time lag (Bhattarai et al., 2021a). Since suspended solids confer protective effect by allowing virus adsorption (Gundy et al., 2008), Balboa et al. confirmed their hypothesis that enveloped virus had higher affinity for biosolids, by identifying higher virus concentrations in thickened and primary sludge than WWTP influent (Balboa et al., 2021). The sparsity of studies utilizing solid samples hitherto, on top of promising results reported by Peccia et al. prompt further investigation into the sample type optimal for SARS-CoV-2 detection (Peccia et al., 2020a). Further evaluation of processing methodologies was not undertaken in this review as it was beyond the expertise of the review authors. In-depth assessment of the optimal methodology is warranted in future studies to guide the adoption of wastewater surveillance. A more recently conducted study on a college campus in Arizona, USA reported a sensitivity of 76.0% and a positive predictive value of 79.8% when comparing wastewater surveillance results with clinical testing (Betancourt et al., 2021b). In Colosi et al.'s analysis of hospital and dormitories, wastewater surveillance was reported to have a sensitivity of 96.2% and specificity of 100% (Colosi et al., 2021a). This suggests that wastewater surveillance can potentially serve as an “early warning system” to monitor trends of SARS-CoV-2 transmission; however, more papers need to look at sensitivity and positive predictive value to allow a meta-analysis providing more robust evidence.

Finally, this review piloted the first tool to assess the risk of bias in wastewater surveillance studies across the domains of study design, outcome measurement and clarity/publication bias. Our risk of bias assessment highlighted the urgent need for more well-established guidance in the aspects of study design, execution and interpretation of wastewater surveillance studies for use globally and to allow robust meta-review. The importance of such an assessment lies in its potential to explain heterogeneity in findings and the ability to grade the strength of evidence presented by studies, which can influence the interpretation of results synthesized (Viswanathan et al., 2017). This assessment tool can 1) provide a platform to guide the consistency of future research design and presentation, which can help strengthen the conclusions made by future systematic reviews; 2) can act as a foundation for the refinement of future risk of bias assessments.

5. Strengths & limitations

This review is limited by the usage of solely qualitative synthesis due to the largely descriptive nature of existing literature. Units in which the viral load in various papers were presented were inconsistent and did not allow for meta-analysis. It is recommended to present it in terms of the daily loads per capita (mg/day/1000 inhabitants) to allow comparisons between cities in different geographic locations, and to be able to normalize the findings (Hata and Honda, 2020). Furthermore, there is also no consistent guidance on the definition of a positive wastewater result, either based on a significantly high viral load cut-off value and/or a consecutive number of days with wastewater sampling tested positive. There is a lack of systematic methods in existing literature to assess association between COVID-19 cases and wastewater signals as well as the accuracy of this wastewater testing over a fixed period of time. Ideally, the association between COVID-19 incidence and wastewater signals should be based on daily regular surveillance of the wastewater and the weekly PCR nasopharyngeal swab test of the susceptible (naïve) population of interest. Though funnel plot analysis for publication bias assessment was not feasible in this review, it would be prudent to assume that most, if not all, studies with negative findings on their wastewater testing and its association with COVID-19 incidences, would be challenging to publish when making interpretation from this review.

Sample positivity was moderately high with respect to the nucleocapsid gene amidst low rates of sample positivity seen among studies overall. While wastewater surveillance omits the factors of time consumption, cost, exposure risk, and avoids the biases of epidemiological indicators arising from limitations in the healthcare system, such as diagnostic testing capacity, symptomatic testing and hospitalization lags, it does not provide a true quantitative estimation of the number of people who are infected in the population. More studies using accurate and validated methods are required along with risk analysis and modelling to understand the dynamics of viral outbreaks. Future studies need to present their findings in a more consistent manner – provide clarity of sampling approach and sampling sites, virus detection and quantification process, use of laboratory controls and genetic targets in addition to mere reporting of sample positivity so as to guide and enable valid comparisons of study design. Guidance should also be provided on analysis methods to enable standardized, statistically robust association of findings with community/clinical data.

6. Conclusion

As countries embrace the emerging SARS-CoV-2 variant of concerns, wastewater surveillance can complement clinical surveillance by providing signs of potential transmission for more active public health responses.

CRediT authorship contribution statement

Junxiong Pang: Conceptualization, Supervision, Writing - Reviewing and Editing. **Shimoni Shah:** Data curation, Methodology,

Writing - Original draft preparation, Writing - Reviewing and Editing. **Sylvia Xiao Wei Gwee:** Data curation, Methodology, Writing - Original draft preparation, Writing - Reviewing and Editing. **Jamie Qiao Xin Ng:** Data curation, Writing - Reviewing and Editing. **Nicholas Lau:** Data curation. **Jiayun Koh:** Data curation.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Data sharing

All data generated in this study are included in this article and its supplementary files.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.150060>.

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