



## Short Communication

# First proof of the capability of wastewater surveillance for COVID-19 in India through detection of genetic material of SARS-CoV-2



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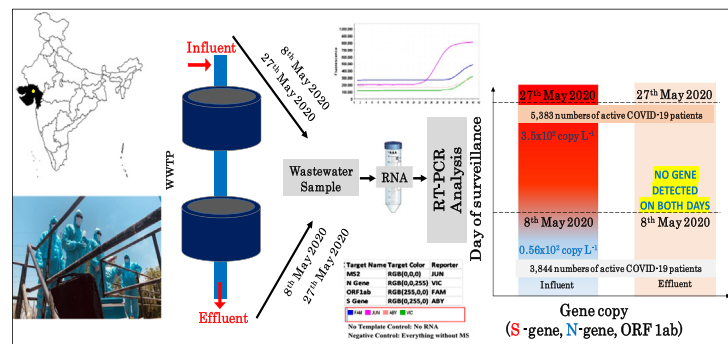
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## HIGHLIGHTS

- First ever report of the presence of gene of SARS-CoV-2 in the wastewater in India.
- $C_T$  value is explicitly indicative of the increase of COVID-19 patient in the vicinity.
- All three i.e. ORF1ab, N and S genes of SARS-CoV-2 were discerned in the influents.
- None of three genes were spotted in the effluent collected on 8 and 27 May 2020.
- Increase in the SARS-CoV-2 genetic loading concurred with active COVID-19 patient.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

## Article history:

Received 18 June 2020

Received in revised form 26 July 2020

Accepted 27 July 2020

Available online 28 July 2020

Editor: Dr. Damia Barcelo

## Keywords:

Coronavirus

COVID-19

Environmental surveillance

Wastewater based epidemiology

Pandemic monitoring

## ABSTRACT

We made the first ever successful effort in India to detect the genetic material of SARS-CoV-2 viruses to understand the capability and application of wastewater-based epidemiology (WBE) surveillance in India. Sampling was carried out on 8 and 27 May 2020 at the Old Pirana Waste Water Treatment Plant (WWTP) at Ahmedabad, Gujarat that receives effluent from Civil Hospital treating COVID-19 patients. All three, i.e. ORF1ab, N and S genes of SARS-CoV-2, were found in the influent with no genes detected in effluent collected on 8 and 27 May 2020. Increase in SARS-CoV-2 genetic loading in the wastewater between 8 and 27 May 2020 samples concurred with corresponding increase in the number of active COVID-19 patients in the city. The number of gene copies was comparable to that reported in untreated wastewaters of Australia, China and Turkey and lower than that of the USA, France and Spain. However, temporal changes in SARS-CoV-2 RNA concentrations need to be substantiated further from the perspectives of daily and short-term changes of SARS-CoV-2 in wastewater through long-term monitoring. The study results SARS-CoV-2 will assist concerned authorities and policymakers to formulate and/or upgrade COVID-19 surveillance to have a more explicit picture of the pandemic curve. While infectivity of SARS-CoV-2 through the excreted viral genetic material in the aquatic environment is still being debated, the presence and detection of genes in wastewater systems makes a strong case for the environmental surveillance of the COVID-19 pandemic.

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## 1. Introduction

The current ongoing global Coronavirus disease (COVID-19) pandemic, caused by the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread to 216 countries and territories, with 7.7 million of the confirmed cases and more than 425,000 deaths worldwide, as of June 12, 2020 (WHO, 2020). The active replication of infectious SARS-CoV-2 particles in enterocytes of human intestine due to expression of ACE2 receptor causes shedding of virus in the faeces (Lamers et al., 2020; Qi et al., 2020). The clinically reported symptoms in COVID-19 patients mainly include cough, difficulty in breathing, fever and diarrhoea (Gao et al., 2020; Kumar et al., 2020a). However, during a previous study of COVID-19 patients, SARS-CoV-2 RNA was detected in faeces more frequently than gastrointestinal symptoms (17%) such as diarrhoea (Cheung et al., 2020). These results suggest a large number of asymptomatic individuals along with symptomatic patients, discharge the virus which ultimately reaches sewage treatment plants (Haramoto et al., 2020). The virus can be shed in faeces for several days, even after the patient stops exhibiting respiratory symptoms (Wu et al., 2020a, 2020b). Zheng et al. (2020), reported detection of SARS-CoV-2 RNA in faeces for a median duration of 22 days. Though the residence time of SARS-CoV-2 virus has not been well studied, evidence from studies reporting detection of SARS-CoV-2 RNA suggest the possibility of detection in wastewater.

Wastewater-based epidemiology (WBE) is a promising approach to understand the status of disease outbreak in a certain catchment by monitoring viral load in the wastewater, as it contains excrement from both symptomatic and asymptomatic individuals (Xagorarakis and O'Brien, 2020; Choi et al., 2018; Yang et al., 2015). WBE was an effective tool during past outbreak of other enteric viruses, such as poliovirus, hepatitis A and norovirus (Hellmér et al., 2014; Asghar et al., 2014; Kitajima et al., 2020; Kumar et al., 2020a, 2020b), it can be used as an early warning tool for the disease outbreak in a community and used to inform the efficacy of the current public health interventions (Ahmed et al., 2020a, 2020b). WBE data can help estimate actual infected population due to the virus, as it also covers asymptomatic and pre-symptomatic the patients, which may be underestimated by clinical surveillance (Bivins et al., 2020; Tang et al., 2020; Wölfel et al., 2020; Zhang et al., 2020; Kumar et al., 2020a).

Detection of, SARS-CoV-2 RNA in wastewater has been reported in Australia, China, France, Israel, Italy, Japan, Netherlands, Spain and the US (Ahmed et al., 2020a, 2020b; Bar-Or et al., 2020; Haramoto et al., 2020; La Rosa et al., 2020; Medema et al., 2020; Nemudryi et al., 2020; Randazzo et al., 2020; Rimoldi et al., 2020; Wu et al., 2020a, 2020b; Wurtzer et al., 2020; Kumar et al., 2020). According to some of these studies, after the number of confirmed cases reached to 1–100 per million population, SARS-CoV-2 RNA was detected in wastewater (Ahmed et al., 2020a; Bar-or et al., 2020; Medema et al., 2020; Nemudryi et al., 2020; Wu et al., 2020a, 2020b; Wurtzer et al., 2020; Kumar et al., 2020). To date of submission of this work, there is no study reporting detection of SARS-CoV-2 in wastewater in India. As of June 12, 2020, the number of confirmed cases in India was 223 per million population. The first case of COVID-19 in India was reported on January 30, 2020 and the number of confirmed cases has reached more than 300,000 as of June 12, 2020 (Ministry of Health and Family Welfare, India, 2020). The state of Gujarat has reported >22,500 confirmed cases of COVID-19, as of June 12, 2020, with >12,000 confirmed cases in Ahmedabad city (Ministry of Health and Family Welfare, India, 2020).

To further understand the capability and potential application of WBE surveillance, we made the first successful detection of genetic material of the SARS-CoV-2 virus in India. We also analysed the temporal variation in genetic material loadings in the same wastewater treatment plant during a lockdown period in India. Finally, we evaluated the effect of traditional treatment systems on SARS-CoV-2 genetic material and aim to assist concerned authorities and policymakers to formulate

and/or upgrade COVID-19 surveillance to include an explicit picture of the pandemic curve.

## 2. Material and methods

### 2.1. Sampling

Wastewater samples were collected on 8 and 27 May 2020 from the Old Pirana Waste Water Treatment Plant (WWTP) at Ahmedabad, Gujarat which is the largest wastewater treatment plant in Asia with a capacity of >180 m<sup>3</sup>/day. The WWTP is equipped with an Upflow Anaerobic Sludge Blanket (UASB) as an advanced process to treat the wastewater. This WWTP is designed to produce treated water with pH, biological oxygen demand (BOD), total suspended solids (TSS), and chemical oxygen demand of 7–8.5, <20 mg.L<sup>-1</sup>, <30 mg.L<sup>-1</sup> and <100 mg.L<sup>-1</sup> respectively. The sampling location for this study was selected based on the fact that Pirana WWTP receives the sewage waste of a government civil hospital treating COVID-19 patient.

Wastewater samples (influent, and final effluents after UASB and aeration pond) were collected on 8 and 27 May 2020 to understand the temporal variation and the effect of wastewater treatment of SARS-CoV-2 RNA. In-situ water quality parameters (pH, temperature, electrical conductivity; EC, dissolved oxygen; DO, oxidation-reduction potential; ORP, and total dissolved solids) of the influent and effluent were measured using a YSI Multiparameter probe (YSI ProDSS handheld with GPS Model No. 626870-2, USA) prior to the sampling. A composite sample was made from three samples simultaneously taken in each location. Samples taken on 8 May were transported in an ice-box and refrigerated at 4 °C till 27 May when the next batch of samples were brought to the laboratory and analysed on the same day. Both days the grab hand sampling were done at 11:30 am using sterile bottles (Tarsons, PP Autoclavable, Wide Mouth Bottle, Cat No. 582240, India) and blanks in the same bottles were analysed to determine if there was any contamination during the transport.

To ensure accuracy and precision, duplicated analyses of the samples were also performed for a raw wastewater, in which the reproducibility was fairly high (average C<sub>T</sub> difference of 1.2). Several blanks were prepared and run to check the cross-contamination, and sensitivity of the protocol, extraction and instrumentation. All analyses were conducted at the Indian Council of Medical Research (ICMR), New Delhi, an approved facility of the Gujarat Biotechnology Research Centre (GBRC).

### 2.2. Method for extraction and detection of viral RNA from sewage samples

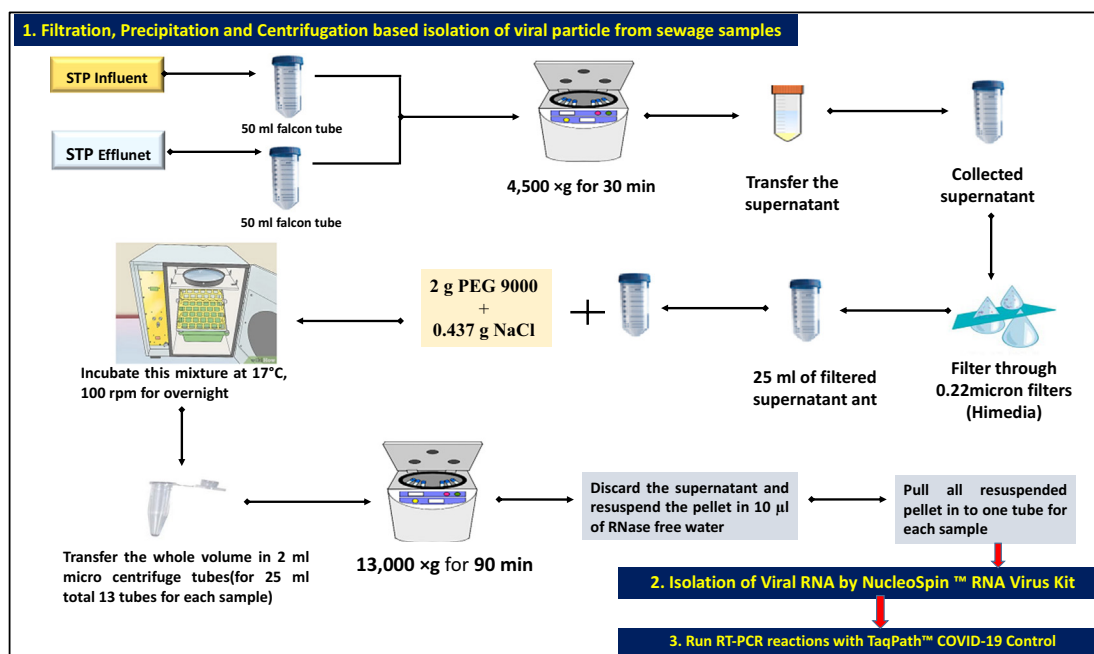
Viral RNAs were isolated from sewage samples using following steps: precipitation of viral particles; viral RNA isolation and quality checking.

#### 2.2.1. Precipitation of viral particle

The sewage samples (50 mL) were centrifuged at 4500 ×g (Model: Sorvall ST 40R, Thermo Scientific) for 30 min followed by filtration of supernatant using 0.22 μm filters (Mixed cellulose esters syringe filter, Himedia). Each sewage filtrate was then concentrated using the poly ethylene glycol (PEG) methods. For this method, PEG 9000 (80 g/L) and NaCl (17.5 g/L) were mixed with 25 mL filtrate and this was incubated overnight at 17 °C and 100 rpm (Model: Incu-Shaker™ 10LR, Benchmark). The following day the mixture was centrifuged at 13000 ×g (Model: Kubota 6500, Kubota Corporation) for 90 min. After centrifugation, the supernatant was discarded and the pellet resuspended in 300 μL RNase-free water. This was further used as a sample for RNA isolation (Fig. 1).

#### 2.2.2. RNA isolation

RNA isolation was carried out using a commercially available kit (NucleoSpin® RNA Virus, Macherey-Nagel GmbH & Co. KG, Germany). Concentrated viral particles (200 μL) were mixed with 10 μL MS2



**Fig. 1.** Illustrative flowchart of the modified polyethylene glycol (PEG) precipitation of centrifugated viral isolation from wastewater samples followed by RNA isolation and reverse transcription polymerase chain reaction (RT-PCR) with 40 amplification cycles.

phage, 20 µL Proteinase K (20 mg/mL) solution and 600 µL of RAV1 buffer containing carrier RNA. Here, MS2 phage was taken as a molecular process inhibition control (MPC; Haramoto et al., 2018) for evaluating the efficiency of nucleic acid extraction and PCR inhibition. It is to be noted that MS2 may naturally occur in wastewater, it is therefore there is possibility that recovered MS2 may consist both the spiked and background viral content. Further steps were carried out as instructed in the product manual (Macherey-Nagel GmbH & Co. KG). Final elution was carried out with 30 µL of elution buffer (provided by kit). RNA concentrations were determined using a Qubit 4 Fluorometer (Invitrogen).

### 2.2.3. Real time PCR for detection of SARS-CoV-2

RNAs were analysed for the detection of ORF1ab, N gene and S gene of SARS-CoV-2 and MS2 (internal process control) by RT-PCR using TaqPath™ Covid-19 RT-PCR Kit (Applied Biosystems). Amplification was performed in a 25 µL reaction mixture containing 7 µL extracted nucleic acids of each samples. Positive control (2 µL) (TaqPath™ COVID-19 Control) and purified negative control (5 µL) were used in case of positive and negative control respectively. Nuclease free water was used as no template control in this study. Further procedures were carried out as described in product manual. RT-PCR experiment consisted of UNG incubation at 25 °C for 2 min, reverse transcription at 53 °C for 10 min and activation at 95 °C for 2 min, followed by 40 cycles, each involving denaturation at 95 °C for 3 s followed by annealing/extension at 60 °C for 30s. The reactions were performed in Applied Biosystems™ 7500 Fast Real Time PCR system (Applied Biosystem), and interpreted as instructed in the manual.

### 2.2.4. Semi-quantitative SARS-CoV-2 gene detection

Although there is no direct correlation of the  $C_T$  value to copy numbers as the kit used for the detection is qualitative assay yet we put an effort to calculate number of gene copies present in a unit volume of the sample. For this the well-established principle of 3.3  $C_T$  change corresponds to 10-fold change has been used. More precisely, 500 copies of SARS-CoV-2 genes were taken as positive control with  $C_T$  of average 26 for all the three genes i.e. ORF1ab, N and S, which were then extrapolated to compare it with sample  $C_T$  values and derive approximate copies of genes in the wastewater sample. The amount of RNA used as

template was multiplied with the enrichment factor to derive an estimated copy numbers for each wastewater sample (Fig. 2).

## 3. Results and discussion

We examined three genes of SARS-CoV-2, ORF1ab, N protein genes and S protein genes, from the influent samples and the final effluent after UASB treatment and aeration pond, both from WWTP Pirana, on May 8 and May 27. The MS2 was added in each sample as MPC as well as in negative control to verify the efficacy of RNA extraction and the absence of inhibitors in the RT-PCR reaction. The  $C_T$  values of MS2 in these samples were all similar: 22.46, 22.35 in the influents on May 8 and May 27, respectively, and 22.4 and 22.2 in the final effluents on May 8 and May 27, respectively, and 22.07 in the negative control. This indicates that there was no significant difference in inhibitory effects by wastewater matrix in RNA extraction as well as on RT-PCR performance. The positive control sample had  $C_T$  values of the three SARS-CoV-2 genes ranging 27.92 to 29.52, while these genes were not detected in the negative control sample.

WWTP samples from both May 8 and May 27 were positive with all of the ORF1ab, N protein genes and S protein genes, which were examined as SARS-CoV-2 genes, with the estimated maximum concentration of  $3.5 \times 10^2$  copies/L (Table 1). To the best of our knowledge, this is the first available proof of detection of SARS-CoV-2 genes in wastewater samples from India. The ORF1ab assay and N protein assay have been used for SARS-CoV-2 RNA detection by RT-PCR from raw wastewater and river water samples in Milano, Italy (Rimoldi et al., 2020). The S protein gene has also been used in evaluating raw wastewater in Italy (La Rosa et al., 2020).

Smaller  $C_T$  values on 27th May suggested that all three genes were more abundant in the May 27 wastewater influent samples than in May 8 samples. This is consistent with increasing infection numbers in the city, the daily new confirmed cases in the previous 10 days of the survey were approximately double, 3844 and 5383 in the case of May 8 and May 27, respectively. Table 1 shows the number of active cases for the city i.e. Ahmedabad and India, obtained by deducting recovered cases from total confirmed cases since 17 March 2020. Consistency between abundance of SARS-CoV-2 genetic materials and number of

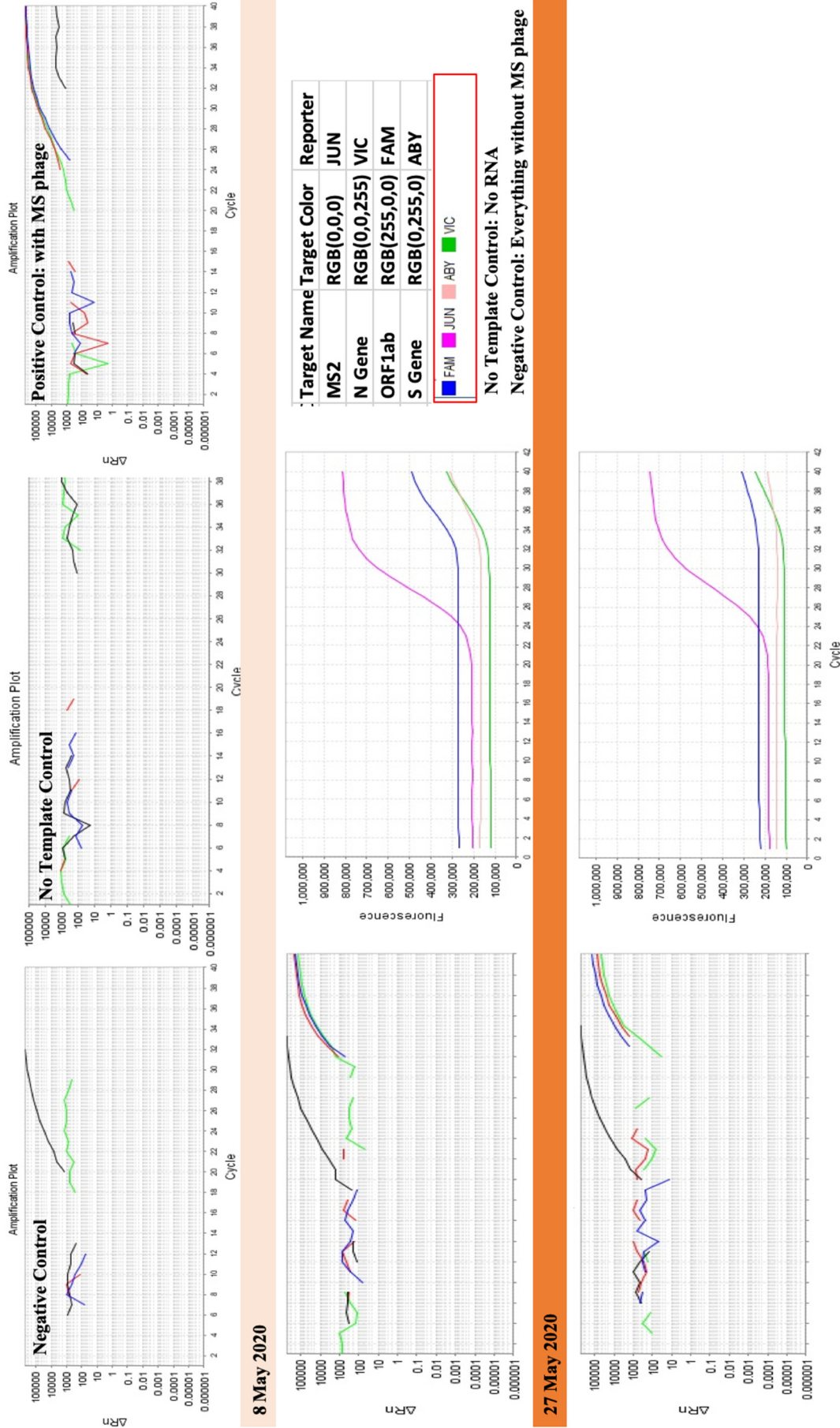


Fig. 2. Amplification plots obtained through RT-PCR illustrating temporal variation through Ct value and Rn value between the samples of 8 and 27 May 2020. Three control samples are also provided.

**Table 1**

Amplification cycles ( $C_T$ ) of raw wastewater and final effluents, along with total number of cases reported positive and discharged since 17th March 2020 in Ahmedabad (AMD) and India (IND).

		ORF1ab	N protein gene	S protein gene	MS2
Raw wastewater	8-May	35.52	35.39	39.56	22.46
	27-May	32.65	34.18	34.83	22.35
Final effluent	8-May	–	–	–	22.40
	27-May	–	–	–	22.20
		Confirmed case AMD IND		Discharged/recovered case AMD IND	
*COVID-19 case Since 17 March 2020	7-May	4912	56,352	985	16,776
	26-May	10,674	150,857	4666	64,291

\*COVID patient number has been enumerated using the government data displayed on the webpage <https://ahmedabadcity.gov.in/portal/web?requestType=ApplicationRH&actionVal=loadCoronaRelatedDtls&queryType=Select&screenId=114> (Accessed on 14th June 2020) <https://www.covid19india.org/> (Accessed on 15th June 2020).

confirmed cases was observed in the previous reports in Australia, France, Italy, Spain and Japan (Ahmed et al., 2020a, 2020b; Hata and Honda, 2020; Randazzo et al., 2020; Wurtzer et al., 2020), showing that WBE is a promising tool for surveillance of COVID-19 spread in a community. In future studies, standards of SARS-CoV-2 RNA should be used for determining actual concentrations of SARS-CoV-2 RNA in wastewater samples and for comparing results among studies.

Final effluent samples taken on May 8 and May 27 were negative ( $C_T$  values >40) with all three SARS-CoV-2 genes examined, showing that the genes were significantly reduced by the UASB treatment and aeration pond. The SARS-CoV-2 genes were shown to decrease during treatments by a secondary treatment and a tertiary treatment including decantation, coagulation, flocculation, sand filtration, disinfection, NaClO, peracetic acid or UV in Spain and Italy (Randazzo et al., 2020; Rimoldi et al., 2020; Kumar et al., 2020e). Our results are the first to suggest the reduction of SARS-CoV-2 RNA after UASB and aeration pond treatment.

We employed the PEG precipitation method for concentrating viruses, which has been used to detect SARS-CoV-2 RNA (Hata and Honda, 2020; La Rosa et al., 2020; Wu et al., 2020a, 2020b). The recovery of spiked MS2 as a MPC was stable in our study ( $C_T$  values ranging from 22.2 to 22.46), showing efficient removal of wastewater matrix potentially inhibiting RNA extraction and RT-PCR. In Hata and Honda (2020), the recovery of indigenous F-phages during PEG concentration was high and stable (57% geometric mean), indicating efficient recovery of SARS-CoV-2 with PEG concentration method. In the future, virus concentration performance of the PEG method should be evaluated against multiple other methods (e.g., ultrafiltration, aluminium hydroxide adsorption-precipitation) in analysing raw wastewater.

Table 2 shows a comparative analysis of protocol and results of detection of SARS-CoV-2 genetic material. Overall, we successfully detected ORF1ab, N protein genes and S protein genes, from Indian wastewater samples by RT-PCR and observed a significant decrease in the final effluents after treatment by UASB and aeration pond. A 10-fold increase in the estimated number of gene copies was observed between 8 and 27 May 2020, i.e.  $5.6 \times 10^3$  copies  $L^{-1}$  and  $3.5 \times 10^2$  copies  $L^{-1}$  corresponding to more than double the number of active COVID-19 patients in Ahmedabad city 4912 and 10,674 individuals on 8 and 27 May, respectively. The estimated number of gene copies was comparable to that reported in untreated wastewaters of Australia, China and Turkey and lower than that of in the USA, France and Spain.

Further, referring to the limitations of the present study owing to lockdown scenario, we recommend that although based on MPC analysis, the efficiency of RNA extraction and RT-PCR is considered high for all the wastewater samples collected for this study, the efficiency of PEG method could have been better established. Further, based on indigenous F-phage analysis Hata and Honda (2020) reported a high efficiency of PEG method in Japanese wastewater, yet an evaluation of sample concentration efficiency, using the whole process control (WPC) together with MPC is recommended (Haramoto et al., 2018). We

recommend longer monitoring with several replicated analyses to evaluate the correlation as well as uncertainties involving RT-PCR (Stuart et al., 2014) and then replace the semi-quantitative method employed in this study with precise copy calculations using suitable methods.

Nevertheless, the bottom line is that the patterns of obtained  $C_T$  values suggest successful detection of SARS-CoV-2 RNA from the wastewater samples, their increasing abundance together with an increase of COVID-19 confirmed cases, and their reduction by UASB treatment and aeration pond. In summary, results demonstrated the capability of wastewater-based epidemiology in Indian settings and strongly advocates that despite the lack of quality sewer infrastructure or other wastewater collection issues, WBE can be applicable and thus we strongly implementing environmental surveillance of the COVID-19 pandemic in India, starting with major cities.

#### 4. Conclusions

While the world is providing high resolution proofs of the WBE concept, India needed indigenous proof of concept and its applicability. In this context, we achieved two major outcomes: i) for the first time in India and top 10 efforts in the world, we isolated SARS-CoV-2 genetic material and detected it during a lockdown period owing to good coordination among the government organizations; ii) temporal variation in  $C_T$  value demonstrated the capability of WBE surveillance in India; and iii) for the third time in the world treated water was analysed for the presence and confirmation of SARS-CoV-2 genetic material. The results were of good resolution and provided significant indication of temporal variation in COVID-19 patient loadings. However, owing to limited samples analysed in this preliminary study, even though the case numbers align with increased RNA concentrations in wastewater, the temporal changes in SARS-CoV-2 RNA concentrations needs to be further investigated from the several perspectives of daily, short-term and long-term changes. Our results demonstrated that a conventional treatment plant is capable of removing genetic materials of SARS-CoV-2, however there may not be the complete elimination. In a country like India where sewer systems are not complete and only a part of the waste is received at WWTPs, it is essential to study each treatment stage to determine the effectiveness of treatment. This will help reduce the commonly perceived fear of the commons pertaining to the effectiveness of treatment plants as well as transmission through wastewater.

#### CRedit authorship contribution statement

**Manish Kumar:** Conceptualization, Visualization, Resources, Methodology, Writing - original draft, Data curation, Writing - review & editing, Supervision. **Arbind Kumar Patel:** Resources, Data curation, Writing - review & editing. **Anil V. Shah:** Supervision, Data curation, Project administration. **Janvi Raval:** Data curation, Writing - review & editing. **Neha Rajpara:** Data curation, Writing - review & editing. **Madhvi Joshi:** Data curation, Writing - review & editing, Supervision.

**Table 2**  
Comparative details of reported molecular detection of SARS-CoV-2 in the wastewater of various countries.

Country	State/city	Water type	Virus concentration method	Positive rate	Maximum concentration (copies/L)	RT-(q) PCR target region	Reference
India	Ahmedabad	Untreated wastewater Treated wastewater	PEG precipitation of centrifuged supernatant	8 May: 100% 27 May: 100% 8 May: 0% 27 May: 0%	$5.6 \times 10^3$ $3.5 \times 10^2$ –	ORF1ab gene S gene N gene	Present study
Australia	Brisbane, Queensland	Untreated wastewater	Electronegative membrane-direct RNA extraction; ultrafiltration	2/9 (22%)	$1.2 \times 10^3$	N gene	(Ahmed et al., 2020a, 2020b)
			Centricon (Merck) ultrafiltration of centrifuged supernatant	N_Sarbecco: 1/9 NIID_2019-nCoV_N: 0/9	1.9	N gene	
The Netherlands	Amsterdam, The Hague, Utrecht, Apeldoorn, Amersfoort, Schiphol, Tilburg	Untreated wastewater	Centricon (Merck) ultrafiltration of centrifuged supernatant	14/24 (58%) N1: 14/24 N2: 0/24 N3: 8/24 E: 5/24	NA	N gene E gene	(Medema et al., 2020)
USA	Massachusetts	Untreated wastewater	PEG precipitation of filtered sample	N1: 4/6 N2: 1/6 N3: 4/6	N1: $10^4$ – $2 \times 10^5$ N2: $3 \times 10^4$ – $10^5$ N3: $10^4$ – $10^5$	N gene	(Wu et al., 2020b)
	Bozeman, Montana	Untreated wastewater	Corning Spin-X ultrafiltration of filtered sample	7/7 (100%) N1: 7/7 N2: 7/7	N1: $10^3$ – $10^5$ N2: $10^3$ – $3 \times 10^5$	N gene	(Nemudryi et al., 2020)
	New Haven, Connecticut	Primary sludge	Direct RNA extraction	44/44	$1.7 \times 10^6$ – $4.6 \times 10^8$	N gene	(Peccia et al., 2020)
France	Paris	Untreated wastewater Treated wastewater Untreated wastewater	Ultracentrifugation	23/23 (100%) 6/8 (75%) NA (100%)	$>10^{7.5}$ $\sim 10^6$ $10^4$ – $10^7$	E gene E gene E gene RdRp gene	(Wurtzer et al., 2020) (Wurtzer et al., 2020)
Italy	Milan and Rome,	Untreated wastewater	PEG/dextran precipitation of centrifuged supernatant	100% 12/12	NA (PCR detection)	ORF1ab gene S gene	(La Rosa et al., 2020)
China	Wuchang Fangcang Hospital	Untreated wastewater Treated wastewater	PEG precipitation of centrifuged supernatant	0/4 77% 7/9	$0.05$ – $1.87 \times 10^5$	ORF1 N gene	(Zhang et al., 2020)
Israel	Various locations	Untreated wastewater	Primary: PEG or Alum precipitation of centrifuged supernatant. Secondary: Amicon ultrafiltration	38% 10/26	NA	E gene	(Bar-Or et al., 2020)
Spain	Murcia	Untreated wastewater	Aluminium flocculation – beef extract precipitation	N1: 21/42 N2: 23/42 N3: 27/42 Secondary: 2/18 Tertiary: 0/12	N1: $1.4 \times 10^5$ N2: $3.4 \times 10^5$ N3: $3.1 \times 10^5$ $<2.5 \times 10^5$	N gene	(Randazzo et al., 2020)
	Ourense	Untreated wastewater Treated wastewater Sludge	Amicon ultrafiltration of centrifuged supernatant Glycine/beef extract elution –centrifugation – filtration – PEG precipitation	Influent: 5/5 Primary effluent: 1/4 Effluent: 0/5 41% 14/34	NA	N gene E gene RdRp gene	(Balboa et al., 2020)
	Valencia	Untreated wastewater Treated wastewater	Aluminium flocculation – beef extract precipitation	80% 12/15 0/9	$10^5$ – $10^6$ 0	N gene	(Randazzo et al., 2020)
Turkey	Istanbul	Untreated wastewater	Amicon ultrafiltration OR PEG precipitation of centrifuged supernatant	77% 7/9	$10^3$ – $10^6$	RdRp gene	(Kocameci et al., 2020)

**Chaitanya G. Joshi:** Data curation, Writing - review & editing, Supervision.

#### 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.

#### Acknowledgement

We acknowledge the unimaginable support from every corner as naming all of them involved or helped during analyses or sampling or protocol development would be difficult. Special mentions are Prof. Sudhir Jain, IIT Gandhinagar; Prof. Keisuke Kuroda, Toyama Prefecture University, Japan; Prof. Patrick J Shea, University of Nebraska-Lincoln USA; Prof. Hiroaki Furumai, the University of Tokyo, Japan; Prof. Prosun Bhattacharya, KTH, Sweden; Prof. AL. Ramanathan, JNU, India; Dr. Sunita

Varjani, GPCB, Ms. Nehal, Ahmedabad Municipal Corporation, and Dr. Neelam M Nathani, GBRC. We appreciate the inputs from the eminent referees and the editor Prof. Damia Barcelo.

## References

- Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., Choi, P.M., Kitajima, M., Simpson, S.L., Li, J., Tschärke, B., Verhagen, R., Smith, W.J.M., Zaugg, J., Dierens, L., Hugenholtz, P., Thomas, K.V., Mueller, J.F., 2020a. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. *Sci. Total Environ.* 728, 138764. <https://doi.org/10.1016/j.scitotenv.2020.138764>.
- Ahmed, W., Bertsch, P.M., Bivins, A., Bibby, K., Farkas, K., Gathercole, A., Haramoto, E., Gyawali, P., Korajkic, A., McMinn, B.R., Mueller, J.F., Simpson, S.L., Smith, W.J.M., Symonds, E.M., Thomas, K.V., Verhagen, R., Kitajima, M., 2020b. Comparison of virus concentration methods for the RT-qPCR-based recovery of murine hepatitis virus, a surrogate for SARS-CoV-2 from untreated wastewater. *Sci. Total Environ.* 739, 139960. <https://doi.org/10.1016/j.scitotenv.2020.139960>.
- Asghar, H., Diop, O.M., Weldegebriel, G., Malik, F., Shetty, S., Bassioni, L. El, Akande, A.O., Maamoun, E. Al, Zaidi, S., Adeniji, A.J., Burns, C.C., Deshpande, J., Oberste, M.S., Lowther, S.A., 2014. Environmental surveillance for polioviruses in the global polio eradication initiative. *J. Infect. Dis.* 210, S294–S303. <https://doi.org/10.1093/infdis/jiu384>.
- Balboa, S., Mauricio-Iglesias, M., Rodríguez, S., Martínez-Lamas, L., Vassallo, F.J., Regueiro, B., Lema, J.M., 2020. The fate of SARS-CoV-2 in wastewater treatment plants points out the sludge line as a suitable spot for incidence monitoring. *medRxiv*, 2020.05.25.20112706 <https://doi.org/10.1101/2020.05.25.20112706>.
- Bar-Or, I., Yaniv, K., Shagan, M., Ozer, E., Erster, O., Mendelson, M., Mannasse, B., Shirazi, R., Kramarsky-Winter, E., Nir, O., Abu-Ali, H., Ronen, Z., Rinott, E., Lewis, Y.E., Friedler, E., Bitkover, E., Paitan, Y., Berchenko, Y., Kushmaro, A., 2020. Regressing SARS-CoV-2 sewage measurements onto COVID-19 burden in the population: a proof-of-concept for quantitative environmental surveillance. *medRxiv* <https://doi.org/10.1101/2020.05.25.20112706>.
- Bivins, A., North, D., Ahmad, A., Ahmed, W., Alm, E., Been, F., ... Buttiglieri, G., 2020. Wastewater-Based Epidemiology: Global Collaborative to Maximize Contributions in the Fight Against COVID-19. *Environ. Sci. Technol.* <https://doi.org/10.1021/acs.est.0c2388>.
- Cheung, K.S., Hung, I.F., Chan, P.P., Lung, K., Tso, E., Liu, R., Ng, Y., Chu, M.Y., Chung, T.W., Tam, A.R., Yip, C.C., Leung, K.-H., Yim-Fong, Fung, A., Zhang, R.R., Lin, Y., Cheng, H.M., Zhang, A.J., To, K.K., Chan, K.-H., Yuen, K.-Y., Leung, W.K., 2020. Gastrointestinal manifestations of SARS-CoV-2 infection and virus load in fecal samples from the Hong Kong cohort and systematic review and meta-analysis. *Gastroenterology* <https://doi.org/10.1053/j.gastro.2020.03.065>.
- Choi, P.M., Tschärke, B.J., Donner, E., O'Brien, J.W., Grant, S.C., Kaserzon, S.L., Mackie, R., O'Malley, E., Crosbie, N.D., Thomas, K.V., Mueller, J.F., 2018. Wastewater-based epidemiology biomarkers: past, present and future. *TrAC - Trends Anal. Chem.* 105, 453–469. <https://doi.org/10.1016/j.trac.2018.06.004>.
- Gao, Q.Y., Chen, Y.X., Fang, J.Y., 2020. 2019 novel coronavirus infection and gastrointestinal tract. *J. Dig. Dis.* 21 (3), 125–126. <https://doi.org/10.1111/1751-2980.12851>.
- Haramoto, E., Kitajima, M., Hata, A., Torrey, J.R., Masago, Y., Sano, D., Katayama, H., 2018. A review on recent progress in the detection methods and prevalence of human enteric viruses in water. *Water Res.* 135, 168–186. <https://doi.org/10.1016/j.watres.2018.02.004>.
- Haramoto, E., Malla, B., Thakali, O., Kitajima, M., 2020. First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan. *medRxiv* <https://doi.org/10.1101/2020.06.04.20122747>.
- Hata, A., Honda, R., 2020. Potential sensitivity of wastewater monitoring for SARS-CoV-2: comparison with norovirus cases. *Environ. Sci. Technol.* 54 (11), 6451–6452. <https://doi.org/10.1021/acs.est.0c02271>.
- Hellmér, M., Paxéus, N., Magnus, L., Enache, L., Arnholm, B., Johansson, A., Bergström, T., Norder, H., 2014. Detection of pathogenic viruses in sewage provided early warnings of hepatitis A virus and norovirus outbreaks. *Appl. Environ. Microbiol.* 80, 6771–6781. <https://doi.org/10.1128/AEM.01981-14>.
- Kitajima, M., Ahmed, W., Bibby, K., Carducci, A., Gerba, C.P., Hamilton, K.A., ... Rose, J.B., 2020. SARS-CoV-2 in wastewater: State of the knowledge and research needs. *Sci. Total Environ.* 739, 139076. <https://doi.org/10.1016/j.scitotenv.2020.139076>.
- Kocameci, B.A., Kurt, H., Sait, A., Sarac, F., Saatici, A.M., Pakdemirli, B., 2020. SARS-CoV-2 detection in Istanbul wastewater treatment plant Sludges. *medRxiv* <https://doi.org/10.1101/2020.05.12.20099358>.
- Kumar, M., Kuroda, K., Dhangar, K., Mazumder, P., Sonne, C., Rinklebe, J., Kitajima, M., 2020. Potential emergence of antiviral-resistant pandemic viruses via environmental drug exposure of animal reservoirs. *Environ. Sci. Technol.* 54 (14), 8503–8505. <https://doi.org/10.1021/acs.est.0c03105>.
- Kumar, M., Patel, A.K., Shah, A.V., Raval, J., Rajpara, N., Joshi, M., Joshi, C.G., 2020. The first proof of the capability of wastewater surveillance for COVID-19 in India through the detection of the genetic material of SARS-CoV-2. *medRxiv*, 2020.06.16.20133215 <https://doi.org/10.1101/2020.06.16.20133215> <https://medrxiv.org/cgi/content/short/2020.06.16.20133215v1>.
- Kumar, M., Taki, K., Gahlrot, R., Sharma, A., Dhangar, K., 2020a. A chronicle of SARS-CoV-2: part-I-epidemiology, diagnosis, prognosis, transmission and treatment. *Sci. Total Environ.* 734, 139278. <https://doi.org/10.1016/j.scitotenv.2020.139278>.
- Kumar, M., Kuroda, K., Dhangar, K., 2020b. The most eagerly awaited summer of the anthropocene: a perspective of SARS-CoV-2 decay and seasonal change. *Groundw. Sustain. Dev.* 11, 100400. <https://doi.org/10.1016/j.gsd.2020.100400>.
- Kumar, M., Thakur, A., Mazumder, P., Kuroda, K., Mphaptra, S., Rinklebe, J., Ramanathan, A.L., Cetecioglu, A., Jain, S., Tyagi, V., Gikas, P., Chakraborty, S., Islam, M.T., Ahmad, A., Shah, A.V., Patel, A.K., Watanabe, T., Vithanage, M., Bibby, K., Kitajima, K., Bhattacharya, P., 2020e. Frontier review on the propensity and repercussions of SARS-CoV-2 migration to aquatic environment. *Hazard. Mater. Lett.* 54 (14), 8503–8505.
- La Rosa, G., Iaconelli, M., Mancini, A., Bonanno, F., Veneri, Bonadonna, L., Lucentini, Suffredini, E., 2020. First detection of SARS-CoV-2 in untreated wastewaters in Italy. *Sci. Total Environ.* 736, 139652. <https://doi.org/10.1016/j.scitotenv.2020.139652>.
- Lamers, M.M., Beumer, J., van der Vaart, J., Kooops, K., Puschhof, J., Breugem, T.L., Ravelli, R.B.G., van Schayck, P., Mykityn, A.Z., Duimel, H.Q., van Donselaar, E., Riesebosch, S., Kuijpers, H.J.H., Schippers, D., van de Wetering, W., de Graaf, M., Koopmans, M., Cuppen, E., Peters, P.J., Haagmans, B.L., Clevers, H., 2020. SARS-CoV-2 productively infects human gut enterocytes. *Science*, eabc1669 <https://doi.org/10.1126/science.abc1669>.
- Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., 2020. Presence of SARS-Coronavirus2 in sewage. *medRxiv* <https://doi.org/10.1101/2020.03.29.20004580>.
- Ministry of Health & Family Welfare, G. of I, 2020. MoEF. URL <https://www.mohfw.gov.in/>. (Accessed 4 July 2020).
- Nemudryi, A., Nemudraia, A., Surya, K., Wiegand, T., Buyukyoruk, M., Wilkinson, R., Wiedenheft, B., 2020. Temporal detection and phylogenetic assessment of SARS-CoV-2 in municipal wastewater. *medRxiv* <https://doi.org/10.1101/2020.04.15.20066746>.
- Peccia, J., Zulli, A., Brackney, DE, Grubaugh, ND, Kaplan, EH, Casanovas-Massana, A, Ko, AI, Malik, AA, Wang, D, Wang, M, Warren, JL, Weinberger, DM, Omer, SB, 2020. SARS-CoV-2 RNA concentrations in primary municipal sewage sludge as a leading indicator of COVID-19 outbreak dynamics. *medRxiv*, 2020.05.19.20105999 <https://doi.org/10.1101/2020.05.19.20105999>.
- Qi, F., Qian, S., Zhang, S., Zhang, Z., 2020. Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses. *Biochem. Biophys. Res. Commun.* 526, 135–140.
- Randazzo, W., Truchado, P., Cuevas-Ferrando, E., Simón, P., Allende, A., Sánchez, G., 2020. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. *Water Res.* 526 (1), 135–140. <https://doi.org/10.1016/j.watres.2020.03.044>.
- Rimoldi, S.G., Stefani, F., Gigantiello, A., Polesello, S., Comandatore, F., Mileto1, D., Maresca, M., Longobardi, C., Mancon, A., Romeri, F., Pagani, C., Moja, L., Gismondo, M.R., Salerno, F., 2020. Presence and vitality of SARS-CoV-2 virus in wastewaters and rivers. *medRxiv* <https://doi.org/10.1101/2020.05.01.20086009>.
- Stuart, J.L., Delpert, J., Lannigan, R., Zahariadis, G., 2014. Determination of real-time polymerase chain reaction uncertainty of measurement using replicate analysis and a graphical user interface with Fieller's theorem. *Can J Infect Dis Med Microbiol* 25 (4), 207–210. <https://doi.org/10.1155/2014/404517>.
- Tang, A., Tong, Z., Wang, H., Dai, Y., Li, K., Liu, J., Wu, W., Yuan, C., Yu, M., Li, P., Yan, J., 2020. Detection of novel coronavirus by RT-PCR in stool specimen from asymptomatic child. *China. Emerg. Infect. Dis.* 26 (6), 1337–1339. <https://doi.org/10.3201/eid2606.200301>.
- Wölfel, R., Corman, V.M., Guggemos, W., Seilmaier, M., Zange, S., Mueller, M.A., Niemeyer, D., Vollmar, P., Rothe, C., Hoelscher, M., Bleicker, T., Bruenink, S., Schneider, J., Ehmann, R., Zwirgmaier, K., Drosten, C., Wendtner, C., 2020. Virological assessment of hospitalized cases of coronavirus disease 2019. *Nature* 581, 465–469. <https://doi.org/10.1038/s41586-020-2196-x>.
- World Health Organization, 2020. Coronavirus disease (COVID-2019) situation report –144. [https://www.who.int/docs/default-source/coronavirus/situation-reports/20200612-covid-19-sitrep-144.pdf?sfvrsn=66f994f\\_2](https://www.who.int/docs/default-source/coronavirus/situation-reports/20200612-covid-19-sitrep-144.pdf?sfvrsn=66f994f_2).
- Wu, Y., Guo, C., Tang, L., Hong, Z., et al., 2020a. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol. Hepatol.* 5, 434–435. [https://doi.org/10.1016/S2468-1253\(20\)30083-2](https://doi.org/10.1016/S2468-1253(20)30083-2).
- Wu, F., Xiao, A., Zhang, J., Gu, X., Lee, W., Kauffman, K., Hanage, J., Matus, M., Ghaeli, N., Endo, N., Duvallet, C., Moniz, K., Erickson, T., Pr, C., Thompson, J., Alm, E., 2020b. SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. *medRxiv* <https://doi.org/10.1101/2020.04.05.20051540>.
- Wurtzer, S., Marechal, V., Mouchel, J.M., Moulin, L., 2020. Time course quantitative detection of SARS-CoV-2 in Parisian wastewaters correlates with COVID-19 confirmed cases. *medRxiv* <https://doi.org/10.1101/2020.04.12.20062679>.
- Xagoraki, I., O'Brien, E., 2020. Wastewater-based epidemiology for early detection of viral outbreaks. In: O'Bannon, D. (Ed.), *Women in Water Quality*. Springer Nature, Switzerland, pp. 75–97. [https://doi.org/10.1007/978-3-030-17819-2\\_5](https://doi.org/10.1007/978-3-030-17819-2_5).
- Yang, Z., Kasprzyk-Hordern, B., Frost, C.G., Estrela, P., Thomas, K.V., 2015. Community sewage sensors for monitoring public health. *Environ. Sci. Technol.* 49 (507), 5845–5846. <https://doi.org/10.1021/acs.est.5b01434>.
- Zhang, W., Du, R.H., Li, B., Zheng, X.S., Yang, X., Lou, H., Wang, Y.Y., Xiao, G.F., Yan, B., Shi, Z.L., Zhou, P., 2020. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg. Microbes Infect.* 9 (1), 386–389. <https://doi.org/10.1080/22221751.2020.1729071>.
- Zheng, S., Fan, J., Feng, B., Lou, B., Zou, Q., Xie, G., Lin, S., Wang, R., Yang, X., Chen, W., Wang, Q., Zhang, D., Liu, Y., Gong, R., Ma, Z., Lu, S., Xiao, Y., Gu, Y., Zhang, J., Yao, H., Xu, K., Lu, X., Wei, G., Zhou, J., Fang, Q., Cai, H., Qiu, Y., Sheng, J., Chen, Y., Liang, T., 2020. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January–March 2020: retrospective cohort study. *BMJ* 369, m1443. <https://doi.org/10.1136/bmj.m1443>.