

Supplementary Table 1. Global studies on SARS-CoV-2 prevalence in wastewater and rivers, according to country, January to August 2020

Country (state/city)	Study design					Main outcomes and remarks		Reference
	Period	Type of water sample	Sampling method (N)	Virus concentration method	Real time RT-PCR ¹ viral targets	Ct ² value range and/or viral load	Main outcomes	
Brazil, Florianopolis	Oct, 2019 - Mar, 2020	• Raw sewage	Grab (N=6)	PEG ³ precipitation and filtration	N1, S, RdRp	5.49 ± 0.02 to 6.68 ± 0.02 gc/L ⁴	<ul style="list-style-type: none"> • RNA was undetectable in October and early November, 2019, but all samples were positive since 27 Nov, 2019 • Early detection: RNA detections preceded the first case in 91 days 	[41]
Brazil, Minas Gerais	13 Apr, 2020 (ongoing)	• Raw sewage and treated effluents (24 catchment points from two basins, including 15 sewage sub-basins, 4 WWTPs ⁵ and 3 hospitals)	Grab and composite (variable)	Membrane filtration and direct extraction	NR ⁶	NR	<ul style="list-style-type: none"> • Viral RNA detection: 29.0-64.0% in initial catchments to 100.0% in the last weeks 	[38]
Brazil, Rio de Janeiro	5 Apr, 2020	• WWTP, Sewage treatment plants (STPs), hospital wastewater and sewers network	10h-composite (N=12)	UC ⁷	N2	Ct 36.3-39.8	<ul style="list-style-type: none"> • Viral RNA detection: 41.7% (5/12) 	[40]
Brazil, Rio Grande do Sul	11 May (ongoing)	<ul style="list-style-type: none"> • WWTP, hospital wastewater, Wastewater Pumping Station • Rivers 	Grab and 24h or 6h composite (N=42)	UC	E, N1, N2	Ct 23.0-38.0 / 6,00E+04 to 6,5E+05 gc/L	<ul style="list-style-type: none"> • Viral RNA detection: 12.5% to 100.0% in wastewater and 44.0% in polluted rivers. • Viral isolation: negative 	[36]
Chile, Santiago	Mar - Jun, 2020	• Influent and effluent wastewater from 2 WWTPs	24h-composite (N=4)	UC	ORF1ab,S, N	Ct 28.1 to 37.7/ up to 5000 genome equivalent/ml	<ul style="list-style-type: none"> • Viral RNA detection along time: 100.0%. • SARS-CoV-2 RNA was not detected during March and April 2020, but SARS-CoV-2 viral load progressively increased from May to June 	[51]

Ecuador, Quito	5 Jun, 2020	<ul style="list-style-type: none"> Rivers, direct discharge of sewage water 	Grab (N=3)	Milk flocculation	N1, N2	2.1x10 ⁵ to 8.5x10 ⁵ gc/L	<ul style="list-style-type: none"> Viral RNA detection: 100.0% (3/3) 	[81]
USA, Connecticut	19 Mar - 1 May, 2020	<ul style="list-style-type: none"> Daily primary sludge samples from WWTP 	Grab (N=44)	Direct method	N1, N2	1.7 x 10 ³ gc/mL-1 to 4.6 x 10 ⁵ gc/mL-1	<ul style="list-style-type: none"> Viral RNA detection: 100.0% Early detection: RNA detection preceded hospital admissions in 1-4 days 	[47]
USA, Massachusetts	18 - 25 Mar, 2020	<ul style="list-style-type: none"> Raw sewage influent 	24hr - composite (N=14)	PEG precipitation with pre-filtration (0.2 um)	S, N1, N2, N3	Ct 33.8-38.4/up to ~250 gc/ml	<ul style="list-style-type: none"> Viral RNA detection: 100.0% (4/4) Early detection: RNA detection preceded the first US known cases 	[44]
USA, Massachusetts	Jan - May, 2020	<ul style="list-style-type: none"> Raw sewage influent (11 composite; 23 grab samples x 4 days) 	Grab and 24hr-composite (n=136)	PEG precipitation with pre-filtration (0.2 um) and UC	S, N1, N2	≤ 15 to 2000 gc/ml	<ul style="list-style-type: none"> Viral titers in wastewater increased exponentially from mid-March to mid-April, declining afterwards Early detection: RNA detection preceded the first cases in 4-10 days 	[45]
USA, Montana	Mar -Jun, 2020	<ul style="list-style-type: none"> Untreated wastewater 	Grab and 24h - composite (N=52)	Sequential filtration (20 um, 5 um, 0.45 um), ultrafiltration	N1, N2	10 ⁴ -10 ⁶ gc/L	<ul style="list-style-type: none"> Viral RNA detection: 50.0% (6/12) Early detection: RNA detection preceded the first cases in 1-2 days 	[48]
USA, New York	6 May and 13 May, 2020	<ul style="list-style-type: none"> Wastewater (11 points WWTP, pump stations, interceptor lines), 2 time points 	Grab and 24h - composite (N=22)	UC	IP2, IP4	42.7 to 112.3 gc/ml	<ul style="list-style-type: none"> Viral RNA detection: 81.1% (18/22) 	[46]
USA, Virginia	Mar-Jul, 2020	<ul style="list-style-type: none"> Raw wastewater influent samples from 9 WWTPs (weekly collected) 	24h – composite (N=198)	InnovaPrep Concentrating Pipette Select and electronegative filtration	N1, N2, N3 (digital RT-PCR)	10 ¹ to 10 ⁴ gc/100 mL ⁻¹	<ul style="list-style-type: none"> Viral RNA detection: 49.5% (98/198). A sharp increase in detections was perceived in mid-March for 3 three weeks, followed by a peak on the beginning of April and a further decline. Since the end of April, a new increase in the detection frequency has been observed. 	[49]
USA, Louisiana	Jan-Apr, 2020	<ul style="list-style-type: none"> Wastewater (untreated, secondary treated and effluents) 	Grab, 24h composite (N=15)	Ultrafiltration and adsorption-elution (electronegative membrane)	N1,N2	3.1×10 ³ - 7.5×10 ³ gc/L	<ul style="list-style-type: none"> Viral RNA detection: 13.0% (2/15) 	[50]

Canada, Ottawa and Gatineau	Apr-Jun, 2020	<ul style="list-style-type: none"> Influent post grit solids (PGS) Primary clarified sludge (PCS) 	Grab, 24-hour composite	Serial filtration and PEG precipitation	N1,N2 E-Sarbeco (qRT-PCR, one-step RT-ddPCR)	1.7 x 10 ³ to 7.8 x 10 ⁴ copies//L (Ottawa) and 6.6 x 10 ⁴ to 3.8 x 10 ⁵ 454 copies/L (Gatineau)	<ul style="list-style-type: none"> Viral RNA detection: 92.7% and 90.6% among PCS samples and 79.2 and 82.3%, among PGS samples for N1 and N2 targets, respectively. RT-qPCR shows higher frequency of detection of N1 and N2 genes in PCS (92.7, 90.6%) as compared to PGS samples (79.2, 82.3%) RT-qPCR shows superior quantification of SARS-CoV-2 PCS compared to RT-ddPCR After normalization, significant correlations were observed between gene copies/L and epidemiological data 	[52]
China	19 - 24 Feb, 2020	<ul style="list-style-type: none"> Sewage (isolation ward sewage; inlets of hospital sewage disinfection pool, preprocessing disinfection pool; outlet) 	Grab (N=5)	NR	NP	Ct 29.3-33.5	<ul style="list-style-type: none"> Viral RNA detection: 60.0% (3/5) in sewage samples (inlet of preprocessing disinfection pool). The sample from the outlet of the last disinfection pool was negative. Viral isolation: negative 	[70]
China	Feb - Mar, 2020	<ul style="list-style-type: none"> Influent and effluent wastewater from septic tanks 	Grab (N=15)	PEG precipitation	ORF1, N	30.1-35.7	<ul style="list-style-type: none"> Viral RNA detection: 63.6% (7/9) in effluents. Undetectable RNA in influents (0/4) 	[71]
China, Wuhan	Mar - Apr, 2020	<ul style="list-style-type: none"> Wastewater (samples from treatment tanks, adjusting tank, raw medical wastewater, sedimentation tank) 	Grab (N=28)	PEG precipitation	ORF1, N	255 to 18,7 gc/L	<ul style="list-style-type: none"> Viral RNA detection: 39.3% (11/28). No SARS-CoV-2 was detected in effluents after disinfection 	[69]
India, Jaipur	3 May and 14 Jun, 2020	<ul style="list-style-type: none"> Influent and effluent wastewater samples and hospitals 	Grab, composite (N=13)	PEG precipitation and direct method	S, E, N RdRp, ORF1ab,	Ct 23.7-36.0	<ul style="list-style-type: none"> Viral RNA detection: 75.0% (6/8) in influents Early detection: RNA detection preceded the increase in reported cases in 10-14 days 	[72]

India, Jaipur	8 and 27 May, 2020	<ul style="list-style-type: none"> Wastewater samples 	Grab (N=4)	PEG precipitation and 96 well filter plate	ORF1ab,N, S	Ct 32.6-35.9/2.4×10 ⁸ gc/L	<ul style="list-style-type: none"> Viral RNA detection: 100.0% in untreated wastewater. None positives in treated wastewater 	[73]
Japan, Ishikawa and Toyama	5 Mar - 23 Apr, 2020	<ul style="list-style-type: none"> Influent wastewater samples 	Grab (N=27)	PEG precipitation	N2, N3	1.2×10 ⁴ to 4.4×10 ⁴ gc/L.	<ul style="list-style-type: none"> Viral RNA detection: 25.9% (7/27). In influents, 20.0% - 57.0%. 	[67]
Japan, Yamanashi	17 Mar and 7 May, 2020	<ul style="list-style-type: none"> Influent and secondary-treated wastewater River 	Grab (N=13)	Electronegative membrane; membrane adsorption-direct RNA extraction	N-Sarbeco, N1, N2, ORF1a, S	Influent: 3.2 to 5.0×10 ⁷ ; Rivers: 1.8 to 4.0×10 ⁵ gc/L	<ul style="list-style-type: none"> Viral RNA detection: 20.0% (1/5) in secondary-treated wastewater. RNA was undetectable in all influent and rivers samples Detections occurred in a low incidence scenario 	[68]
Pakistan, Islamabad	20 Mar - 28 April, 2020	<ul style="list-style-type: none"> Sewage 	Grab (N=78)	Centrifugation and PEG-dextran precipitation	ORF1ab, ORF-1, N, E	Ct 32.0-38.0	<ul style="list-style-type: none"> Viral RNA detection: 27.0% (21/78) 	[91]
Bangladesh, Noakhali	10 Jul – 29 Aug, 2020	<ul style="list-style-type: none"> Wastewater (sewage waste tank, passage drain, and toilets) 	Grab (N=16)	Flitration and PEG precipitation	ORF1ab, N	Ct 20.4-40-7	<ul style="list-style-type: none"> Viral RNA detection: 75.0% (12/16) 	[92]
Australia, Queensland	20 Mar – 1 Apr, 2020	<ul style="list-style-type: none"> Untreated wastewater 	Grab and composite (N=9)	Electronegative membrane; ultrafiltration	N-Sarbeco, NIID_2019 -nCOV_N	Ct 37-40/1.9 to 12 gc/100mL	<ul style="list-style-type: none"> Viral RNA detection: 22.2% (2/9) 	[74]
France, Paris	Mar - Apr, 2020	<ul style="list-style-type: none"> Raw sewage influent 	Grab (N=27)	UC	E-Sarbeco	5.10 ⁴ gc/L - 3.10 ⁶ gc/L	<ul style="list-style-type: none"> Viral RNA detection: 100.0% (27/27). After a 2-log exponential increase, viral loads reached a peak, followed by a marked decrease. Early detection: RNA detection preceded the exponential growth of the epidemic 	[60]
France, Paris	5 Mar - 7 Apr, 2020.	<ul style="list-style-type: none"> Raw and treated wastewater 	Grab (N=31)	UC	E	10 ⁴ -10 ⁷ eq/L	<ul style="list-style-type: none"> Viral RNA detection: 100.0% in raw wastewater and 75.0% (6/8) in treated wastewater samples. Early detection: RNA detection preceded the exponential growth of the epidemic 	[59]

France, Montpellier	May-Jul, 2020	<ul style="list-style-type: none"> • Effluent wastewater (WWTP) 	24h – composite (N=7)	Membrane filtration	N1, N3	10 ² -10 ⁴ gc/ml	<ul style="list-style-type: none"> • A fifty-fold increase in SARS-CoV-2 RNA genome copies was observed since mid-June samples, about a month after the end of lockdown Early detection: RNA detection preceded the increase of new COVID-19 cases in 2-3 weeks 	[61]
Italy, Milan and Monza	14 and 22 Apr, 2020	<ul style="list-style-type: none"> • Raw and treated wastewater • Rivers 	Grab (N=8)	Glass fiber filtration and membrane filtration	N, ORF1ab, E	NR	<ul style="list-style-type: none"> • Viral RNA detection: 100.0% in raw wastewater and river samples. RNA was undetectable in all treated wastewater showed negative results. • Viral isolation: negative 	[82]
Italy, Milan and Rome	3 Feb - 2 Apr, 2020	<ul style="list-style-type: none"> • Raw sewage 	24h - composite (N=12)	PEG-dextran precipitation	ORF1ab, S, RdRP	NR	<ul style="list-style-type: none"> • Viral RNA detection: 50.0% (6/12) • Positive in Milan was collected a few days after the first case. 	[62]
Italy, Milan/Lombardy, Turin/Piedmont and Bologna/Emilia Romagna	9 Oct, 2019 - 28 Feb, 2020	<ul style="list-style-type: none"> • Influent wastewater samples and retrospective samples from 5 WWTPs 	24h - composite (N=40)	WHO modified two-phase (PEG-dextran)	E, RdRp, E-Sarbeco, ORF1ab	Undetectable to 5.6 × 10 ⁴ gc/L, most results 10 ² -10 ³ gc/L.	<ul style="list-style-type: none"> • Early detection: RNA detection preceded the first reported cases. SARS-CoV-2 was in circulation in Northern Italy at the end of 2019. In Milan and Turin, the earliest dates back to December 18, 2019 and, in Bologna, to January 29, 2020 	[63]
Netherlands, Amsterdam, Hague, Utrecht, Apeldoorn, Tilburg and Schiphol airport	5 Feb - 25 Mar, 2020	<ul style="list-style-type: none"> • Sewage 	24h - composite (N=30)	Ultrafiltration (Centricon)	N1, N2, N3,E	29.9-37.8/ 2.6–30 gc/mL (early) to 790–2200 gc/ml	<ul style="list-style-type: none"> • Viral RNA detection: 68.9%. • Early detection: RNA detection preceded the first reported case in 6 days 	[54]
Netherlands, Amsterdam	Feb - Apr, 2020	<ul style="list-style-type: none"> • Wastewater 	24h - composite (NR)	NR	NR	NR	<ul style="list-style-type: none"> • Early detection: RNA detection preceded the first cases in 4 days 	[93]

Spain Murcia, Totana, Lorca, Cartagena, Cieza, Molina de Segura	12 Mar - 14 Apr, 2020	<ul style="list-style-type: none"> Influent, secondary and tertiary treated effluent water 	Grab (N=72)	Adsorption-precipitation (aluminum hydroxide)	N1, N2, N3	Ct 34.0-37.8 / Untreated wastewater: 5.1 ± 0.3 log ₁₀ gc/L-5.5 ± 0.3 log ₁₀ gc/L	<ul style="list-style-type: none"> Viral RNA detection: 83.0% (35/42) in influent samples and 11% (2/18) in secondary treated water samples. RNA was undetectable in tertiary effluent samples (0/12). Early detection: RNA detection preceded the reported cases in 12–16 days 	[56]
Spain, Valencia	12 Feb - 14 Apr, 2020	<ul style="list-style-type: none"> Wastewater (WWTPs) 	Grab (N=24)	Flocculation precipitation	N1, N2	Ct 34.0-37.8 5.22 and 5.99gc/L	<ul style="list-style-type: none"> Viral RNA detection: 80.0% (12/15) Early detection: RNA detection preceded the first declared case in Spain in 1 day 	[55]
Spain, Barcelona	13 Apr - 25 May, 2020	<ul style="list-style-type: none"> Raw sewage samples from WWTP; frozen archival samples from 2018 (Jan-Mar), 2019 (Jan, Mar, Sep-Dec) and 2020 (Jan-Mar) 	Grab and 24h - composite (N=28)	PEG precipitation	RdRp, IP2, IP4, E, E- Charité, N1, N2	2 to 5 log ₁₀ gc/L	<ul style="list-style-type: none"> Early detection: RNA detection preceded the declaration of the first case in 41 days 	[57]
Spain, Santiago de Compostela	6 Apr - 21 Apr, 2020	<ul style="list-style-type: none"> Wastewater and sludge 	Grab and 24h - composite (N=50)	Ultrafiltration; PEG precipitation	RdRP, N, E	Ct 33.7-39.8 / Influent: 7.5 to 15 gc/ml. Sludge: 10 to 40 gc/ml	<ul style="list-style-type: none"> Viral RNA detection: 40.0% (6/15) in wastewater and 40.0% (14/35) in sludge 	[58]
Germany, North Westphalia	8 April, 2020	<ul style="list-style-type: none"> Wastewater and sludge 	24h - composite	Ultrafiltration	RdRP,M, N, E	Ct 32- 37/Influent: 3.0 and 20 gc/ml Effluent: 2.7 to 37 gc/ml	<ul style="list-style-type: none"> Viral RNA detection: 100.0% but some positives were not confirmed by Sanger sequencing. In influents, SARS-CoV-2 RNA gc/ml was one log unit higher in the solid phase (25 vs.1.8 gc/mL) The total load of gene equivalents in wastewater correlated with the cumulative and the acute number of COVID-19 cases reported in the respective catchment areas Viral isolation: negative 	[65]

Czech Republic	Apr - Jun, 2020	• Raw wastewater (33 WWTPs)	24h - composite (N=112)	Direct flocculation	ORF1ab, E	Ct 34-40	• Viral RNA detection: 11.6%	[94]
Turkey, Istanbul	7 May, 2020	• Wastewater, primary and activated sludge	Grab (N=9)	PEG precipitation	RdRp	Ct 33.5 to 35.8 / 1.17x10 ⁴ to 4.02x10 ⁴ gc/ml	• Viral RNA detection: 100.0% (9/9). Viral concentration was similar in PS and WAS samples	[66]
Israel, Tel Aviv	NR	• Raw sewage, hospital effluents	Grab and 24h - composite (N=26)	PEG precipitation or alum precipitation; ultrafiltration	E	Ct 32.8-38.03	• Viral RNA detection: 38.4%	[75]

¹ RT-PCR, reverse-transcription followed by polymerase chain reaction; ² Ct, cycle threshold; ³ PEG, polyethylene glycol; ⁴ gc, genome copies; ⁵ WWTPs, wastewater treatment plants;

⁶ NR, not reported; ⁷ UC, ultracentrifugation