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Detection and survival of SARS in human stool, urine, wastewater and sludge

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Abstract

The COVID-19 pandemic has revealed many knowledge gaps with implications toward the speed and nature of our response to contain, assess risk, and mitigate. The routine discharge of treated and untreated wastewater into rivers and coastal waters has placed SARS-CoV-2 viability in wastewater at the centre of an emerging hazard and potential risk to water industry workers and the public who come into contact with sewage-impacted water. Here we provide a review of the SARS-CoV-1 and SARS-CoV-2 primary literature that presents the evidence base pertaining to the key questions of whether the virus is shed in stool and urine, is recoverable, and infectious in wastewater and sludge. We discuss the challenges posed by the current literature base and the extent to which the current evidence is fit for the purpose of informing robust human and environmental risk assessments.

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INTRODUCTION

The COVID-19 pandemic is a global crisis that is infecting millions with Severe Acute Respiratory Syndrome (SARS-CoV-2) resulting in a case fatality ratio of between 0.5% and10% (Coronavirus disease (COVID-2019) situation reports; Mortality Analyses - Johns Hopkins Coronavirus Resource Center). Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses, which are presumed to have initially been transmitted from an animal reservoir to humans, possibly via an amplifying host, first in 2002 when they caused the disease Severe Acute Respiratory Syndrome (Ksiazek et al., 2003) and most recently in 2019 (Li et al., 2020b). The emergence of SARS-CoV-1 highlighted the potential that, as well as being present in the respiratory system, it could also be shed faecally. The faecal-oral and faecal-respiratory route of transmission were first described in 2003, where 329 residents of Amoy Gardens, a private housing estate in Hong Kong, were infected by SARS-CoV-1 shed by a single patient into faulty sewage pipelines (Hong Kong Special Administrative Region Department of Health, 2003; Yu et al., 2004). The chain of events in Amoy Gardens led to the aerosolization of contaminated faeces, resulting in the death of 42 people.

The relevance of faecal shedding of SARS speaks to several larger issues, such as: 1) How important is the faecal-oral route for transmission; 2) Does wastewater represent a risk to human infection?; 3) Does treated wastewater represent a risk to human infection?; 4) Where are the high-risk areas for exposure to infectious SARS originating from the faecal route? To answer these policy-relevant questions necessitates a thorough review of the literature with a focus on three questions:

1) What is the evidence for SARS-CoV-1 and SARS-CoV-2 detection in human stool or urine?
2) What is the evidence for SARS-CoV-1 and SARS-CoV-2 detection in wastewater?
3) What is the evidence for infectious SARS-CoV-1 and SARS-CoV-2 in stool, urine or wastewater?

We took a conservative view on evidence gathering by exclusively focusing on SARS-CoV-1 and SARS-CoV-2, thereby excluding similarly enveloped viruses that are occasionally used as proxies for more pathogenic viruses.

METHODS

A literature review was conducted on each of the study questions. Inclusion criteria were broad, including 1) only primary research (i.e., no reviews); 2) minimally, English language abstract of sufficient detail for relevant data extraction; 3) only human coronavirus studies; 4) only SARS-CoV-1 or SARS-CoV-2; 5) lab and field-derived samples; 6) all publications including abstracts and preprints; and 7) wastewater is inclusive of wastewater treatment plants (WWTPs). Papers were included in this review up until a cut-off date of June 1, 2020.

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Six data extraction tables were generated from the literature focusing primarily on the number of samples examined and the number of positive samples for CoV-1 or CoV-2 in the relevant matrix, i.e., stool, urine, wastewater (Supplementary Tables). Culture-based analysis of CoV-1 and CoV-2 on human cell lines was used as the definitive determination of virus survival and infectivity in the relevant matrix. Summaries of these tables are presented within the paper.

RESULTS

Evidence for detection of SARS in stool

Studies focused on the detection of SARS in stool and urine had a mixture of designs, with some analysing samples from hundreds of anonymous hospitalised patients only once ("Samples" in Table 1), while others focused on a small cohort of patients who might have been resampled over a period of time ("Patients" in Table 1). Critically, it was occasionally ambiguous whether samples were from the same patient or unique patients(this is noted in the data extraction table, when relevant (Supp. Tables 1-4)). We reported data from studies in terms of numbers of samples per study and/or numbers of patients. Several studies were ambiguous about the origin of the samples and a best guess was made given the available information.

SARS-CoV1. Primary research was surveyed for data on the recovery of SARS-CoV-1 from human stool and urine (Table 1; Supp. Table 1). We identified n=15 papers addressing SARS-CoV-1 in stool and urine. Specifically, all 15 papers examined SARS-CoV-1 in stool, while six also examined SARS-CoV-1 in urine (Table 3; Supp. Table 1). The majority of papers (11/15) used reverse-transcriptase polymerase-chain-reaction (RT-PCR) for detection of SARS-CoV-1, and the remaining used real-time reverse-transcriptase quantitative polymerase-chain-reaction (RT-qPCR) (4/15) which quantifies viral RNA from the sample. Two studies used both RT-PCR and RT-qPCR, which accounts for the overlap in study cohorts.

The studies reported recovery of CoV-1 in 51% of patient studies and 55% of stool samples, equating to 234/457 patients and 607/1109 samples (Table 1). Two studies, Poon et al. (2004) and Hung et al. (2004), used both qPCR and PCR. The frequency of CoV-1 detection by RT-PCR was 22/37 (59%) as compared with moderately higher detection rates by RT-qPCR of 26/37 (70%) (Poon et al., 2004). Hung et al. (2004) reported CoV-1 positive rates by RT-PCR of 42/94 (46%), with substantially higher detection rates by RT-qPCR of 82/94 (87%).

SARS-CoV-2. Primary research was surveyed starting from publications from the second SARS outbreak in late 2019 (Table 1). We identified n=27 papers addressing SARS-CoV-2 in stool and urine. Specifically, all 27 papers examined SARS-CoV-2 in stool, while 13 also examined SARS-CoV-2 in urine (Table 3; Supp. Table 2). More than half (18/27) employed RT-PCR for detection of SARS-CoV-2, with the remaining using RT-qPCR (10/27).

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The studies reported recovery of CoV-2 in 51% of patient studies and 52% of stool samples, equating to 258/510 patients and 171/332 samples (Table 1). Notably, the detection rates of CoV-1 and CoV-2 in stool were comparable, despite the much lower sample numbers reported in the literature for CoV-2. The higher number of studies but lower sample sizes might be a reflection of the current trend in science to publish small studies, rapidly; an issue we will pick up again in the discussion. None of the studies employed droplet digital PCR (Suo et al., 2020; Dong et al., 2020), which might be expected to increase the detection rate further.

Isolating the studies that focused on the detection of SARS-CoV-2 in the stool of children (Table 1, indicated by *) reveals a higher detection rate in the stool of children (26/31; 84%)when compared to adult-only studies (258/510; 51%). Given the small study sizes, particularly for children viral shedding, it is not possible to conclude that children more frequently shed SARS-CoV-2 than adults; more research is needed.

Table 1. Detection of SARS from Stool (Adults & Children)

	CoV-1					CoV-2			
References (CoV-1)	**Patients**		**Samples**		**References (CoV-2)**	**Patients**		**Samples**	
	Positive	Total	Positive	Total		Positive	Total	Positive	Total
(Zhai et al., 2004)			60	326	(Holshue et al., 2020)	1	1		
(Ren et al., 2003)	29	46			(Wang et al., 2020)			44	153
(Vabret et al., 2006)	2	6			(Zhang et al., 2020a)	5	14		
(Peiris et al., 2003)	65	67			(Tang et al., 2020)	1	1*		
(Poon et al., 2004)			22	37	(Kam et al., 2020)	1	1*		
(Poon et al., 2004)			26	37	(Chen et al., 2020b)	11	28		
(Chan et al., 2004)			348	386	(Ling et al., 2020)	11	66		
(Leung et al., 2003)			20	124	(Young et al., 2020)	4	8		
(Hung et al., 2004)			42	94	(Xiao et al., 2020b)	39	71		
(Hung et al., 2004)			82	94	(Xu et al., 2020)	8	10*		
(He et al., 2004)	58	101			(Zhang et al., 2020a)	5	14		
(Study group of SARS, 2004)	21	177			(Xing et al., 2020)	3	3*		
(Liu et al., 2004)	56	56			(Kujawski et al., 2020)	7	10		
(Wong et al., 2003)	3	4			(Wölfel et al., 2020)	9	9	57	59

(Wang et al., 2005)			7	11	(Wu et al., 2020b)	8	10*		
					(Cai et al., 2020)	5	6*		
					(Chan et al., 2020)	0	3		
					(Zhang et al., 2020b)	10	12		
					(Lo et al., 2020)			46	79
					(Lescure et al., 2020)	2	5	11	22
					(Chen et al., 2020a)	13	19	18	74
					(Wu et al., 2020c)	41	74		
					(Pan et al., 2020)	9	17		
					(Zhang et al., 2020c)	9	16		
					(Zhang et al., 2020c)	4	15		
					(Zheng et al., 2020)	55	93		
					(Xiao et al., 2020a)	12	28		
Totals	234	457	607	1109		258	510	171	332
Percentages	51%		55%			51%		52%	

Red text indicates studies that employed RT-qPCR

* Indicates child-focused study

Evidence for detection of SARS in urine

SARS-CoV-1. The frequency of CoV-1 detection in urine across patient studies (n=2) was 42% (31/74), when compared to sample-based studies (n=4) which was 22% (81/367) (Table 2; Supp. Table 1). One study using both RT-qPCR and RT-PCR demonstrated similar detection rates: RT-qPCR (32/111) and RT-PCR (29/111) (Hung et al., 2004). The CoV-1 recovery rate from urine, 22-42%, was lower than that from stool, 51-55%, with only one study unable to detect CoV-1 by RT-PCR or RT-qPCR.

SARS-CoV-2. The frequency of CoV-2 detection in urine across patient studies (n=10) was 4% (7/179), in comparison to sample-based studies (n=4), which was 0% (0/27) (Table 2; Supp. Table 2). Sample numbers for CoV-2 (n=206) were half those reported for CoV-1 (n=441), with substantially lower recoveries of CoV-2 in urine than CoV-1 (22%). Studies on the detection of SARS-CoV-2 in urine tended to be smaller in size than those from the first SARS pandemic, possibly indicating an emphasis on speed to publication over study size. Despite the lower sample numbers, it is clear that CoV-2 is not as readily recovered from urine as was CoV-1. Only three studies detecting CoV-2 in urine focused on children (indicated by * in Table 2); of these three, none were able to detect CoV-2. Given the small study sizes and few studies, overall, it is not possible to conclude that children less-frequently

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shed SARS-CoV-2 than adults; more research is needed. Evidence of viral shedding in urine will be important for parameterising wastewater-based epidemiology studies.

Table 2. Detection of SARS in Urine In Adults & Children

	CoV-1					CoV-2			
References (CoV-1)	Patients		Samples		References (CoV-2)	Patients		Samples	
	Positive	Total	Positive	Total		Positive	Total	Positive	Total
(Peiris et al., 2003)	31	74			(Wang et al., 2020)			0	72
(Chan et al., 2004)			20	124	(Tang et al., 2020)	0	1*		
(Hung et al., 2004)			32	111	(Kam et al., 2020)	0	1*		
(Hung et al., 2004)			29	111	(Ling et al., 2020)	4	58		
(Study group of SARS, 2004)	26	177			(Young et al., 2020)	0	8		
(Wang et al., 2005)			0	21	(Kujawski et al., 2020)	0	10		
					(Wölfel et al., 2020)			0	27
					(Cai et al., 2020)	0	3*		
					(Chan et al., 2020)	0	3		
					(Zhang et al., 2020b)	2	23		
					(Lo et al., 2020)			0	49
					(Lescure et al., 2020)	0	5	0	13
					(Zheng et al., 2020)	1	67		
Totals	31	74	81	367		7	179	0	27
Percentages	42%		22%			4%		0%	

Red text indicates studies that employed RT-qPCR

* Indicates child-focused study

Evidence for detection of SARS in wastewater

SARS-CoV1. Primary data on the detection of SARS-CoV-1 from wastewater was found in eight papers from 2004 and 2005 (Supp. Table 5), authored by teams exclusively from Tianjin Institute of Environment and Health, China and the Institute of Hygiene and Environmental Medicine, Academy of Military Medical Sciences, Tianjin, China (Figure 1). All of the (n=19) samples across the four studies tested positive for CoV-1 using RT-PCR protocols. Several of these papers were written in such a way as to make them difficult to interpret.

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SARS-CoV-2. Primary data on the detection of SARS-CoV-2 from wastewater and sludge was found in thirteen papers (Table 3; Supp. Table 6) from a geographically diverse range of laboratories (Figure 2). Of these thirteen papers that examined CoV-2 in wastewater and/or sludge, eleven exclusively used RT-qPCR, two exclusively used RT-PCR and one used both techniques. Reviews and commentaries on the methods used to detect SARS-CoV-2 in wastewater have been recently published precluding the need to review them here (La Rosa et al., 2020a; Silverman and Boehm, 2020; Hill et al., 2020; Kitajima et al., 2020; Amirian, 2020; Naddeo and Liu, 2020). There are many methodological considerations to quantitatively recover CoV-2 from wastewater. This remains an on-going area for international development and refinement as wastewater-based epidemiology for COVID-19 develops into a standard tool in the toolbox for public health surveillance.

Table 3. Recovery of SARS-CoV-2 in Wastewater.

Study	Positive for CoV-2	Samples Tested	Notes
(Wu et al., 2020a)	10	14 (raw sewage)	
(Ahmed et al., 2020)	2	9 (raw sewage)	Pumping station: negative for CoV-2
(Medema et al., 2020)	10	13 (raw sewage)	Detection rate varied depending on primer
(Wurtzer et al., 2020)	23 6	23 (raw sewage) 8 (treated sewage)	
(Nemudryi et al., 2020)	5 2	5 (raw composite sample) 2 (raw grab sample)	
(Randazzo et al., 2020)	36 0	42 (raw sewage) 42 (treated sewage)	
(La Rosa et al., 2020b)	6	12 (raw sewage)	
(Bar Or et al., 2020)	3 1 3 3	17 (raw sewage) 2 (hospital sewage) 4 (sewer network) 3 (isolation facility)	
(Lodder and de Roda Husman, 2020)	1	3 (raw sewage)	
(Alpaslan Kocamemi et al., 2020a)	5 2	7 (raw sewage) 2 (sewer network)	
(Alpaslan Kocamemi et al., 2020b)	2 7	2 (primary sludge) 7 (activated sludge)	
(Rimoldi et al., 2020)	4 0 3	6 (raw sewage) 6 (treated sewage) 4 (river water)	First study to report CoV-2 detection in river water

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(Peccia et al., 2020)	36	36 (primary sludge)	
Totals (n=studies) Raw sewage = 12 Treated sewage = 3 Sludge = 2 River = 1	 116 6 49 3	 280 (raw sewage) 62 (treated sewage) 57 (sludge) 4 (river)	CoV2+ 41.4% 9.7% 49.9% 75%

Evidence of Infectious SARS in stool, urine and wastewater

SARS-CoV1. Primary data on the recovery of infectious SARS-CoV-1 in stool and urine was reported in seven papers and four additional papers in wastewater (Supp. Tables 3 and 5). All of the researchers on CoV-1 infectivity were based in a small number of laboratories in China (Beijing (x1), Tianjin (x4)) or Honk Kong (x1) (Figure 1)). In all cases, the methods used to recover CoV-1 were poorly described, used no positive controls, and employed low or no replication.

Recovery of infectious CoV-1 was relatively low, 67/210 (31.9%) as compared to recovery in stool, though comparable to recoveries from urine. Given the poor description of methods used for recovery, it is impossible to know whether infectious CoV-1 is abundant but loses infectivity during processing for the assay, whether the assay is sensitive to inhibitors found in the virus extract, or if infectious CoV-1 is relatively rare within these matrices.

Wang et al., (2005b) spiked stool and wastewater with CoV-1, incubated at either 4°C or 20°C and repeatedly attempted to recover infectious virus from the matrix. At 20°C the authors recovered infectious virus from wastewater and domestic sewage after 2d, whereas recovery was possible up to 14d at 4°C. CoV-1 remained infectious in stool stored at 20°C for 3d and urine for 17d. Hung et al., (2004) was the only study to examine urine for infectious CoV-1 and was only successful in demonstrating infectivity from 1/20 samples.

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Figure 1. Geographic distribution of research teams contributing to the SARS-CoV-1 evidence base.

SARS-CoV-2. Primary data on the recovery of infectious SARS-CoV-2 in stool (n=4), urine (n=1), wastewater (n=1) and rivers (n=1) was reported in six papers (Table 6). Across these six studies, five reported on samples from a total of 13 patients. A total of 21 samples were acquired from these 13 patients, from which 5 tested positive for infectious CoV-2. Similar to the CoV-1 papers, methodologies from the majority of these published papers were very sparse. Patient numbers remained small in these studies, e.g., Sun et al. (2020), Ziao et al. (2020), making it difficult to use the available data for risk assessment purposes.

Methodological considerations around infectivity assays further complicates the determination of how numerous infectious CoV-2 is in wastewater. Rimoldi et al., (2020) reported on the infectivity of CoV-2 from WWTP influent and effluent, and two rivers. Of the 16 samples assayed, none of the samples were positive for infectious CoV-2. The authors reported using 2 ml of the environmental sample for the infectivity assay. The smaller the assay volume the more difficult it will be to recover CoV-2, a fraction of which will be infectious. There is a general lack of positive controls in these assays, which would have been able to signal inhibition of the virus or VERO E6 cells; as such, negative infectivity results must be cautiously interpreted.

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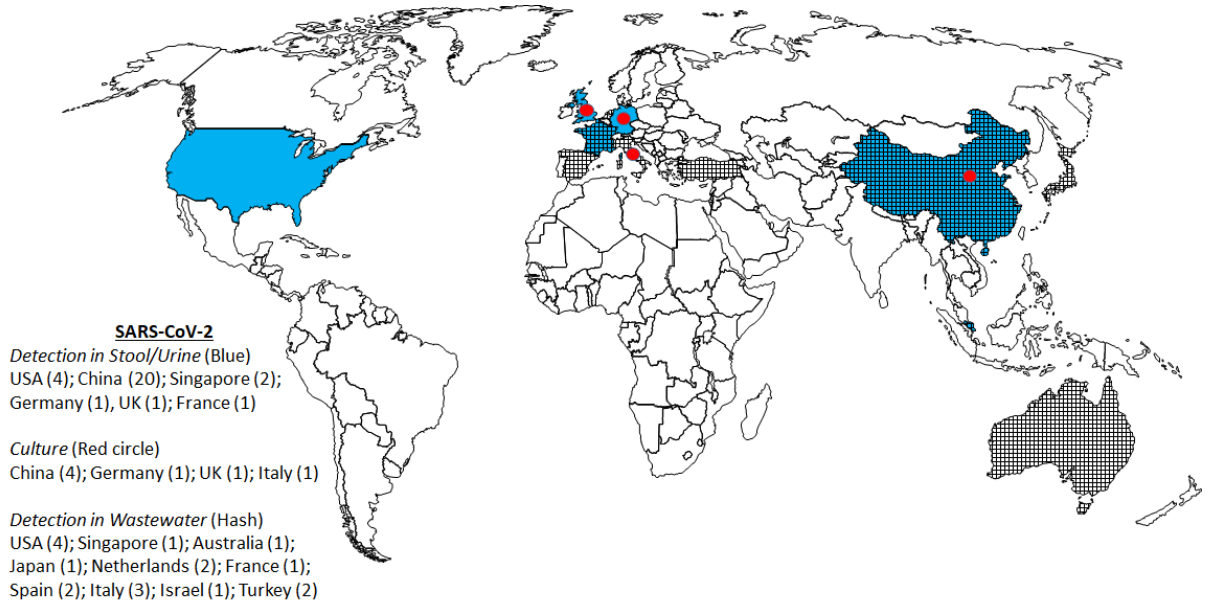
Figure 2. Geographic distribution of research teams contributing to the SARS-CoV-1 evidence base.



Reflections on the Evidence

Global Research Community. Teams publishing during the SARS-CoV-1 pandemic were typically from one nation, and most frequently China (Figure 1). Of the 29 CoV-1 papers across all topics, only three contained authors affiliated with a country other than China, USA (x1) and France (x2).

The prevalence of papers from China continued during the start of the SARS-CoV-2 pandemic, with 20 of the 29 papers examining SARS-CoV-2 in stool and urine having had authors with an affiliation in China. Unlike papers detecting SARS-CoV-1 in wastewater, a diverse array of countries have contributed to the detection of CoV-2 in wastewater (Figure 2). The global interest in wastewater based epidemiology of COVID-19 appears to be the driver for the widespread interest.

Four of the seven papers examining the infectivity of SARS-CoV-2 had affiliations from China, with Germany, UK and Italy each leading on a manuscript. At present, five months into the COVID-19 pandemic, there are nearly as many publications on the infectivity of CoV-2 in stool, urine and wastewater as there had been for the entire CoV-1 pandemic and beyond--a period of over 16 years.

Use of Pre-print manuscripts. A pandemic tests all aspects of society and the scientific community is certainly not immune. The systems of publication, the ethics of research and the rigour of the scientific method can all be pushed to the limit when ignorance is at risk and decisions must be made on very little evidence. Perhaps the best example of this is the UK Government's use of a preprint in medrXiv (Li et al., 2020a) in their first COVID-19 action plan (Department of Health and Social Care, 2020) for the following statement "Illness is less

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common and usually less severe in younger adults". It is clear that preprint servers are playing an important role in hastening information dissemination.

Many of the publications included in this review pertaining to CoV-2 are submissions to a preprint server. We have chosen to accept this data as evidence in much the same spirit as the UK Government - 'useful' but not gospel. There is debate about whether this approach is valid (Smyth et al., 2020). However, given the research questions asked in this review, we felt that the risk was in the interpretation of the data not the acknowledgement of its existence. To that end, we provide our perspective on the evidence.

Evidence of SARS-CoV-1 and SARS-CoV-2 in human stool or urine. In answer to this question, SARS-CoV-1 and SARS-CoV-2 have been recovered from human stool. Of the 457 patients found within all the CoV-1 papers, 51% were positive for CoV-1. A larger number of patients were assayed for CoV-2 (n=510), yielding a similar recovery of CoV-2 in stool, 51%. Urine assayed for CoV-1 in 74 patients were positive 42% of the time, while only 4% of urine from 179 patients were positive for CoV-2. When investigating child-focused studies, 26 of 31 patients were positive. In addition to the low study size, the high CoV-2+ rate might be a result of the majority of child-focused studies employing RT-qPCR which would yield higher detection rates. The methods used across papers were varied and often poorly described making it impossible to make broader conclusions from the evidence, beyond the fact that SARS-CoV-1 and CoV-2 will often be found in stool and less frequently in urine, particularly CoV-2, where recovery was much lower than in CoV-1.

Evidence of SARS-CoV-1 and SARS-CoV-2 in wastewater. There were only four papers reporting detection of SARS-CoV-1 from wastewater, however, due to difficulties in understanding the methods used in the studies, they do not represent a strong foundation of knowledge. In contrast, there is no shortage of evidence demonstrating detection of SARS-CoV-2 in wastewater. Of the thirteen papers, nine of these were still in the preprint stage at the time of writing, with one paper in 'Correspondence' format--indicating a lower level of peer review and reduced methodological information. Despite the majority of papers being at the preprint stage, there is evidence of SARS-CoV-2 detection in wastewater. Notably, none of the CoV-1 papers used RT-qPCR, while all but one publication used RT-qPCR in the CoV-2 papers.

It is observed that the scientists examining SARS in wastewater had been medical-facing labs, particularly during the CoV-1 pandemic. It is proposed that the wastewater-based epidemiology application of such data was beyond the disciplinary scope of these teams and as such, the methods were not as exhaustively reported as similar manuscripts on CoV-2, which have been written by scientists with an eye towards surveillance applications (Amirian, 2020; Carducci et al., 2020; Kitajima et al., 2020; La Rosa et al., 2020a).

Evidence of infectious SARS-CoV-1 and SARS-CoV-2 in stool, urine or wastewater. The evidence in the literature is considerably less quantitative and less reproducible as compared to papers answering the other questions. In few cases have the authors sufficiently reported

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on the methodology used for isolating the SARS from the media to allow for the procedure to be replicated. There were few examples of positive controls used to demonstrate that the recovery process did not inhibit the virus or the VERO E6 cells used for CoV-1 and CoV-2 culture. The lack of replicability, no method development for optimising live virus recovery from stool, urine and wastewater, and no controls makes any data interpretation tenuous. Wang et al., (2004) assayed infectivity of CoV-1 from wastewater 12 times and was able to recover infectious virus each time. The authors subsequently assayed the wastewater after a disinfection step and were only able to recover infectious virus from 1 of 12 samples. Unfortunately, this understanding is gained from the English language abstract with the main study in a Chinese language paper. As such, there is evidence of infectious virus, but the evidence is sparse and in need of substantial methodological improvements.

Reflections on the Evidence. Possibly the most contentious of our editorial decisions was to include papers for which only an English-language abstract was available, e.g., He et al. (2004) and Study group of SARS (2004). The lack of opportunity to critique the methodology and interpretation in these papers compares favourably to the difficulty in interpreting many of the full papers that were available. Incidentally, these abstracts were clearer in their presentation of data than many of the papers we reviewed. A more rigorous review of papers during a pandemic is clearly needed to improve the clarity of papers that have executed valuable studies and weed out those studies that are fundamentally flawed.

As previously mentioned, the methods in many papers are inadequate for assessing whether the data is representative. For example, not only are methods pertaining to the recovery of the virus for infectivity lacking, but the RT-qPCR descriptions are not compliant with MIQE Guidelines (Bustin et al., 2009), reagents are very often not mentioned, QA-QC cut-offs are often missing or set too high, i.e., Ct cutoff for ‘detection limits’ are frequently set at <40 (Wang et al., 2020). The ambiguity around molecular methods limits the gains that can be made by these early studies. Had this been a meta-analysis with rigorous exclusion criteria, i.e., MIQE guidelines, there would be virtually no studies to review.

Application of Evidence for Risk Assessing. The shedding of CoV-2 in stool and urine is critical data needed to assess the risks from wastewater to water industry and others who might come into contact with untreated wastewater (i.e., combined sewer overflow, stormwater). Recent wastewater -based epidemiology efforts to understand carriage of COVID-19 within the community using viral RNA recovered from wastewater necessitates detailed evidence of viral shedding. Viral shedding is thought to be sensitive to age, contra-indication, ethnicity, and gender. Hence, although some of the studies reported on age, gender and comorbidities, more of the studies that reported on detection of CoV in stool/urine would have benefited by having this meta-data included in the study. Disease progression appears different in children to that of adults with this group often being only mildly affected (Dong et al., 2020) and so it follows that the kidneys of children may be affected less frequently, if at all. The mildness of infection in children and indeed that some of these patients are asymptomatic as in the 20/74 infected children in the study by (Wu et al., 2020b), which also demonstrated the presence of faecal shedding at least 42 days after diagnosis, supports the

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need for further investigation in this area. Infectivity in faecal matter was not tested in the studies presented here. Given that the COVID-19 pandemic is impacting over 7 billion people, the data used to model and understand risk is from a minuscule percentage of the world's population--most importantly, from only six countries (Figure 2).

All but two of the papers (Peccia et al., 2020; Alpaslan Kocamemi et al., 2020b) examining detection of CoV-2 in wastewater used the liquid waste, not solids. However, solids have been shown to contain higher quantities of CoV-2 and, as such, might offer greater sensitivity for approaches such as wastewater-based epidemiology.

Application of the evidence. The immediate application of the evidence presented in this review is to inform risk assessments to workers and the public who might come into contact with stool, urine, wastewater and sewage-impacted surface water. Countries and cities where sewage systems are routinely used for waste disposal will introduce risks to the public during times of flooding, accidental releases (e.g., pumping stations), blocked sewage pipes, and combined sewage overflows. Future work will reveal the extent to which SARS infectivity is reduced in wastewater transit to and within WWTPs. At the time of writing this remains an open question for which precautions would prudently be taken to limit exposure. The downstream risks from SARS in wastewater that prematurely enters the environment (as described above), could be the risk to humans by open swimming, bathing waters, and recreation. Wild mammals (e.g., rodents), many of which are likely susceptible to SARS-CoV-2 (Chen, 2020; Shi et al., 2020) might act as reservoirs of CoV-2 with the potential to cycle back to humans. Evidence for virus survival in natural water resources is likely to depend on four key conditions: (i) water temperature; (ii) light availability; (iii) level of organic matter; and (iv) predation (Wartecki and Rzymski, 2020). As such, future research will need to explore the role of these factors within a hazard characterisation and risk assessment framework.

Biosolids produced from sewage containing SARS-CoV-2 remains a source of CoV-2 (Balboa et al., 2020) that will eventually be spread onto land. The journey that sludge takes before going to land might also expose wildlife to CoV-2. At the time of writing, the risks from CoV-2 in biosolids have not been explored, therefore activities should consider additional precautions to limit human and wildlife exposure.

World Health Organisation Guidance. World Health Organisation (WHO) reported in 2003 that "the "faecal droplet" route may have been one of several modes of transmission in Hong Kong during the SARS outbreak in early 2003." (WHO | Inadequate plumbing systems likely contributed to SARS transmission; Inadequate plumbing likely contributed to spread of SARS in Hong Kong – WHO | | UN News) The press release further states that "proper plumbing ... is a significant tool in stopping faecal droplet transmission of disease." The WHO's early recognition of a likely faecal-oral or faecal-respiratory route of infection did not appear to inform a precautionary approach ahead of the SARS-CoV-2 pandemic.

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As of March 3, 2020 and until the next Technical Brief at the end of April, the World Health Organisation proposed: "...there is no evidence on the survival of COVID-19 virus in drinking water or sewage" (Technical Brief: Water, sanitation, hygiene and waste management for COVID-19, 2020). This statement by the WHO is contrary to the evidence presented in this review. Combined with the evidence for transmission of SARS in aerosolised sewage (Ng, 2003), it might have been more prudent for the WHO to begin the COVID-19 pandemic with a precautionary approach regarding the infectivity of SARS-CoV-2 in wastewater.

The WHO Technical Brief further states:

> "While persistence in drinking-water is possible, there is no current evidence that surrogate human coronaviruses are present in surface or groundwater sources or transmitted through contaminated drinking water...The presence of the COVID-19 virus has not been detected in drinking water supplies and based on current evidence the risk to water supplies is low" (Technical Brief: Water, sanitation, hygiene and waste management for COVID-19, 2020).

This review reveals evidence to justify a different starting position from the WHO. The evidence confirms that SARS-CoV-2: 1) can persist in wastewater; 2) should be easily removed during drinking water treatment, and 3) might be expected to persist within drinking water distribution systems where connections between sewage and drinking water are suspected or known. To our knowledge there are no studies attempting to quantify SARS-CoV-1 or SARS-CoV-2 in drinking water distribution systems, ideally, examining scenarios with suboptimal disinfection residuals to capture a realistic, worst-case scenario of sewage ingress. The connectivity between sewage and drinking water distribution systems is not uncommon across high income countries (e.g., Finland (Laine et al., 2011), UK (Stuart et al., 2012) as well as middle and low-income countries (Lee and Schwab, 2005; Karkey et al., 2016)). The WHO might have taken this opportunity to empower governments to fund the research needed to quantify the risk and improve the guidance. The phrase used by the WHO "there is no (current) evidence" is often read by non-scientists as a dismissal of risk, whereas the reader should be left with the message that *the absence of evidence is not the evidence of absence*. Other phrases need to be examined to more effectively communicate an absence of evidence to a non-scientific audience.

The WHO continues: "There is no evidence to date that COVID-19 virus has been transmitted via sewerage systems, with or without wastewater treatment." As stated earlier, there is indeed evidence from the Amoy Gardens work in 2003 that sewage was a vehicle for the transmission of SARS-CoV-1. Moreover, evidence gathered in 2020 has indicated infectious virus is recoverable from wastewater. Given the frequent discharge of untreated sewage into the environment, globally (Kay et al., 2008; Olds et al., 2018; Honda et al., 2020), and the subsequent hazard this poses, the WHO might have been better placed to explicitly encourage governments to fund the research needed to inform and improve guidance.

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The WHO follows: “Furthermore, there is no evidence that sewage and wastewater treatment workers contracted SARS...in 2003.” Again, the *absence of evidence is not evidence of absence*. Aerosolisation of bacteria, fungi and viruses with WWTPs is well documented in the literature (Vantarakis et al., 2016; Bauer et al., 2002; Carducci, 2000; Teltsch et al., 1980). Previous research on occupational contact with wastewater or sludge demonstrated significant differences in the sera-recovery of parainfluenza virus type 1 and adenovirus antigens between those workers with direct exposure to those with sporadic or no exposure (Iftimovici et al., 1980). Whereas studies on hepatitis A, E and *Helicobacter pylori* were less conclusive of an increased risk of infection to workers from sewage (Glas et al., 2001; Jeggli et al., 2004). The lack of research on this question constrains our ability to understand whether workers directly exposed to aerosolised sewage are at risk of infection. Moreover, there might be reason to speculate that otherwise healthy workers with routine exposure to sewage can be protected from the severe effects of COVID-19 in much the same way that it is thought that children who have had frequent and recent coronavirus exposures could mount a more robust defence against SARS-CoV-2. Given the lack of data to understand this question, a precautionary approach must be adopted. The WHO might have been better placed to encourage governments to fund the research needed to generate such evidence to inform and improve guidance.

On April 23, 2020, over three months after the start of the COVID-19 pandemic, WHO guidance changed (Interim Guidance: Water, sanitation, hygiene, and waste management for the COVID-19 virus, 2020). The update incorporated more evidence and adopted a more precautionary approach. Notably, the new guidance states “Faecal sludge and wastewater from health facilities should never be released on land…” This guidance is not practical as all hospital effluent in the UK is treated within combined wastewater treatment plants for which the sludge is ultimately amended to land. The risk posed by sludge amended to land indeed remains an open question for which additional research will be needed.

Given the leadership role that the WHO plays in guiding global public health policy, an earlier recognition of knowledge gaps might have more rapidly focused the global research effort on those issues for which ‘there is no evidence’, thereby supporting the development of a more relevant, evidence-based and actionable guidance document.

Conclusion

The motivation for this review was: 1) to inform on the rationale and likely success of wastewater epidemiology approaches for estimating COVID-19 carriage in a population 2) to inform the hazard and risk assessment for CoV-2 exposure in the workplace as well as in the wider environment, to wildlife, companion animals and humans. The literature available to review was sufficiently abundant to acknowledge several conclusions:

1) CoV-2 will be detectable in wastewater where there are ‘sufficient numbers’ of active and convalescing cases.
2) CoV-2 is infectious in stool and urine and, as such, will remain infectious in wastewater for an undefined period of time. It is not clear from the evidence whether

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wastewater poses a genuine risk of infection to workers or the public by a faecal-oral or faecal-respiratory route. The risk of transmission to wildlife will also need to be explored as their chronic exposure to treated and untreated wastewater might greatly elevate the risk of infection.

3) It is unclear from the evidence whether CoV-2 RNA is released from treated wastewater and whether any of the viral RNA that is detected comes from infectious CoV-2.
4) Efforts to estimate COVID-19 cases from wastewater-acquired CoV-2 RNA will benefit greatly from large studies of viral shedding across ethnicities, gender and age groups. Models will need to account for virus shedding in urine if the rates of shedding in urine approximates that seen in CoV-1 infected individuals.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

The manuscript was conceived, researched and written by ACS and RW.

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References

Bibliography

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J. W., Choi, P. M., Kitajima, M., Simpson, S. L., Li, J., et al. (2020). 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. doi:10.1016/j.scitotenv.2020.138764.

Alpaslan Kocamemi, B., Kurt, H., Hacioglu, S., Yarali, C., Saatci, A. M., and Pakdemirli, B. (2020a). First Data-Set on SARS-CoV-2 Detection for Istanbul Wastewaters in Turkey. *medRxiv*. doi:10.1101/2020.05.03.20089417.

Alpaslan Kocamemi, B., Kurt, H., Sait, A., Sarac, F., Saatci, A. M., and Pakdemirli, B. (2020b). SARS-CoV-2 Detection in Istanbul Wastewater Treatment Plant Sludges. *medRxiv*. doi:10.1101/2020.05.12.20099358.

Amirian, E. S. (2020). Potential Fecal Transmission of SARS-CoV-2: Current Evidence and Implications for Public Health. *Int. J. Infect. Dis.* doi:10.1016/j.ijid.2020.04.057.

Balboa, S., Mauricio-Iglesias, M., Rodríguez, S., Martínez-Lamas, L., Vasallo, F. J., Regueiro, B., and 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*. doi:10.1101/2020.05.25.20112706.

Bar Or, I., Yaniv, K., Shagan, M., Ozer, E., Erster, O., Mendelson, E., Mannasse, B., Shirazi, R., Kramarsky-Winter, E., Nir, O., et al. (2020). Regressing SARS-CoV-2 sewage measurements onto COVID-19 burden in the population: a proof-of-concept for quantitative environmental surveillance. *medRxiv*. doi:10.1101/2020.04.26.20073569.

Bauer, H., Fuerhacker, M., Zibuschka, F., Schmid, H., and Puxbaum, H. (2002). Bacteria and fungi in aerosols generated by two different types of wastewater treatment plants. *Water Res.* 36, 3965–3970. doi:10.1016/s0043-1354(02)00121-5.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., et al. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* 55, 611–622. doi:10.1373/clinchem.2008.112797.

Cai, J., Xu, J., Lin, D., Yang, Z., Xu, L., Qu, Z., Zhang, Y., Zhang, H., Jia, R., Liu, P., et al. (2020). A Case Series of children with 2019 novel coronavirus infection: clinical and epidemiological features. *Clin. Infect. Dis.* doi:10.1093/cid/ciaa198.

Carducci, A. (2000). Assessing airborne biological hazard from urban wastewater treatment. *Water Res.* 34, 1173–1178. doi:10.1016/S0043-1354(99)00264-X.

Carducci, A., Federigi, I., Liu, D., Thompson, J. R., and Verani, M. (2020). Making Waves: Coronavirus detection, presence and persistence in the water environment: State of the art and knowledge needs for public health. *Water Res.* 179, 115907.

doi:10.1016/j.watres.2020.115907.

Chan, J. F.-W., Yuan, S., Kok, K.-H., To, K. K.-W., Chu, H., Yang, J., Xing, F., Liu, J., Yip, C. C.-Y., Poon, R. W.-S., et al. (2020). A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 395, 514–523. doi:10.1016/S0140-6736(20)30154-9.

Chan, K. H., Poon, L. L. L. M., Cheng, V. C. C., Guan, Y., Hung, I. F. N., Kong, J., Yam, L. Y. C., Seto, W. H., Yuen, K. Y., and Peiris, J. S. M. (2004). Detection of SARS coronavirus in patients with suspected SARS. *Emerging Infect. Dis.* 10, 294–299. doi:10.3201/eid1002.030610.

Chen, C., Gao, G., Xu, Y., Pu, L., Wang, Q., Wang, L., Wang, W., Song, Y., Chen, M., Wang, L., et al. (2020a). SARS-CoV-2-Positive Sputum and Feces After Conversion of Pharyngeal Samples in Patients With COVID-19. *Ann. Intern. Med.* doi:10.7326/M20-0991.

Chen, H. (2020). Susceptibility of ferrets, cats, dogs, and different domestic animals to SARS-coronavirus-2. *BioRxiv*. doi:10.1101/2020.03.30.015347.

Chen, W., Lan, Y., Yuan, X., Deng, X., Li, Y., Cai, X., Li, L., He, R., Tan, Y., Deng, X., et al. (2020b). Detectable 2019-nCoV viral RNA in blood is a strong indicator for the further clinical severity. *Emerg. Microbes Infect.* 9, 469–473. doi:10.1080/22221751.2020.1732837.

Coronavirus disease (COVID-2019) situation reports Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports [Accessed March 30, 2020].

Department of Health and Social Care (2020). Policy paper: Coronavirus action plan: a guide to what you can expect across the UK. Available at: https://www.gov.uk/government/publications/coronavirus-action-plan/coronavirus-action-plan-a-guide-to-what-you-can-expect-across-the-uk [Accessed May 31, 2020].

Dong, Y., Mo, X., Hu, Y., Qi, X., Jiang, F., Jiang, Z., and Tong, S. (2020). Epidemiological characteristics of 2143 pediatric patients with 2019 coronavirus disease in China. *Pediatrics*. doi:10.1542/peds.2020-0702.

Dong, lianhua, Zhou, J., Niu, C., Wang, Q., Pan, Y., Wang, X., zhang, Y., yang, J., Liu, M., zhao, Y., et al. (2020). Highly accurate and sensitive diagnostic detection of SARS-CoV-2 by digital PCR. *medRxiv*. doi:10.1101/2020.03.14.20036129.

Glas, C., Hotz, P., and Steffen, R. (2001). Hepatitis A in workers exposed to sewage: a systematic review. *Occup. Environ. Med.* 58, 762–768. doi:10.1136/oem.58.12.762.

He, Z., Dong, Q., Song, S., He, L., and Zhuang, H. (2004). Detection for severe acute respiratory syndrome (SARS) coronavirus RNA in stool of SARS patients. *Zhonghua Yu Fang Yi Xue Za Zhi* 38, 90–91.

Hill, K., Zamyadi, A., Deere, D., Vanrolleghem, P. A., and Crosbie, N. (2020). SARS-CoV-2 known and unknowns, implications for the water sector and wastewater-based

epidemiology tosupport national responses worldwide: early review of global experiences with the COVID-19 pandemic. *Water Qual Res J Can*. doi:10.2166/wqrj.2020.100.

Holshue, M. L., DeBolt, C., Lindquist, S., Lofy, K. H., Wiesman, J., Bruce, H., Spitters, C., Ericson, K., Wilkerson, S., Tural, A., et al. (2020). First case of 2019 novel coronavirus in the united states. *N. Engl. J. Med.* 382, 929–936. doi:10.1056/NEJMoa2001191.

Honda, R., Tachi, C., Yasuda, K., Hirata, T., Noguchi, M., Hara-Yamamura, H., Yamamoto-Ikemoto, R., and Watanabe, T. (2020). Estimated discharge of antibiotic-resistant bacteria from combined sewer overflows of urban sewage system. *npj Clean Water* 3, 15. doi:10.1038/s41545-020-0059-5.

Hong Kong Special Administrative Region Department of Health (2003). Outbreak of Severe Acute Respiratory Syndrome (SARS) at Amoy Gardens, Kowloon Bay, Hong Kong Main Findings of the Investigation. Hong Kong, China.

Hung, I. F. N., Cheng, V. C. C., Wu, A. K. L., Tang, B. S. F., Chan, K. H., Chu, C. M., Wong, M. M. L., Hui, W. T., Poon, L. L. M., Tse, D. M. W., et al. (2004). Viral loads in clinical specimens and SARS manifestations. *Emerging Infect. Dis.* 10, 1550–1557. doi:10.3201/eid1009.040058.

Iftimovici, R., Iacobescu, V., Copelovici, Y., Dincă, A., Iordan, L., Niculescu, R., Teleguţă, L., and Chelaru, M. (1980). Prevalence of antiviral antibodies in workers handling wastewater and sludge. *REV. ROUM. MED. SER. VIROL.* 31, 187–189.

Inadequate plumbing likely contributed to spread of SARS in Hong Kong – WHO | | UN News Available at: https://news.un.org/en/story/2003/09/80662-inadequate-plumbing-likely-contributed-spread-sars-hong-kong-who [Accessed June 8, 2020].

Interim Guidance: Water, sanitation, hygiene, and waste management for the COVID-19 virus (2020). World Health Organization.

Jeggli, S., Steiner, D., Joller, H., Tschopp, A., Steffen, R., and Hotz, P. (2004). Hepatitis E, Helicobacter pylori, and gastrointestinal symptoms in workers exposed to waste water. *Occup. Environ. Med.* 61, 622–627. doi:10.1136/oem.2003.011411.

Kam, K.-Q., Yung, C. F., Cui, L., Lin Tzer Pin, R., Mak, T. M., Maiwald, M., Li, J., Chong, C. Y., Nadua, K., Tan, N. W. H., et al. (2020). A Well Infant with Coronavirus Disease 2019 (COVID-19) with High Viral Load. *Clin. Infect. Dis.* doi:10.1093/cid/ciaa201.

Karkey, A., Jombart, T., Walker, A. W., Thompson, C. N., Torres, A., Dongol, S., Tran Vu Thieu, N., Pham Thanh, D., Tran Thi Ngoc, D., Voong Vinh, P., et al. (2016). The ecological dynamics of fecal contamination and salmonella typhi and salmonella paratyphi A in municipal kathmandu drinking water. *PLoS Negl. Trop. Dis.* 10, e0004346. doi:10.1371/journal.pntd.0004346.

Kay, D., Crowther, J., Stapleton, C. M., Wyer, M. D., Fewtrell, L., Edwards, A., Francis, C. A., McDonald, A. T., Watkins, J., and Wilkinson, J. (2008). Faecal indicator organism concentrations in sewage and treated effluents. *Water Res.* 42, 442–454. doi:10.1016/j.watres.2007.07.036.

Kitajima, M., Ahmed, W., Bibby, K., Carducci, A., Gerba, C. P., Hamilton, K. A., Haramoto, E., and Rose, J. B. (2020). SARS-CoV-2 in wastewater: State of the knowledge and research needs. *Science of The Total Environment*, 139076. doi:10.1016/j.scitotenv.2020.139076.

Ksiazek, T. G., Erdman, D., Goldsmith, C. S., Zaki, S. R., Peret, T., Emery, S., Tong, S., Urbani, C., Comer, J. A., Lim, W., et al. (2003). A novel coronavirus associated with severe acute respiratory syndrome. *N. Engl. J. Med.* 348, 1953–1966. doi:10.1056/NEJMoa030781.

Kujawski, S. A., Wong, K. K., Collins, J. P., Epstein, L., Killerby, M. E., Midgley, C. M., Abedi, G. R., Ahmed, N. S., Almendares, O., Alvarez, F. N., et al. (2020). First 12 patients with coronavirus disease 2019 (COVID-19) in the United States. *medRxiv*. doi:10.1101/2020.03.09.20032896.

La Rosa, G., Bonadonna, L., Lucentini, L., Kenmoe, S., and Suffredini, E. (2020a). Coronavirus in water environments: Occurrence, persistence and concentration methods - A scoping review. *Water Res.* 179, 115899. doi:10.1016/j.watres.2020.115899.

La Rosa, G., Iaconelli, M., Mancini, P., Bonanno Ferraro, G., Veneri, C., Bonadonna, L., and Lucentini, L. (2020b). First detection of sars-cov-2 in untreated wastewaters in italy. *medRxiv*. doi:10.1101/2020.04.25.20079830.

Laine, J., Huovinen, E., Virtanen, M. J., Snellman, M., Lumio, J., Ruutu, P., Kujansuu, E., Vuento, R., Pitkänen, T., Miettinen, I., et al. (2011). An extensive gastroenteritis outbreak after drinking-water contamination by sewage effluent, Finland. *Epidemiol. Infect.* 139, 1105–1113. doi:10.1017/S0950268810002141.

Lee, E. J., and Schwab, K. J. (2005). Deficiencies in drinking water distribution systems in developing countries. *J. Water Health* 3, 109–127. doi:10.2166/wh.2005.0012.

Lescure, F.-X., Bouadma, L., Nguyen, D., Parisey, M., Wicky, P.-H., Behillil, S., Gaymard, A., Bouscambert-Duchamp, M., Donati, F., Le Hingrat, Q., et al. (2020). Clinical and virological data of the first cases of COVID-19 in Europe: a case series. *Lancet Infect. Dis.* doi:10.1016/S1473-3099(20)30200-0.

Leung, W. K., To, K.-F., Chan, P. K. S., Chan, H. L. Y., Wu, A. K. L., Lee, N., Yuen, K. Y., and Sung, J. J. Y. (2003). Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection. *Gastroenterology* 125, 1011–1017. doi:10.1016/s0016-5085(03)01215-0.

Ling, Y., Xu, S.-B., Lin, Y.-X., Tian, D., Zhu, Z.-Q., Dai, F.-H., Wu, F., Song, Z.-G., Huang, W., Chen, J., et al. (2020). Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. *Chin. Med. J.* doi:10.1097/CM9.0000000000000774.

Liu, W., Tang, F., Fontanet, A., Zhan, L., Zhao, Q.-M., Zhang, P.-H., Wu, X.-M., Zuo, S.-Q., Baril, L., Vabret, A., et al. (2004). Long-term SARS coronavirus excretion from patient cohort, China. *Emerging Infect. Dis.* 10, 1841–1843. doi:10.3201/eid1010.040297.

Li, J., Li, S., Cai, Y., Liu, Q., Li, X., Zeng, Z., Chu, Y., Zhu, F., and Zeng, F. (2020a).

Epidemiological and Clinical Characteristics of 17 Hospitalized Patients with 2019 Novel Coronavirus Infections Outside Wuhan, China. *medRxiv*. doi:10.1101/2020.02.11.20022053.

Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., Ren, R., Leung, K. S. M., Lau, E. H. Y., Wong, J. Y., et al. (2020b). Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. *N. Engl. J. Med.* 382, 1199–1207. doi:10.1056/NEJMoa2001316.

Lodder, W., and de Roda Husman, A. M. (2020). SARS-CoV-2 in wastewater: potential health risk, but also data source. *Lancet Gastroenterol. Hepatol.* doi:10.1016/S2468-1253(20)30087-X.

Lo, I. L., Lio, C. F., Cheong, H. H., Lei, C. I., Cheong, T. H., Zhong, X., Tian, Y., and Sin, N. N. (2020). Evaluation of SARS-CoV-2 RNA shedding in clinical specimens and clinical characteristics of 10 patients with COVID-19 in Macau. *Int. J. Biol. Sci.* 16, 1698–1707. doi:10.7150/ijbs.45357.

Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., and Brouwer, A. (2020). Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in The Netherlands. *Environ. Sci. Technol. Lett.* doi:10.1021/acs.estlett.0c00357.

Mortality Analyses - Johns Hopkins Coronavirus Resource Center Available at: https://coronavirus.jhu.edu/data/mortality [Accessed June 7, 2020].

Naddeo, V., and Liu, H. (2020). Editorial Perspectives: 2019 novel coronavirus (SARS-CoV-2): what is its fate in urban water cycle and how can the water research community respond? *Environ. Sci.: Water Res. Technol.* doi:10.1039/D0EW90015J.

Nemudryi, A., Nemudraia, A., Surya, K., Wiegand, T., Buyukyoruk, M., Wilkinson, R., and Wiedenheft, B. (2020). Temporal detection and phylogenetic assessment of SARS-CoV-2 in municipal wastewater. *medRxiv*. doi:10.1101/2020.04.15.20066746.

Ng, S. K. C. (2003). Possible role of an animal vector in the SARS outbreak at Amoy Gardens. *Lancet* 362, 570–572. doi:10.1016/S0140-6736(03)14121-9.

Olds, H. T., Corsi, S. R., Dila, D. K., Halmo, K. M., Bootsma, M. J., and McLellan, S. L. (2018). High levels of sewage contamination released from urban areas after storm events: A quantitative survey with sewage specific bacterial indicators. *PLoS Med.* 15, e1002614. doi:10.1371/journal.pmed.1002614.

Pan, Y., Zhang, D., Yang, P., Poon, L. L. M., and Wang, Q. (2020). Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect. Dis.* 20, 411–412. doi:10.1016/S1473-3099(20)30113-4.

Peccia, J., Zulli, A., Brackney, D. E., Grubaugh, N. D., Kaplan, E. H., Casanovas-Massana, A., Ko, A. I., Malik, A. A., Wang, D., Wang, M., et al. (2020). SARS-CoV-2 RNA concentrations in primary municipal sewage sludge as a leading indicator of COVID-19 outbreak dynamics. *medRxiv*. doi:10.1101/2020.05.19.20105999.

Peiris, J. S. M., Chu, C. M., Cheng, V. C. C., Chan, K. S., Hung, I. F. N., Poon, L. L. M., Law, K. I., Tang, B. S. F., Hon, T. Y. W., Chan, C. S., et al. (2003). Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet* 361, 1767–1772. doi:10.1016/s0140-6736(03)13412-5.

Poon, L. L. M., Chan, K. H., Wong, O. K., Cheung, T. K. W., Ng, I., Zheng, B., Seto, W. H., Yuen, K. Y., Guan, Y., and Peiris, J. S. M. (2004). Detection of SARS coronavirus in patients with severe acute respiratory syndrome by conventional and real-time quantitative reverse transcription-PCR assays. *Clin. Chem.* 50, 67–72. doi:10.1373/clinchem.2003.023663.

Randazzo, W., Truchado, P., Ferrando, E. C., Simon, P., Allende, A., and Sanchez, G. (2020). SARS-CoV-2 RNA titers in wastewater anticipated COVID-19 occurrence in a low prevalence area. *medRxiv*. doi:10.1101/2020.04.22.20075200.

Ren, Y., Ding, H., Wu, Q., Chen, W., Chen, D., Bao, Z., Yang, L., Zhao, C., and Wang, J. (2003). Detection of SARS-CoV RNA in stool samples of SARS patients by nest RT-PCR and its clinical value. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* 25, 368–371.

Rimoldi, S. G., Stefani, F., Gigantiello, A., Polesello, S., Comandatore, F., Mileto, D., Maresca, M., Longobardi, C., Mancon, A., Romeri, F., et al. (2020). Presence and vitality of SARS-CoV-2 virus in wastewaters and rivers. *medRxiv*. doi:10.1101/2020.05.01.20086009.

Shi, J., Wen, Z., Zhong, G., Yang, H., Wang, C., Huang, B., Liu, R., He, X., Shuai, L., Sun, Z., et al. (2020). Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science*. doi:10.1126/science.abb7015.

Silverman, A. I., and Boehm, A. B. (2020). Systematic review and meta-analysis of the persistence and disinfection of human coronaviruses and their viral surrogates in water and wastewater. *Environ. Sci. Technol. Lett.* doi:10.1021/acs.estlett.0c00313.

Smyth, A. R., Rawlinson, C., and Jenkins, G. (2020). Preprint servers: a “rush to publish” or “just in time delivery” for science? *Thorax*. doi:10.1136/thoraxjnl-2020-214937.

Stuart, M., Lapworth, D., Crane, E., and Hart, A. (2012). Review of risk from potential emerging contaminants in UK groundwater. *Sci. Total Environ.* 416, 1–21. doi:10.1016/j.scitotenv.2011.11.072.

Study group of SARS (2004). Quantitative detection of SARS-CoV RNA in excreta and oropharyngeal washing fluid from convalescence patients with severe acute respiratory syndrome. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* 26, 251–254.

Suo, T., Liu, X., Guo, M., Feng, J., Hu, W., Yang, Y., Zhang, Q., Wang, X., Sajid, M., Guo, D., et al. (2020). ddPCR: a more sensitive and accurate tool for SARS-CoV-2 detection in low viral load specimens. *medRxiv*. doi:10.1101/2020.02.29.20029439.

Tang, A., Tong, Z.-D., Wang, H.-L., Dai, Y.-X., Li, K.-F., Liu, J.-N., Wu, W.-J., Yuan, C., Yu, M.-L., Li, P., et al. (2020). Detection of Novel Coronavirus by RT-PCR in Stool Specimen from Asymptomatic Child, China. *Emerging Infect. Dis.* 26, 1337–1339. doi:10.3201/eid2606.200301.

Technical Brief: Water, sanitation, hygiene and waste management for COVID-19 (2020). World Health Organisation.

Teltsch, B., Shuval, H. I., and Tadmor, J. (1980). Die-away kinetics of aerosolized bacteria from sprinkler application of wastewater. *Appl. Environ. Microbiol.* 39, 1191–1197.

Vabret, A., Dina, J., Gouarin, S., Petitjean, J., Corbet, S., and Freymuth, F. (2006). Detection of the new human coronavirus HKU1: a report of 6 cases. *Clin. Infect. Dis.* 42, 634–639. doi:10.1086/500136.

Vantarakis, A., Paparrodopoulos, S., Kokkinos, P., Vantarakis, G., Fragou, K., and Detorakis, I. (2016). Impact on the quality of life when living close to a municipal wastewater treatment plant. *J. Environ. Public Health* 2016, 8467023. doi:10.1155/2016/8467023.

Wang, W., Xu, Y., Gao, R., Lu, R., Han, K., Wu, G., and Tan, W. (2020). Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *JAMA*. doi:10.1001/jama.2020.3786.

Wang, X.-W., Li, J.-S., Guo, T.-K., Zhen, B., Kong, Q.-X., Yi, B., Li, Z., Song, N., Jin, M., Wu, X.-M., et al. (2005). Excretion and detection of SARS coronavirus and its nucleic acid from digestive system. *World J. Gastroenterol.* 11, 4390–4395. doi:10.3748/wjg.v11.i28.4390.

Wartecki, A., and Rzymski, P. (2020). On the Coronaviruses and Their Associations with the Aquatic Environment and Wastewater. *Water (Basel)* 12, 1598. doi:10.3390/w12061598.

WHO | Inadequate plumbing systems likely contributed to SARS transmission Available at: https://www.who.int/mediacentre/news/releases/2003/pr70/en/ [Accessed March 1, 2020].

Wölfel, R., Corman, V. M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M. A., Niemeyer, D., Jones, T. C., Vollmar, P., Rothe, C., et al. (2020). Virological assessment of hospitalized patients with COVID-2019. *Nature* 581, 465–469. doi:10.1038/s41586-020-2196-x.

Wong, P.-N., Mak, S.-K., Lo, K.-Y., Tong, G. M. W., Wong, Y., Watt, C.-L., and Wong, A. K. M. (2003). Clinical presentation and outcome of severe acute respiratory syndrome in dialysis patients. *Am. J. Kidney Dis.* 42, 1075–1081. doi:10.1016/j.ajkd.2003.08.005.

Wurtzer, S., Marechal, V., Mouchel, J.-M., and Moulin, L. (2020). Time course quantitative detection of SARS-CoV-2 in Parisian wastewaters correlates with COVID-19 confirmed cases. *medRxiv*. doi:10.1101/2020.04.12.20062679.

Wu, F., Xiao, A., Zhang, J., Gu, X., Lee, W. L., Kauffman, K., Hanage, W., Matus, M., Ghaeli, N., Endo, N., et al. (2020a). SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. *medRxiv*. doi:10.1101/2020.04.05.20051540.

Wu, Q., Xing, Y., Shi, L., Li, W., Gao, Y., Pan, S., Wang, Y., Wang, W., and Xing, Q. (2020b). Clinical Characteristics of 74 Children with Coronavirus Disease 2019. *medRxiv*. doi:10.1101/2020.03.19.20027078.

Wu, Y., Guo, C., Tang, L., Hong, Z., Zhou, J., Dong, X., Yin, H., Xiao, Q., Tang, Y., Qu, X.,

et al. (2020c). Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol. Hepatol.* 5, 434–435. doi:10.1016/S2468-1253(20)30083-2.

Xiao, F., Sun, J., Xu, Y., Li, F., Huang, X., Li, H., Zhao, J., Huang, J., and Zhao, J. (2020a). Infectious SARS-CoV-2 in Feces of Patient with Severe COVID-19. *Emerging Infect. Dis.* 26. doi:10.3201/eid2608.200681.

Xiao, F., Tang, M., Zheng, X., Liu, Y., Li, X., and Shan, H. (2020b). Evidence for Gastrointestinal Infection of SARS-CoV-2. *Gastroenterology* 158, 1831-1833.e3. doi:10.1053/j.gastro.2020.02.055.

Xing, Y., Ni, W., Wu, Q., Li, W., Li, G., Tong, J., Song, X., and Xing, Q. (2020). Prolonged presence of SARS-CoV-2 in feces of pediatric patients during the convalescent phase. *medRxiv*. doi:10.1101/2020.03.11.20033159.

Xu, Y., Li, X., Zhu, B., Liang, H., Fang, C., Gong, Y., Guo, Q., Sun, X., Zhao, D., Shen, J., et al. (2020). Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. *Nat. Med.* 26, 502–505. doi:10.1038/s41591-020-0817-4.

Young, B. E., Ong, S. W. X., Kalimuddin, S., Low, J. G., Tan, S. Y., Loh, J., Ng, O.-T., Marimuthu, K., Ang, L. W., Mak, T. M., et al. (2020). Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore. *JAMA*. doi:10.1001/jama.2020.3204.

Yu, I. T. S., Li, Y., Wong, T. W., Tam, W., Chan, A. T., Lee, J. H. W., Leung, D. Y. C., and Ho, T. (2004). Evidence of airborne transmission of the severe acute respiratory syndrome virus. *N. Engl. J. Med.* 350, 1731–1739. doi:10.1056/NEJMoa032867.

Zhai, J., Briese, T., Dai, E., Wang, X., pang, X., Du, Z., Liu, H., Wang, J., Wang, H., Guo, Z., et al. (2004). Real-time polymerase chain reaction for detecting SARS coronavirus, Beijing, 2003. *Emerging Infect. Dis.* 10, 300–303. doi:10.3201/eid1002.030799.

Zhang, J., Wang, S., and Xue, Y. (2020a). Fecal specimen diagnosis 2019 Novel Coronavirus-Infected Pneumonia. *J. Med. Virol.* doi:10.1002/jmv.25742.

Zhang, N., Gong, Y., Meng, F., Bi, Y., Yang, P., and Wang, F. (2020b). Virus shedding patterns in nasopharyngeal and fecal specimens of COVID-19 patients. *medRxiv*. doi:10.1101/2020.03.28.20043059.

Zhang, W., Du, R.-H., Li, B., Zheng, X.-S., Yang, X.-L., Hu, B., Wang, Y.-Y., Xiao, G.-F., Yan, B., Shi, Z.-L., et al. (2020c). Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg. Microbes Infect.* 9, 386–389. doi:10.1080/22221751.2020.1729071.

Zheng, S., Fan, J., Yu, F., Feng, B., Lou, B., Zou, Q., Xie, G., Lin, S., Wang, R., Yang, X., et al. (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. doi:10.1136/bmj.m1443.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1: Detection of SARS-CoV-1 from stool and urine.

Reference	Sample type: stool/urine/ anal swab	CoV Detection (RT-qPCR) (Data on length of shedding if available)	CoV Detection (RT-PCR) (Data on length of shedding if available)	Notes	Country
(Zhai et al., 2004)	Stool		n= 326 patients; n = 326 samples Days after onset: CoV+ Stool/Total 1-10d: 10/37 (27.0%) 11-20d: 19/71 (26.8%) 21-30d: 12/77 (15.6%) 31-40d: 12/67 (17.9%) >40d: 7/74 (9.5%) 18% of fecal samples contained SARS-CoV RNA >31–40 days after onset of symptoms	Methods missing, unclear if number of patients equals number of samples or whether people were resampled.	USA, China
(Ren et al., 2003)	Stool		n = 46 patients, n = 103 samples n = 29/46 (63.0%) CoV+ Duration of positive cases 31.76 +/- 10.78 days (12-64 d)	Abstract only	China
(Vabret et al., 2006)	Stool		n = 6 patients (5 Children, 1 Adult) n= 6 samples n = 2/6 (33.3%) CoV+		France
(Peiris et al., 2003)	Stool & Urine		n = 75 patients, n= 67 samples Stool: 65/67 (97.0%) CoV+ on day 14 Urine: 31/74 (41.9%) CoV+ on day 14		China

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(Poon et al., 2004)	Stool	Subsample of 37 seropositive patients compared RT-qPCR & PCR n = 37 samples n = 26/37 (70%) CoV+ Days after onset: CoV+/Total 1–3 : 4/6 (66.7%) 4–6 : 12/15 (80.0%) 7–10: 10/16 (62.5%)	n = 44 samples from seropositive CoV+ patients n = 25/44 (56.8%) CoV+ Days after onset: CoV+/Total (ORF1b region) 1–3 : 2/8 (25.0%) 4–6 : 10/17(58.8%) 7–10: 13/19 (68.4%) Subsample of 37 seropositive patients compared RT-qPCR & PCR n = 37 samples n = 22/37 (59%) CoV+ Days after onset: CoV+/Total 1–3 : 2/6 (33.3%) 4–6 : 10/15 (66.7%) 7–10: 10/16 (62.5%)	Samples collected within 10 days of disease onset. Detection rate in stool increased as disease progressed Method is unclear.	China
(Chan et al., 2004)	Stool Urine		n = 386 patients CoV+ <u>Stool:</u> 5/25 (20.0%) CoV+ before day 5 (serologically confirmed case) <u>Urine:</u> 0/15 (0%) CoV+ before day 5 (serologically confirmed case) n = 1/184 (<1%) CoV+ in presumed CoV- patients. <u>Stool</u> Samples up until day 5, 0% CoV+ (from graph) Samples day 11-12 over 90% (348 samples) CoV+ (from graph) Samples after day 30 approx 10% CoV+ (39 samples) (from graph) <u>Urine</u>	Data extracted from Figure.	China

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			Up to day 7, 0% CoV+ Day 7 - 8 under 10% CoV+ Day 11 - 12, >40% <50% CoV+ After day 30 approx 5% CoV+		
(Leung et al., 2003)	Stool		n = 124 patients n = 20/124 (16.1%) CoV+ Viral RNA detected in stool up to 73 days (10 weeks) after onset of symptoms.		China
(Hung et al., 2004)	Stool Urine	Stool: n = 94 samples n= 82/94 (87.2%) CoV+ Mean viral load in log10 copies/mL (SD) n=82: Stool: 7.0 (2.1) -diarrhea: 7.5 -no diarrhea: 5 Urine: n = 111 urine samples n= 32/111 (28.8%) CoV+ Mean viral load in log10 copies/mL (SD), n=32: Urine: 4.4 (1.3)	Stool: n = 94 samples n = 42/94 (44.7%) RT-PCR Urine: n = 111 urine samples n = 29/111 (27%) RT-PCR n = 1/20 (5%) CoV+culture	Day 10 to 15 after onset of symptoms	China
(He et al., 2004)	Stool	Days after onset of fever: CoV+/total patients:: 10-55d: 58/101 (57.4%) 10-19d: 8/8 (100%) 20-29d: 21/31 (67.7%) 30-39d: 27/57 (47.4%) 40-55d: 2/5 (40.0%)		Abstract only Showed the viral load to be highest in the acute phase	China

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(Study group of SARS, 2004)	Stool Urine	n = 531 samples from n = 177 SARS antibody positive patients n = 26/177 (14.7%) positive in urine n = 21/177 (11.9%) positive in stool The quantity of SARS-CoV RNA in samples was 100-47,000 copies/ml No significant difference was found among urine and stool.		Abstract only. Convalescent Patients	China
(Liu et al., 2004)	Stool		n = 56 patients, n = 514 stool samples n= 56/56 patients CoV+ within the first 20 days. 4/56 (7.1%) CoV+ >100 days after disease onset. The median (range) duration between onset of symptoms and first positive RT-PCR test result was 6 (3–10) days for stool. Duration of virus excretion in stool n = 27 (16-126) days. Duration was marked by the first of three consecutive negative tests for SARS-CoV RNA.	Coexisting illness or conditions were associated with longer viral excretion in stools. Methods unclear.	China, France
(Wong et al., 2003)	Stool		n = 4 patients 3/4 (75.0%) CoV+, Days 2 - 9		China
(Wang et al., 2005a)	Stool Urine		n = 11 samples from active patients n = 10 samples from recovered patients Stool: 7/11 (63.6%) CoV+ in active infections 0/10 (0%) stool positive for viral RNA in recovered patients Urine: 0/21 (0%) urine positive for viral RNA		China

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Supplementary Table 2: Detection of SARS-CoV-2 from stool and urine.

Reference	Sample type: stool/urine/anal swab	CoV Detection (RT-qPCR) (Data on length of shedding if available)	CoV Detection (RT-PCR) (Data on length of shedding if available)	Notes	Country
(Holshue et al., 2020)	Stool	n = 1 patient 100% CoV+ on day 6 after infection		First report of COVID in U.S.	USA
(Wang et al., 2020)	Stool Urine	Stool: n = 153 specimens 44/153 (28.7%) CoV+ Urine: n = 72 specimens 0/72 (0%) CoV+		Ct <40 +ve result	China
(Zhang et al., 2020a)	Stool		n = 14 patients 5/14 (35.7%) CoV+		China
(Tang et al., 2020)	Stool Urine		n = 1 patient (Child) Stool: 6/8 (75%) samples CoV+ Urine 0/1 (0%) samples CoV+ Day 1 after infection: ORF1ab Ct 26.3; nucleoprotein Ct 27.6 Day 2 ORF1ab Ct 31.4; nucleoprotein Ct 30.6 Day 3: ORF1ab Ct 27.0; nucleoprotein Ct 27.0		China
(Kam et al., 2020)	Stool Urine	n = 1 patient (infant) From day of admission to hospital Stool, n=2			Singapore

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		n=1/2 CoV+ Urine, n=1 n= 0-1 CoV+			
(Chen et al., 2020b)	Anal Swab	n = 28 patients n = 11/28 (39.2%) CoV+ Day 10: Ct 24+39			China
(Ling et al., 2020)	Stool & Urine		n = 66 patients Stool: 11/66 (16.7%) CoV+ 43/55 (78.1%) CoV+ longer than in throat swabs (median 2.0 (1.0-4.0) days). Urine: 4/58 (6.9%) CoV+ 3/4 (75.0%) CoV+ after throat swabs turned negative	Methods are unclear	China
(Young et al., 2020)	Stool Urine	Stool: 4/8 (50.0%) patients CoV+ Urine: 0/8 (0%) CoV+			Singapore
(Xiao et al., 2020b)	Stool		n = 71 patients Stool: n = 39/71 (53.4%) CoV+ 17/39 (43.6%) remained positive after showing negative respiratory results.		China
(Xu et al., 2020)	Anal Swab	n = 10 children,			China, USA

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		8/10 children CoV+ in stool Rectal swabs remained CoV+ until day 27 on average			
(Zhang et al., 2020a)	Stool		n = 14 patients Stool: 5/14 (35.7%) CoV+	When CoV- stool samples, also CoV- for oropharyngeal swabs.	China
(Xing et al., 2020)	Stool	n = 3 patients (children) <u>Patients 1 and 2:</u> Pt 1: CoV+ day 4 to 23 inclusive Pt 2: CoV+ day 4 to 33 inclusive Pt 1: discharge day 27 Pt 2: discharge day 26 <u>Patient 3:</u> Pt 3: CoV+ day 25 to 30 after admission.		PREPRINT	China
(Kujawski et al., 2020)	Stool Urine	n = 10 patients Stool: n = 7/10 (70.0%) CoV+ Most CoV+ when CoV+ in respiratory tract. CoV+ up to day 25 (Median 14 days) Urine: n = 0/10 (0%) urine CoV+		PREPRINT First 12 positive patients in US Serial testing to determine duration RNA detection and viral shedding ongoing.	USA
(Wölfel et al., 2020)	Stool Urine	n = 9 patients Stool: n=59 samples		PREPRINT	Germany, UK

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		9/9 (100%) patients CoV+ 57/59 CoV+ samples Last CoV+ swab day 28 Urine: n = 0/27 (0%) samples CoV+ (From Graph)		Stool and sputum samples RNA CoV+ over three weeks in 6/9 patients in spite of full resolution of symptoms. Methods state RT-PCR was used, but quantification was presented indicating RT-qPCR Data extracted from Figure.	
(Wu et al., 2020b)	Stool	n = 10 children, Serologically CoV+ Stool n = 8/10 (80.0%) patients CoV+		PREPRINT	China
(Cai et al., 2020)	Stool (method unstated) Urine	n = 6 children 3 - 5 days after illness onset Stool: 5/6 (83.3%) patients CoV+ Urine: 0/6 (0%) CoV+		PREPRINT	China
(Chan et al., 2020)	Stool Urine		n = 3 patients Stool: 0/3 (0%) patients CoV+ Urine: 0/3 (0%) patients CoV+		China
(Zhang et al.,	Stool	Stool:		PREPRINT	China

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2020b)	Urine	n = 12 patients, n = 51 samples 10/12 (83.3%) patients CoV+ stool 2/23 (8.7%) patients CoV+ 16 and 21 days after hosp.admission. Median Duration of Shedding 22 days fecal Urine: n = 23 patients 2/23 (8.7%) CoV+		Results were unclear.	
(Lo et al., 2020)	Stool & Urine	n = 10 patients Stool samples 46/79 (58%) CoV+ Patient 1: 1/8 (12.5%) CoV+ Patient 2: 7/10 (70.0%) CoV+ Patient 3: 4/6 (66.7%) CoV+ Patient 4: 3/3 (100%) CoV+ Patient 5: 5/8 (62.5%) CoV+ Patient 6: 4/6 (66.7%) +1 inconclusive CoV+ Patient 7: 5/8 (62.5%) +1 inconclusive CoV+ Patient 8: 6/8 (75.0%) CoV+ Patient 9: 10/10 (100%) CoV+ Patient 10: 1/12 (8.3%) +9 inconclusive CoV+ Ct <= 35 is positive test Ct > 38 negative test Ct 36 to 38 required confirmation by retesting and was reported as inconclusive. Urine: n=0/49 (0%) CoV+			China

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(Lescure et al., 2020)	Stool Urine	n = 2 patients Stool: Patient 4: viral load max 6.8 log10 copies/g Patient 5: viral load max 8.1 log10 copies/g	n = 5 patients Stool: n = 2/5 patients CoV+ n = 11/22 (50%) samples CoV+ Patient 1: 0/6 (0%) CoV+ Patient 2: 0/1 (0%) CoV+ Patient 3: 0/4 (0%) CoV+ Patient 4: 6/6 (100%) CoV+ Patient 5: 5/5 (100%) CoV+ Urine: 0/5 (0%) Patients CoV+ n = 0/13 (0%) samples CoV+	PREPRINT RT-PCR used as a screening test followed by RT-qPCR for testing viral loading	France
(Chen et al., 2020a)	Stool	n = 19 patients, n = 74 faecal samples Stool samples taken after first negative pharyngeal/sputum sample n = 13/19 (68.4%) patients CoV+ n = 18/74 (24.3%) samples CoV+ Patient 1: 1/2 (20.0%) Patient 2: 1/4 (25.0%) Patient 3: 1/7 (14.3%) Patient 4: 1/6 (16.7%) Patient 5: 1/3 (33.3%) Patient 6: 2/3 (66.6%) Patient 7: 2/8 (25.0%) Patient 8: 1/2 (50%) Patient 9: 0/3 (0.0%) Patient 10: 0/2 (0.0%) Patient 11: 1/6 (16.7%) Patient 12: 0/6 (0.0%) Patient 13: 0/5 (0.0%) Patient 14: 0/2 (0.0%) Patient 15: 0/2 (0.0%) Patient 16-18: N/A Patient 19: 2/2 (100%) Patient 20: 1/1 (100%)		Low detection in stool due to sample collection following first CoV- pharyngeal or sputum test.	China

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		Patient 21: 1/2 (50%) Patient 22: 3/3 (100%)			
(Wu et al., 2020c)	Stool	n = 98 patients CoV+ (nasopharyngeal) Stool n = 41/74 (55.4%) patients CoV+ Nasopharyngeal CoV+ mean 16.7 days Stool CoV+ mean 27.9 days		CORRESPONDENCE Fecal samples CoV+ after NP samples CoV-	China, USA
(Pan et al., 2020)	Stool	Stool n = 17 patients (Day 0-13 post onset) 9/17 (52.9%) CoV+ 550 copies to 1.21 x 10^5 copies/ml		CORRESPONDENCE Data fairly sparse. No methodology	China
(Zhang et al., 2020c)	Anal Swab	n =15 patients after 0 days treatment 4/15 (27%) CoV+ by RT-qPCR Ct Patient 3: 19.5 Patient 4: 30.2 Patient 5: 33.1 Patient 9: 33.6 n= 16 patients after 10 days treatment (day 5) 9/16 CoV+ by RT-qPCR Ct Patient 5: 33.1 Patient 6: 31.4 Patient 7: 30.2 Patient 8: 33.1			China

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		Patient 10: 23.8 Patient 13: 17.8 Patient 14: 25.5 Patient 15: 30.0 Patient 16: 27.5			
(Zheng et al., 2020)	Stool Urine		n = 96 patients CoV+ Stool n = 55/93 (59%) CoV+ Urine n = 1/67 (0.1%) CoV+	CoV+ if Ct threshold <= 38.0 Median duration virus in stool 22 days Median duration virus in resp 18 days CoV+ in urine in severe case only	China
(Xiao et al., 2020a)	Stool	n = 28 patients n = 12/28 CoV+ One patient from the 28 Ct			China

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Supplementary Table 3: Culture of SARS-CoV-1 from stool/urine or wastewater.

Reference	Sample type: stool/urine/anal swab	SARS-CoV-1 culture	Notes	Country
(Leung et al., 2003)	Stool	Undefined number of attempts to culture. None were successful.		China
(Wang et al., 2005c)	Spiked Stool Urine Wastewater	Positive Virus Detection: Temp = 20C 309th Hospital wastewater: 2d (n = 3/9 (33.3%) samples CoV+) Domestic sewage: 2d (n = 3/9 (33.3%) samples CoV+) Temp = 4C 309th Hospital wastewater: 14d (n = 9/9 (100%) samples CoV+) Domestic sewage: 14d (n = 9/9 (100%) samples CoV+) Temp = 20C Stool: 3d (n = 9/30 (30.0%) samples CoV+) Urine: 17d (n = 20/20 (100%) samples CoV+)		China
(Hung et al., 2004)	Stool Urine	Stool: n = 1/20 (5.0%) CoV+ culture stool Urine: n = 1/20 (5.0%) CoV+ culture urine		China
(Liu et al., 2004)	Stool	n = 0/12 (0%) CoV+ isolation from RT-PCR–positive stool specimens > 6 weeks after disease onset.		China, France
(Wang et al., 2005b)	Sewage	"All sewage samples tested for the presence of infectious SARS-CoV in cell culture were negative"	Methods not detailed enough.	China
(Wang et al., 2004)	Sewage 2 x Hospital	n = 12 samples 0/12 (0%) CoV+ infectious virus	Chinese paper, English abstract	China
(Wang et al., 2005d)	Sewage: Hospital	Temp: 4C CoV+ infectious virus: 14 days (n = 9/9 (100%) samples CoV+)	CoV+ RNA detection in 20C	China

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		Temp: 20C CoV+ infectious virus: 2 days (n = 3/9 (33.3%) samples CoV+)	samples for 8 days.	
(Wang et al., 2005a)	Stool Urine Sewage	n = 21 samples n = 11 samples from active patients n = 10 samples from recovered patients 0/21 Stool positive for infectious SARS-CoV-1 0/21 Urine positive for infectious SARS-CoV-1 n = 12 sewage samples (over 7 days and 2 hospitals) 0/12 sewage samples positive for infectious SARS-CoV-1	Methodology not clear	China

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Supplementary Table 4: Culture of SARS-CoV-2 from stool/urine or wastewater.

Reference	Sample type	CoV culture	Notes	Country
(Zhang et al., 2020d)	Stool	n = 1 patient 100% CoV+	First paper to demonstrate recovery of infectious virus from stool. Electron microscopy verification.	China
(Wölfel et al., 2020)	Anal swab	n = 13 samples from n = 4 patients over 6 - 12 weeks 0/13 (0%) CoV-2+	Samples containing <10^6 copies/mL (or copies per sample) never yielded an isolate. However, CoV+ cultures obtained from oral or nasopharyngeal swabs (16.7%) and sputum (83.3%)	Germany, UK
(Wang et al., 2020)	Stool	n = 4 patients 2/4 (50%) CoV-2+	Verified intact virus by electron microscope	China
(Sun et al., 2020)	Urine	n = 1 patient 1/1 (100%) CoV-2+	CoV+ on day 12 post infection up until day 42. RT-PCR positive urine specimens (Ct 34) from day 12 p.i. was serially diluted in infection media and inoculated onto Vero E6 cells. Cytopathic effects were clearly observed after 3 days.	China
(Rimoldi et al., 2020)	3 x WWTW (Influent and Effluent) 2 x Rivers (WWTW A & B discharge to Lambro River WWTW C discharges to Lambro Meridionale River)	n = 16 samples (Over two different days) 0/16 (0%) CoV-2+	PREPRINT No positive cultures detected 48 and 72 hrs after inoculation.	Italy
(Xiao et al., 2020a)	Stool	n = 3 patients	Detection of virus particles using transmission electron microscopy after 72 hours	China

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		2/3 cultures CoV+		

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Supplementary Table 5: Detection of SARS-CoV-1 from wastewater.

Reference	Wastewater Source[1]	Method of CoV detection	CoV Detection	Notes	Country
(Wang et al., 2005c)	Hospital wastewater Domestic sewage	RT-PCR	Temp: 20C 1x Hospital wastewater CoV+ 1 x Domestic sewage CoV+		China
(Wang et al., 2005b)	2 x Hospitals 1 x Housing estate	RT PCR	Confirmed presence of CoV+ in hospital sewage	Methods/Results unclear	China
(Wang et al., 2004)	Hospital sewage	RT-PCR	n = 12/12 (100%)	1/10 positive in sewage after disinfection	China
(Wang et al., 2005d)	2 x Hospitals	RT-PCR	Confirmed presence of CoV+ in hospital sewage	Methods/Results unclear	China

[1] Description of the nature of the dataset collected: virus spiked into lab-created wastewater (i.e., Lab) or natural abundance of CoV in wastewater collected from plumbing/wastewater/river (i.e., Environment).

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Supplementary Table 6: Detection of SARS-CoV-2 from wastewater

Reference	Wastewater Source[1]	Method of CoV Detection	CoV Detection	Notes	Country
(Wu et al., 2020a)	WWTP x 1 (From two catchments)	RT-qPCR	n = 10/14 (71.4%) samples 7/10 hitting all three primers with an average Ct for all samples below 40	Post 1st US SARS-CoV-2 case	USA, Singapore
(Ahmed et al., 2020)	2 x WWTP (A & B) 1 x Pumping station	RT-qPCR	n = 9 wastewater samples tested (WWTP A,B; Pumping Station) n = 2/9 (22.2 %) samples WWTP B CoV+ Pumping station and WWTP A CoV-		USA, Australia, Japan
(Medema et al., 2020a) and (Medema et al., 2020b)	7 x WWTP in 5 cities (2 large and 3 medium size) 1 x airport	RT-PCR	3 weeks prior to epidemic: 0/6 CoV+ Week 1 of epidemic: n = 4/6 (66.6%) CoV+ Week 3 of epidemic: n = 6/7 (85.7%) CoV+ Detection varied by primer: n = 5/7 (71.4%) CoV+ N3 n = 4/7 (57.1%) CoV+ E primer/probe		The Netherlands
(Wurtzer et al., 2020)	3 x WWTP (Parisian area)	RT- qPCR	n = 23/23 (100%) raw sewage CoV+	PREPRINT CoV+ quantity in WWTP effluent is 100 x lower than	France

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			n = 6/8 (75%) treated sewage CoV+	influent.	
(Nemudryi et al., 2020)	1 x WWTP influent	RT-PCR RT-qPCR	n = 7 sampling days over 17 days 5/5 (100%) CoV+ composite sampling days Viral abundance: N1: 100 to 1700 viral genomes/L N2: 100 to 500 viral genomes/L 2/2 CoV+ grab sampling days Viral abundance: N1: 8,000 to 9,000 viral genomes/L N2: 9,0000 to 23,000	PREPRINT SARS-CoV-2 detected over the entire time course. Viral RNA (N1) steadily decreased over the last week. Viral genomes/L deduced from Figure.	USA

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(Randazzo et al., 2020)	6 x WWTP in two cities Influent Secondary Treatment Tertiary Treatment	RT-qPCR	n = 42 influent samples n = 18 secondary treatment samples n = 12 tertiary treatment samples Untreated wastewater: 5.29log genomic copies/l Influent: n = 36/42 (85.7%) CoV+ 12% samples CoV+ Ct 37 - 40 29% samples CoV+ Ct 34 - 37 Secondary /Tertiary treatment n =0/42 (0%) CoV+ (Ct <40)	PREPRINT	Spain
(La Rosa et al., 2020)	2 x WWTPs in Milan 1 x WWTP in Rome	RT-PCR	n = 12 composite influent 6/12 (50%) CoV+	PREPRINT	Italy

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(Bar Or et al., 2020)	17 x WWTW (Influent) 2 x Hospital Effluent (In sewer) 3 x Isolation facilities (In sewer)	RT-qPCR	n = 17 WWTW samples (influent) 3/17 (17.6%) CoV+ (Ct <40) (Ct = 38.5, 34.7, 37.0) n = 2 Hospital sewer network 1/2 CoV+ (Ct<40) (Ct = 33.2) n = 4 Sewer network 3/4 CoV+ (Ct<40) (Ct 37.24, 35.57, 33.75) n = 3 Isolation facilities sewer network 3/3 CoV+ (Ct<40) (Ct 38.03, 35.51, 32.76)	PREPRINT	Israel
(Alpaslan Kocamemi et al., 2020a)	7 X WWTW (Influent) 2 x Manholes	RT-qPCR	n = 5/7 (71.4%) WWTW CoV+ (Cq 38.37, 37.23, 38.82, 39.18, 39.54) n = 2/2 Manholes CoV+ (Cq 35.91, 34.67) n = 5/7 (71.4%) WWTW Viral genome detected (titre/l) 8.26 E+03 1.80 E+04 4.95 E+03 3.73 E+03 2.89 E+03 n = 2/2 Manholes Viral	PREPRINT SARS-CoV-2 titres greater in manhole sewage to that for WWTW	Turkey

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			genome detected (titre/l) 4.49 E+04 9.33 E+04		
(Lodder and de Roda Husman, 2020)	1 x WWTW	RT-qPCR	n = unknown. At least 3 Weekly 24hr samples n= 1/3 CoV+	CORRESPONDENCE No methodology Wastewater sample CoV+ve 4 days after 1st CoV+ person in NL	The Netherlands

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(Alpaslan Kocamemi et al., 2020b)	7 x WWTW (2 Primary Sludge; 7 Waste Activated Sludge)	RT-qPCR Copy Numbers of Genome	n = 2/2 Primary Sludge CoV+ (Cq 35.96, 34.71) n = 7/7 Waste Activated Sludge CoV+ (Cq 35.67, 35.00, 34.98, 34.74, 34.61, 34.11, 33.52) Primary Sludge Viral genome detected (titre/l) 1.41E+03 8.60E+02 Waste Activated Sludge Viral genome detected (titre/l) 1.17E+04 1.62E+04 1.64E+04 1.91E+04 1.95E+04 3.08E+04 4.02E+04	PREPRINT	Turkey

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(Rimoldi et al., 2020)	3 x WWTW (Influent and Effluent) 2 x Rivers (WWTW A & B discharge to Lambro River WWTW C discharges to Lambro Meridionale River)	RT-qPCR	<u>1st Day Sampling</u> n = 3/3 CoV+ Raw Sewage n = 0/3 CoV+ Treated Sewage n = 2/2 CoV+ River Samples (Lambro and Lambro Meridonale Rivers) <u>2nd Day Sampling</u> n = 1/3 CoV+ Raw Sewage *(CoV+ From WWTW B which discharges to Lambro River)* n = 0/3 CoV+ Treated Sewage n = 1/2 CoV+ River Samples *(Lambro River)*	PREPRINT No Cq results Second day sampling raw and river samples CoV+ for discharge from WWTW B to Lambro River	Italy
(Peccia et al., 2020)	Primary Sludge 1 x WWTW	RT-qPCR	n = 36 samples taken over 36 days n = 36/36 (100%) CoV+	PREPRINT Solids content of sludge - 2.6 - 5% Over 96.5% all CoV+ samples Ct less than 38 Ct 38 - 40 deemed CoV+ only if detection occurred with virus nucleocapsids N1 and N2 primer sets and both replicates.	USA

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., et al. (2020). 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. doi:10.1016/j.scitotenv.2020.138764.

Alpaslan Kocamemi, B., Kurt, H., Hacioglu, S., Yarali, C., Saatci, A. M., and Pakdemirli, B. (2020a). First Data-Set on SARS-CoV-2 Detection for Istanbul Wastewaters in Turkey. medRxiv. doi:10.1101/2020.05.03.20089417.

Alpaslan Kocamemi, B., Kurt, H., Sait, A., Sarac, F., Saatci, A. M., and Pakdemirli, B. (2020b). SARS-CoV-2 Detection in Istanbul Wastewater Treatment Plant Sludges. medRxiv. doi:10.1101/2020.05.12.20099358.

Bar Or, I., Yaniv, K., Shagan, M., Ozer, E., Erster, O., Mendelson, E., Mannasse, B., Shirazi, R., Kramarsky-Winter, E., Nir, O., et al. (2020). Regressing SARS-CoV-2 sewage measurements onto COVID-19 burden in the population: a proof-of-concept for quantitative environmental surveillance. medRxiv. doi:10.1101/2020.04.26.20073569.

Cai, J., Xu, J., Lin, D., Yang, Z., Xu, L., Qu, Z., Zhang, Y., Zhang, H., Jia, R., Liu, P., et al. (2020). A Case Series of children with 2019 novel coronavirus infection: clinical and epidemiological features. Clin. Infect. Dis. doi:10.1093/cid/ciaa198.

Chan, J. F.-W., Yuan, S., Kok, K.-H., To, K. K.-W., Chu, H., Yang, J., Xing, F., Liu, J., Yip, C. C.-Y., Poon, R. W.-S., et al. (2020). A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 395, 514–523. doi:10.1016/S0140-6736(20)30154-9.

Chan, K. H., Poon, L. L. L. M., Cheng, V. C. C., Guan, Y., Hung, I. F. N., Kong, J., Yam, L. Y. C., Seto, W. H., Yuen, K. Y., and Peiris, J. S. M. (2004). Detection of SARS coronavirus in patients with suspected SARS. Emerging Infect. Dis. 10, 294–299. doi:10.3201/eid1002.030610.

Chen, C., Gao, G., Xu, Y., Pu, L., Wang, Q., Wang, L., Wang, W., Song, Y., Chen, M., Wang, L., et al. (2020a). SARS-CoV-2-Positive Sputum and Feces After Conversion of Pharyngeal Samples in Patients With COVID-19. Ann. Intern. Med. doi:10.7326/M20-0991.

Chen, W., Lan, Y., Yuan, X., Deng, X., Li, Y., Cai, X., Li, L., He, R., Tan, Y., Deng, X., et al. (2020b). Detectable 2019-nCoV viral RNA in blood is a strong indicator for the further clinical severity. Emerg. Microbes Infect. 9, 469–473. doi:10.1080/22221751.2020.1732837.

He, Z., Dong, Q., Song, S., He, L., and Zhuang, H. (2004). Detection for severe acute respiratory syndrome (SARS) coronavirus RNA in stool of SARS patients. Zhonghua Yu Fang Yi Xue Za Zhi 38, 90–91.

Holshue, M. L., DeBolt, C., Lindquist, S., Lofy, K. H., Wiesman, J., Bruce, H., Spitters, C., Ericson, K., Wilkerson, S., Tural, A., et al. (2020). First case of 2019 novel coronavirus

in the united states. N. Engl. J. Med. 382, 929–936. doi:10.1056/NEJMoa2001191.

Hung, I. F. N., Cheng, V. C. C., Wu, A. K. L., Tang, B. S. F., Chan, K. H., Chu, C. M., Wong, M. M. L., Hui, W. T., Poon, L. L. M., Tse, D. M. W., et al. (2004). Viral loads in clinical specimens and SARS manifestations. Emerging Infect. Dis. 10, 1550–1557. doi:10.3201/eid1009.040058.

Kam, K.-Q., Yung, C. F., Cui, L., Lin Tzer Pin, R., Mak, T. M., Maiwald, M., Li, J., Chong, C. Y., Nadua, K., Tan, N. W. H., et al. (2020). A Well Infant with Coronavirus Disease 2019 (COVID-19) with High Viral Load. Clin. Infect. Dis. doi:10.1093/cid/ciaa201.

Kujawski, S. A., Wong, K. K., Collins, J. P., Epstein, L., Killerby, M. E., Midgley, C. M., Abedi, G. R., Ahmed, N. S., Almendares, O., Alvarez, F. N., et al. (2020). First 12 patients with coronavirus disease 2019 (COVID-19) in the United States. medRxiv. doi:10.1101/2020.03.09.20032896.

La Rosa, G., Iaconelli, M., Mancini, P., Bonanno Ferraro, G., Veneri, C., Bonadonna, L., and Lucentini, L. (2020). First detection of sars-cov-2 in untreated wastewaters in italy. medRxiv. doi:10.1101/2020.04.25.20079830.

Lescure, F.-X., Bouadma, L., Nguyen, D., Parisey, M., Wicky, P.-H., Behillil, S., Gaymard, A., Bouscambert-Duchamp, M., Donati, F., Le Hingrat, Q., et al. (2020). Clinical and virological data of the first cases of COVID-19 in Europe: a case series. Lancet Infect. Dis. doi:10.1016/S1473-3099(20)30200-0.

Leung, W. K., To, K.-F., Chan, P. K. S., Chan, H. L. Y., Wu, A. K. L., Lee, N., Yuen, K. Y., and Sung, J. J. Y. (2003). Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection. Gastroenterology 125, 1011–1017. doi:10.1016/s0016-5085(03)01215-0.

Ling, Y., Xu, S.-B., Lin, Y.-X., Tian, D., Zhu, Z.-Q., Dai, F.-H., Wu, F., Song, Z.-G., Huang, W., Chen, J., et al. (2020). Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. Chin. Med. J. doi:10.1097/CM9.0000000000000774.

Liu, W., Tang, F., Fontanet, A., Zhan, L., Zhao, Q.-M., Zhang, P.-H., Wu, X.-M., Zuo, S.-Q., Baril, L., Vabret, A., et al. (2004). Long-term SARS coronavirus excretion from patient cohort, China. Emerging Infect. Dis. 10, 1841–1843. doi:10.3201/eid1010.040297.

Lodder, W., and de Roda Husman, A. M. (2020). SARS-CoV-2 in wastewater: potential health risk, but also data source. Lancet Gastroenterol. Hepatol. doi:10.1016/S2468-1253(20)30087-X.

Lo, I. L., Lio, C. F., Cheong, H. H., Lei, C. I., Cheong, T. H., Zhong, X., Tian, Y., and Sin, N. N. (2020). Evaluation of SARS-CoV-2 RNA shedding in clinical specimens and clinical characteristics of 10 patients with COVID-19 in Macau. Int. J. Biol. Sci. 16, 1698–1707. doi:10.7150/ijbs.45357.

Medema, G., Heijnen, L., Elsinga, G., and Italiaander, R. (2020a). Presence of SARS-Coronavirus-2 in Sewage. Available at: https://www.medrxiv.org/content/10.1101/2020.03.29.20045880v1.full.pdf [Accessed

April 5, 2020].

Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., and Brouwer, A. (2020b). Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in The Netherlands. Environ. Sci. Technol. Lett. doi:10.1021/acs.estlett.0c00357.

Nemudryi, A., Nemudraia, A., Surya, K., Wiegand, T., Buyukyoruk, M., Wilkinson, R., and Wiedenheft, B. (2020). Temporal detection and phylogenetic assessment of SARS-CoV-2 in municipal wastewater. medRxiv. doi:10.1101/2020.04.15.20066746.

Pan, Y., Zhang, D., Yang, P., Poon, L. L. M., and Wang, Q. (2020). Viral load of SARS-CoV-2 in clinical samples. Lancet Infect. Dis. 20, 411–412. doi:10.1016/S1473-3099(20)30113-4.

Peccia, J., Zulli, A., Brackney, D. E., Grubaugh, N. D., Kaplan, E. H., Casanovas-Massana, A., Ko, A. I., Malik, A. A., Wang, D., Wang, M., et al. (2020). SARS-CoV-2 RNA concentrations in primary municipal sewage sludge as a leading indicator of COVID-19 outbreak dynamics. medRxiv. doi:10.1101/2020.05.19.20105999.

Peiris, J. S. M., Chu, C. M., Cheng, V. C. C., Chan, K. S., Hung, I. F. N., Poon, L. L. M., Law, K. I., Tang, B. S. F., Hon, T. Y. W., Chan, C. S., et al. (2003). Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet 361, 1767–1772. doi:10.1016/s0140-6736(03)13412-5.

Poon, L. L. M., Chan, K. H., Wong, O. K., Cheung, T. K. W., Ng, I., Zheng, B., Seto, W. H., Yuen, K. Y., Guan, Y., and Peiris, J. S. M. (2004). Detection of SARS coronavirus in patients with severe acute respiratory syndrome by conventional and real-time quantitative reverse transcription-PCR assays. Clin. Chem. 50, 67–72. doi:10.1373/clinchem.2003.023663.

Randazzo, W., Truchado, P., Ferrando, E. C., Simon, P., Allende, A., and Sanchez, G. (2020). SARS-CoV-2 RNA titers in wastewater anticipated COVID-19 occurrence in a low prevalence area. medRxiv. doi:10.1101/2020.04.22.20075200.

Ren, Y., Ding, H., Wu, Q., Chen, W., Chen, D., Bao, Z., Yang, L., Zhao, C., and Wang, J. (2003). Detection of SARS-CoV RNA in stool samples of SARS patients by nest RT-PCR and its clinical value. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 25, 368–371.

Rimoldi, S. G., Stefani, F., Gigantiello, A., Polesello, S., Comandatore, F., Mileto, D., Maresca, M., Longobardi, C., Mancon, A., Romeri, F., et al. (2020). Presence and vitality of SARS-CoV-2 virus in wastewaters and rivers. medRxiv. doi:10.1101/2020.05.01.20086009.

Study group of SARS (2004). Quantitative detection of SARS-CoV RNA in excreta and oropharyngeal washing fluid from convalescence patients with severe acute respiratory syndrome. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 26, 251–254.

Sun, J., Zhu, A., Li, H., Zheng, K., Zhuang, Z., Chen, Z., Shi, Y., Zhang, Z., Chen, S.-B., Liu, X., et al. (2020). Isolation of Infectious SARS-CoV-2 from Urine of a COVID-19 Patient. Emerg. Microbes Infect., 1–8. doi:10.1080/22221751.2020.1760144.

Tang, A., Tong, Z.-D., Wang, H.-L., Dai, Y.-X., Li, K.-F., Liu, J.-N., Wu, W.-J., Yuan, C., Yu, M.-L., Li, P., et al. (2020). Detection of Novel Coronavirus by RT-PCR in Stool Specimen from Asymptomatic Child, China. Emerging Infect. Dis. 26, 1337–1339. doi:10.3201/eid2606.200301.

Vabret, A., Dina, J., Gouarin, S., Petitjean, J., Corbet, S., and Freymuth, F. (2006). Detection of the new human coronavirus HKU1: a report of 6 cases. Clin. Infect. Dis. 42, 634–639. doi:10.1086/500136.

Wang, W., Xu, Y., Gao, R., Lu, R., Han, K., Wu, G., and Tan, W. (2020). Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA. doi:10.1001/jama.2020.3786.

Wang, X.-W., Li, J.-S., Guo, T.-K., Zhen, B., Kong, Q.-X., Yi, B., Li, Z., Song, N., Jin, M., Wu, X.-M., et al. (2005a). Excretion and detection of SARS coronavirus and its nucleic acid from digestive system. World J. Gastroenterol. 11, 4390–4395. doi:10.3748/wjg.v11.i28.4390.

Wang, X.-W., Li, J.-S., Guo, T.-K., Zhen, B., Kong, Q.-X., Yi, B., Li, Z., Song, N., Jin, M., Xiao, W.-J., et al. (2005b). Concentration and detection of SARS coronavirus in sewage from Xiao Tang Shan Hospital and the 309th Hospital. J. Virol. Methods 128, 156–161. doi:10.1016/j.jviromet.2005.03.022.

Wang, X.-W., Li, J.-S., Guo, T.-K., Zhen, B., Kong, Q.-X., Yi, B., Li, Z., Song, N., Jin, M., Xiao, W.-J., et al. (2004). Detection of RNA of SARS coronavirus in hospital sewage. Zhonghua Yu Fang Yi Xue Za Zhi 38, 257–260.

Wang, X.-W., Li, J.-S., Jin, M., Zhen, B., Kong, Q.-X., Song, N., Xiao, W.-J., Yin, J., Wei, W., Wang, G.-J., et al. (2005c). Study on the resistance of severe acute respiratory syndrome-associated coronavirus. J. Virol. Methods 126, 171–177. doi:10.1016/j.jviromet.2005.02.005.

Wang, X. W., Li, J., Guo, T., Zhen, B., Kong, Q., Yi, B., Li, Z., Song, N., Jin, M., Xiao, W., et al. (2005d). Concentration and detection of SARS coronavirus in sewage from Xiao Tang Shan Hospital and the 309th Hospital of the Chinese People's Liberation Army. Water Sci. Technol. 52, 213–221. doi:10.2166/wst.2005.0266.

Wölfel, R., Corman, V. M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M. A., Niemeyer, D., Jones, T. C., Vollmar, P., Rothe, C., et al. (2020). Virological assessment of hospitalized patients with COVID-2019. Nature 581, 465–469. doi:10.1038/s41586-020-2196-x.

Wong, P.-N., Mak, S.-K., Lo, K.-Y., Tong, G. M. W., Wong, Y., Watt, C.-L., and Wong, A. K. M. (2003). Clinical presentation and outcome of severe acute respiratory syndrome in dialysis patients. Am. J. Kidney Dis. 42, 1075–1081. doi:10.1016/j.ajkd.2003.08.005.

Wurtzer, S., Marechal, V., Mouchel, J.-M., and Moulin, L. (2020). Time course quantitative detection of SARS-CoV-2 in Parisian wastewaters correlates with COVID-19 confirmed cases. medRxiv. doi:10.1101/2020.04.12.20062679.

Wu, F., Xiao, A., Zhang, J., Gu, X., Lee, W. L., Kauffman, K., Hanage, W., Matus, M.,

Ghaeli, N., Endo, N., et al. (2020a). SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. medRxiv. doi:10.1101/2020.04.05.20051540.

Wu, Q., Xing, Y., Shi, L., Li, W., Gao, Y., Pan, S., Wang, Y., Wang, W., and Xing, Q. (2020b). Clinical Characteristics of 74 Children with Coronavirus Disease 2019. medRxiv. doi:10.1101/2020.03.19.20027078.

Wu, Y., Guo, C., Tang, L., Hong, Z., Zhou, J., Dong, X., Yin, H., Xiao, Q., Tang, Y., Qu, X., et al. (2020c). Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. Lancet Gastroenterol. Hepatol. 5, 434–435. doi:10.1016/S2468-1253(20)30083-2.

Xiao, F., Sun, J., Xu, Y., Li, F., Huang, X., Li, H., Zhao, J., Huang, J., and Zhao, J. (2020a). Infectious SARS-CoV-2 in Feces of Patient with Severe COVID-19. Emerging Infect. Dis. 26. doi:10.3201/eid2608.200681.

Xiao, F., Tang, M., Zheng, X., Liu, Y., Li, X., and Shan, H. (2020b). Evidence for Gastrointestinal Infection of SARS-CoV-2. Gastroenterology 158, 1831-1833.e3. doi:10.1053/j.gastro.2020.02.055.

Xing, Y., Ni, W., Wu, Q., Li, W., Li, G., Tong, J., Song, X., and Xing, Q. (2020). Prolonged presence of SARS-CoV-2 in feces of pediatric patients during the convalescent phase. medRxiv. doi:10.1101/2020.03.11.20033159.

Xu, Y., Li, X., Zhu, B., Liang, H., Fang, C., Gong, Y., Guo, Q., Sun, X., Zhao, D., Shen, J., et al. (2020). Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. Nat. Med. 26, 502–505. doi:10.1038/s41591-020-0817-4.

Young, B. E., Ong, S. W. X., Kalimuddin, S., Low, J. G., Tan, S. Y., Loh, J., Ng, O.-T., Marimuthu, K., Ang, L. W., Mak, T. M., et al. (2020). Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore. JAMA. doi:10.1001/jama.2020.3204.

Zhai, J., Briese, T., Dai, E., Wang, X., pang, X., Du, Z., Liu, H., Wang, J., Wang, H., Guo, Z., et al. (2004). Real-time polymerase chain reaction for detecting SARS coronavirus, Beijing, 2003. Emerging Infect. Dis. 10, 300–303. doi:10.3201/eid1002.030799.

Zhang, J., Wang, S., and Xue, Y. (2020a). Fecal specimen diagnosis 2019 Novel Coronavirus-Infected Pneumonia. J. Med. Virol. doi:10.1002/jmv.25742.

Zhang, N., Gong, Y., Meng, F., Bi, Y., Yang, P., and Wang, F. (2020b). Virus shedding patterns in nasopharyngeal and fecal specimens of COVID-19 patients. medRxiv. doi:10.1101/2020.03.28.20043059.

Zhang, W., Du, R.-H., Li, B., Zheng, X.-S., Yang, X.-L., Hu, B., Wang, Y.-Y., Xiao, G.-F., Yan, B., Shi, Z.-L., et al. (2020c). Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg. Microbes Infect. 9, 386–389. doi:10.1080/22221751.2020.1729071.

Zhang, Y., Chen, C., Zhu, S., Shu, C., Wang, D., Song, J., Song, Y., Zhen, W., Feng, Z., Wu, G., et al. (2020d). Isolation of 2019-nCoV from a Stool Specimen of a Laboratory-Confirmed Case of the Coronavirus Disease 2019 (COVID-19). CCDC Weekly 2.

Zheng, S., Fan, J., Yu, F., Feng, B., Lou, B., Zou, Q., Xie, G., Lin, S., Wang, R., Yang, X., et al. (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. doi:10.1136/bmj.m1443.