Detection and survival of SARS-coronavirus 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 and mitigate risk. 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 Severe Acute Respiratory Syndrome coronavirus primary literature that presents the evidence base pertaining to the key questions of whether the SARS-CoV-1 and SARS-CoV-2 is shed in stool and urine, is recoverable, and infectious in wastewater. 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.

Introduction

The COVID-19 pandemic is a global crisis that is infecting millions with Severe Acute Respiratory Syndrome coronavirus-2 (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 SARS (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 was 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 aerosolisation of contaminated faeces, resulting in the infection and death of 42 people.

The relevance of faecal shedding of SARS-CoV 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-CoV 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?

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.

Six data extraction tables were generated from the literature focusing primarily on the number of samples examined and the number of positive samples for SARS-CoV-1 or CoV-2 in the relevant matrix, i.e., stool, urine, wastewater (Supp. Tables). Culture-based analysis of SARS-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-CoV 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 unclear about the origin of the samples making it difficult to interpret.

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 SARS-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 SARS-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) detected SARS-CoV-1 in42/94 (46%) by RT-PCR, 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).

The studies reported recovery of SARS-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 SARS-CoV-1 and SARS-CoV-2 in stool were comparable, despite the much lower sample numbers reported in the literature for SARS-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-CoV 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-CoV in urine

SARS-CoV-1. SARS-CoV-1 was detected in 42% (31/74) urine samples across n=2 patient studies. In studies that did not indicate patient origin (n=4), 22% of samples (81/367) detected SARS-CoV-1 from urine (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. SARS-CoV-2 was detected in 4% (7/179) of n=10 patient-based studies examining urine. An even lower detection rate of 0% (0/27) was reported in the n=4 sample-based studies (Table 2; Supp. Table 2). Sample numbers for SARS-CoV-2 (n=206) were half those reported for SARS-CoV-1 (n=441), with substantially lower recoveries of SARS-CoV-2 in urine than SARS-CoV-1. Studies on the detection of SARS-CoV-2 in urine tended to be smaller in size than those from the first SARS-CoV pandemic, possibly indicating an emphasis on speed to publication over study size. Despite the lower sample numbers, it is clear that SARS-CoV-2 is not as readily recovered from urine as was SARS-CoV-1. Only three studies detecting SARS-CoV-2 in urine focused on children (indicated by * in Table 2); of these three, none were able to detect SARS-CoV-2. Given the small study sizes and few studies, overall, it is not possible to conclude that children less-frequently 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-CoV 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-CoV 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 SARS-CoV-1 using RT-PCR protocols. Several of these papers were written in such a way as to make them difficult to interpret.

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 SARS-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; Farkas et al., 2020; Cahill and Morris, 2020). There are many methodological considerations to recover SARS-CoV-2 from wastewater quantitatively. 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
(Peccia et al., 2020)	36	36 (primary sludge)	
Totals (n=studies) Raw sewage = 12	116	280 (raw sewage)	CoV2+ 41.4%

Treated sewage = 3	6	62 (treated sewage)	9.7%
Sludge = 2	49	57 (sludge)	49.9%
River = 1	3	4 (river)	75%

Evidence of Infectious SARS-CoV 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 SARS-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.

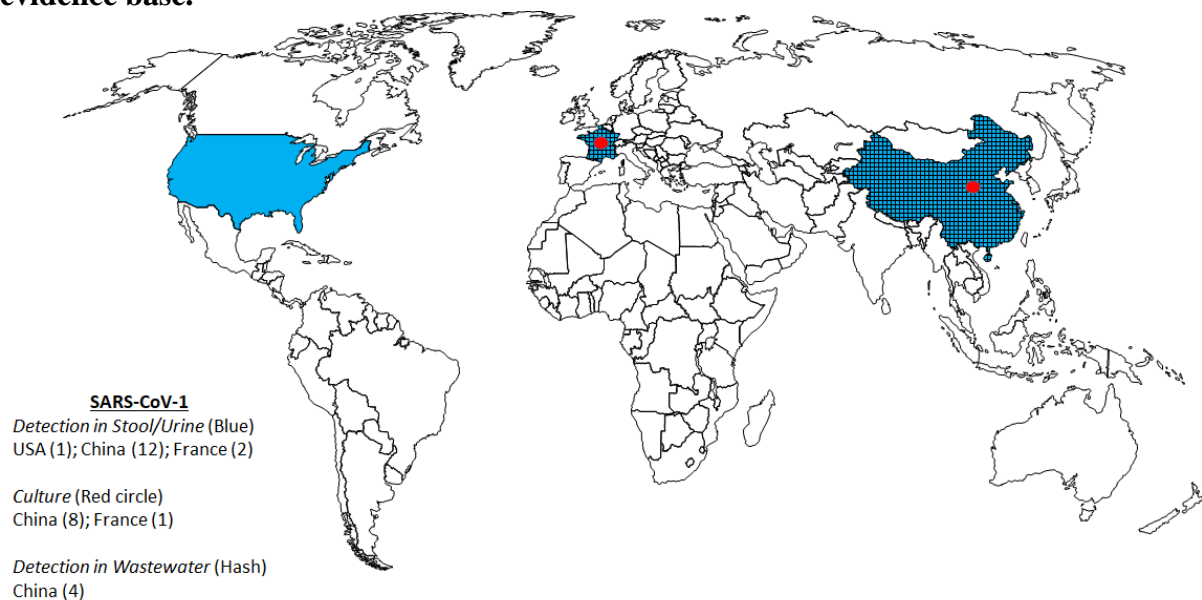
Recovery of infectious SARS-CoV-1 was relatively low, 67/210 (31.9%) as compared to recovery in stool, though comparable to recoveries from urine. Given the poor description of methods used for recovery, it is impossible to know whether infectious SARS-CoV-1 is abundant but loses infectivity during processing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

Wang et al., (2005b) spiked stool and wastewater with SARS-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 14 d at 4°C. SARS-CoV-1 remained infectious in stool stored at 20°C for 3 d and urine for 17 d. 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.

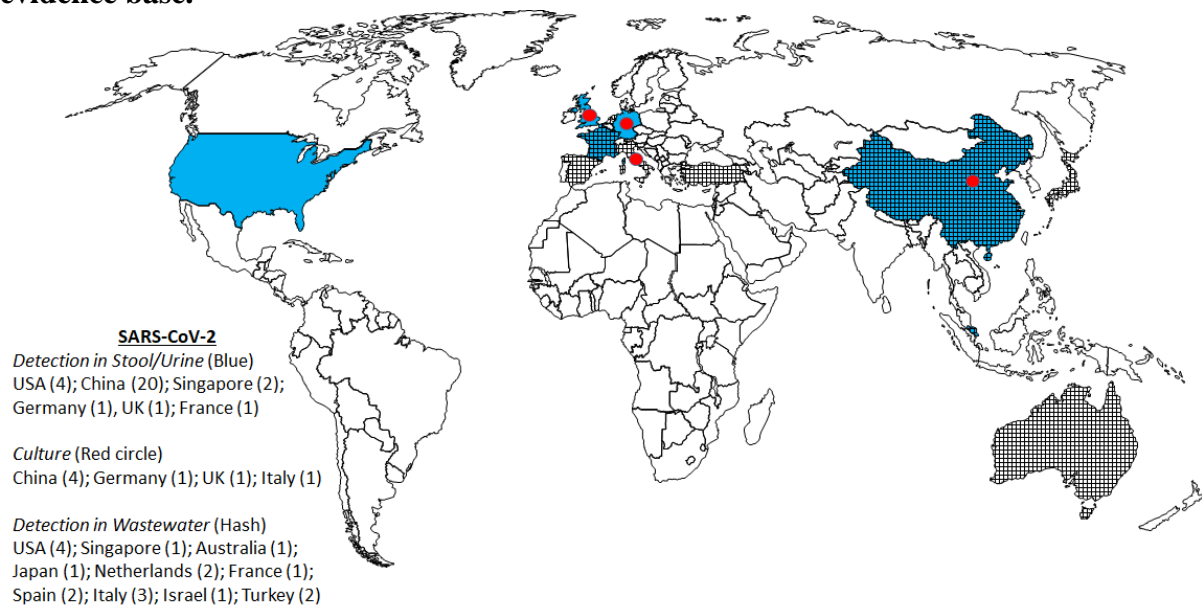
Figure 1. Geographic distribution of research teams contributing to the SARS-CoV-1 evidence base.

SARS-CoV-1
Detection in Stool/Urine (Blue)
USA (1); China (12); France (2)

Culture (Red circle)
China (8); France (1)

Detection in Wastewater (Hash)
China (4)

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 five tested positive for infectious CoV-2. Similar to the SARS-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 complicate the determination of infectious SARS-CoV-2 in wastewater. Rimoldi et al., (2020) reported on the infectivity of SARS-CoV-2 from WWTP influent and effluent, and two rivers. Of the 16 samples assayed, none of the samples were positive for infectious SARS-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 SARS-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.

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 dominated by laboratories in China (Figure 1). Of the 29 SARS-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 SARS-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 SARS-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 Preprint 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 decisions must be made despite very little scientific 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 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 SARS-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 SARS-CoV-1 papers, 51% were positive for SARS-CoV-1. A larger number of patients were assayed for SARS-CoV-2 (n=510), yielding a similar recovery of SARS-CoV-2 in stool, 51%. Urine assayed for SARS-CoV-1 in 74 patients were positive 42% of the time, while only 4% of urine from 179 patients were positive for SARS-CoV-2. When investigating child-focused studies, 26 of 31 patients were positive. In addition to the low study size, the high SARS-CoV-2 detection 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 SARS-CoV-2, where recovery was much lower than in SARS-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. Despite the majority of papers being at the preprint stage, there is strong evidence of SARS-CoV-2 detection in wastewater. Notably, none of the SARS-CoV-1 papers used RT-qPCR, while all but one publication used RT-qPCR in the SARS-CoV-2 papers.

It is observed that the scientists examining SARS-CoV in wastewater were often medical-facing labs, particularly during the SARS-CoV-1 pandemic. It is proposed that the wastewater-based epidemiology (WBE) application of such data was beyond the disciplinary scope of these teams and as such, the omission of methods that would be relevant to WBE made these papers of limited use for the abundance of WBE-focused scientists during the SARS-CoV-2 pandemic (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 on the methodology used for isolating the SARS-CoV 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 SARS-CoV-1 and SARS-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 mirrors 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 peer-review of papers during a pandemic is clearly needed to improve the quality and utility of valuable studies and more effectively 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 cut-off 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 WBE 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 need for further investigation in this area. Infectivity in faecal matter was not tested in the studies presented here. It is worth not losing sight of the fact that the data used to model and understand risk from SARS-CoV 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 SARS-CoV-2 in wastewater used 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-CoV 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-CoV 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 SARS-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 SARS-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 SARS-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.

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-CoV 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, no studies are 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 by highlighting the need for research 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.

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). 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. 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.

CONCLUSIONS

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 SARS-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) SARS-CoV-2 will be detectable in wastewater where there are sufficient numbers of active and convalescing cases.
2) SARS-CoV-2 is infectious in stool and urine and, as such, will remain infectious in wastewater for an undefined period. It is not clear from the evidence whether 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 routinely released from treated wastewater and whether any of the viral RNA that is detected comes from infectious SARS-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 also need to account for virus shedding in urine if the rates of SARS-CoV-2 shedding in urine approximates that seen in SARS-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.

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