


Absence of virological and epidemiological evidence that SARS-CoV-2 poses COVID-19 risks from environmental fecal waste, wastewater and water exposures

Mark D. Sobsey 

Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC 27599-7431, USA
E-mail: mark_sobsey@unc.edu

ABSTRACT

This review considers evidence for infectious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) presence and COVID-19 infection and illness resulting from exposure to environmental fecal wastes and waters. There is no documented evidence that (1) infectious, replication-capable SARS-CoV-2 is present in environmental fecal wastes, wastewater or water, and (2) well-documented epidemiological evidence of COVID-19 infection, illness or death has never been reported for these exposure media. COVID-19 is transmitted mainly by direct personal contact and respiratory secretions as airborne droplets and aerosols, and less so by respiratory-secreted fomites via contact (touch) exposures. While SARS-CoV-2 often infects the gastrointestinal tract of infected people, its presence as infectious, replication-capable virus in environmental fecal wastes and waters has never been documented. There is only rare and unquantified evidence of infectious, replication-capable SARS-CoV-2 in recently shed feces of COVID-19 hospital patients. The human infectivity dose–response relationship of SARS-CoV-2 is unknown, thereby making it impossible to estimate evidence-based quantitative health effects assessments by quantitative microbial risk assessment methods requiring both known exposure assessment and health effects assessment data. The World Health Organization, Water Environment Federation, US Centers for Disease Control and Prevention and others do not consider environmental fecal wastes and waters as sources of exposure to infectious SARS-CoV-2 causing COVID-19 infection and illness.

Key words: absence, COVID-19, exposure, SARS-CoV-2, wastewater, water

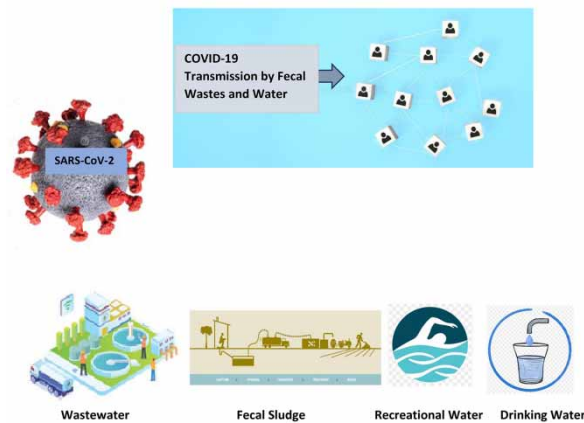
HIGHLIGHTS

- No infectious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been found in fecal wastes or waters.
- There is no epidemiological evidence of COVID-19 infection, illness or death from exposures via environmental fecal wastes or waters.
- Health risk assessments for COVID-19 by quantitative microbial risk assessment are not possible.
- There is no evidence of infectious SARS-CoV-2 in fecal wastes and waters or COVID-19 infection, illness and death attributable to such exposure media.
- Additional and coordinated efforts are recommended to further seek infectious, replication-capable SARS-CoV-2 in environmental fecal wastes and water using state-of-the-science methods.

GRAPHICAL ABSTRACT

Question:

Is infectious SARS-CoV-2 in wastewater, fecal sludge, recreational water and drinking water?



Answer: NO!

INTRODUCTION AND BACKGROUND

The purpose of this report is to identify, review and consider any documented evidence that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of the COVID-19 pandemic, is present in and poses human health risks from exposure to environmental fecal wastes (fecal sludge), wastewaters (sewage), biosolids and fecally contaminated environmental waters used for drinking, irrigation, recreation and other beneficial purposes. Based on an extensive review of the existing literature and my analysis and interpretation of it, no documented evidence exists for infectious SARS-CoV-2 presence in environmental fecal wastes and water or COVID-19 disease causation from these exposure and transmission sources. According to the World Health Organization, there are more than 415,709 scientific publications on SARS-CoV-2 and COVID-19 as of November 26, 2021 (WHO 2021a). Of those many published papers, only two reported efforts to detect infectious replication-capable SARS-CoV-2 in wastewater and water, and such viruses were not found (Rimoldi *et al.* 2020; Albert *et al.* 2021). It is likely that other laboratories, including some known to this author, have been and are continuing to examine wastewater and related fecal wastes for infectious, replication-capable SARS-CoV-2, but none so far have reported positive detection. Furthermore, there are no epidemiologically rigorous studies that attribute COVID-19 infection, illness or death to such environmental fecal waste or water exposure sources. Such key findings are presented in this report.

SARS-COV-2 VIRUS AND ITS EXPOSURE SOURCES: EXPOSURE ASSESSMENT

SARS-CoV-2 is a member of the *Coronaviridae* family and is genetically related to other coronaviruses causing respiratory illnesses in humans and as well as other animal hosts ranging from bats to mammals and birds (Ghai *et al.* 2021). SARS-CoV-2 causes the respiratory disease called COVID-19 (an acronym standing for **coronavirus disease of 2019**). The virus is similar to other coronaviruses causing human infection and illness, including the 2002–2003 severe acute respiratory syndrome (SARS) virus, the Middle East Respiratory Virus (MERS), first recognized in 2012, and several coronaviruses causing upper respiratory illnesses referred to as the common cold and first recognized in the 1960s. Other coronaviruses that cause infection and sometimes illness in livestock, companion animals and laboratory animals were recognized as early as the 1930s. There are also coronaviruses of wild animals such as bats, birds and rodents, with bats being the main reservoir host and source of viruses infecting humans and other animals. SARS-CoV-2 was first detected in humans in Wuhan, China in late 2019, but the origin of the virus and how it first got to humans remain uncertain (WHO 2021b).

Like other coronaviruses, the SARS-CoV-2 virion is about 120 nm in diameter and has an outer lipoprotein envelope (Bar-on *et al.* 2020; Yang *et al.* 2020). It is comprised of an internal nucleic acid genome of single-stranded plus-sense RNA, which is associated with an internal nucleocapsid (N) protein covered by an outermost lipid bilayer (an envelope) containing several proteins or glycoproteins, namely (1) a membrane glycoprotein, (2) a small membrane glycoprotein and (3) a

spike (S) glycoprotein that extends outward from the virus surface. The spike protein binds to host cells via the cell's angiotensin-converting enzyme 2 (ACE-2) receptor, resulting in the penetration of host cells. Virus replication then proceeds, with further spread by progressively more infection of cells in various host tissues and organs, primarily the lungs but other tissues and organs as well.

Despite the wealth of data collected and published since the beginning of the pandemic, with more than 415,709 reports by late November 2021, there is still no credible and robust scientific evidence for the presence of infectious and replication-capable SARS-CoV-2 in environmental sources of feces, wastewater, biosolids, fecal sludge or fecally contaminated waters. This assessment is supported by communications from other investigators (Jones *et al.* 2020; Haas *et al.* 2021).

For the known and better-documented routes of SARS-CoV-2 exposure and transmission by direct physical contact, respiratory droplets and aerosols, fomites, urine and possibly ocular (eye) secretions, the quality of available virological and epidemiological evidence is still considered weak (Aiello *et al.* 2020; Brönimann *et al.* 2020; Heneghan *et al.* 2021a, 2021b; Onakpoya *et al.* 2021). Nevertheless, it is clear from available virological and epidemiological evidence that sources of SARS-CoV-2 are primarily from direct personal contact or exposure to virus-containing, environmental media of respiratory origin shed from people infected with COVID-19. These exposure media are (1) airborne droplets and perhaps aerosol particles, (2) fomites containing respiratory secretions from which viruses get transferred via touching (e.g., hand contact) or another direct contact to a susceptible person who then transfers them to their mouth, nose or eye to initiate infection and less frequently, (3) urine and (4) ocular secretions. The fomite exposure route has been considered less common than the airborne particle route (Goldman 2020; CDC 2021; Chen 2021; Lewis 2021; Mondelli *et al.* 2021; Onakpoya *et al.* 2021). However, recent evidence of relatively high concentrations of infectious SARS-CoV-2 virus in and on fomites in hospitalized patient's rooms, including surfaces, door handles and knobs, used nasal tissues and cell phones in patient rooms, suggests that fomite exposure risk may be more important than previously thought (Lin *et al.* 2021).

Recently, Jefferson *et al.* (2021) proposed a 4-tier hierarchical framework of evidence for any proposed exposure and transmission route of a respiratory virus and SARS-CoV-2 in particular. Their analysis is based on systematically reviewing and synthesizing 378 primary studies for an evidence-based update of SARS-CoV-2 modes of transmission. Their analysis revealed significant methodological shortcomings in nearly all reported studies, with a lack of standardization in design, conduct, testing and reporting of SARS-CoV-2 transmission. Further details are provided below.

Most of the evidence for virus presence in clinical and environmental samples is based on detection of viral RNA by reverse transcription (RT)-polymerase chain reaction (PCR) amplification methods (RT-PCR). Few studies report the presence and concentrations of infectious, replication-capable SARS-CoV-2 in such samples. This may be due to the need for such analysis to be done in biosafety level 3 laboratories having high-level containment and other protective measures to contain infectious agents like SARS-CoV-2 that are transmitted through the air to cause potentially lethal infections.

It is important to know that the presence and concentrations of infectious SARS-CoV-2 in human clinical specimens and environmental samples are not predictable by the presence and concentrations of the viral RNA. This is because the ratios of viral RNA concentrations to infectious virus concentrations are very low and highly variable, typically only 0–1 infectious unit per 1,000 to >100,000 RNA genome copies. The high variability in this ratio depends in part on the source and type of clinical or environmental sample, the stage of infection in the human host and the duration of time the sample has been in the environment. It is also important to know that the duration and magnitude of shedding of SARS-CoV-2 RNA by COVID-19-infected people is much longer and higher than that of infectious, replication-capable virus (Widders *et al.* 2020; van Kampen *et al.* 2021). Viral RNA shedding continues typically for days to weeks after apparent cessation (based on lack of detectability) of infectious virus shedding. Also, the longer the period of time the sample is in the environment relative to when it was shed by an infected host, the greater the opportunity for virus die-off or inactivation by environmental stressors.

Based on a systematic review of the literature, of 77 reported primary studies for evidence of infectious SARS-CoV-2 presence in fecal samples from COVID-19-infected humans, little evidence was found (Heneghan *et al.* 2021b). Only six studies attempted virus culture, with only one observing viral cytopathogenic effects (CPE) in cell culture and none reporting or attempting serial viral culture positivity as confirmatory evidence. The available evidence for infectious, replication-competent SARS-CoV-2 presence in feces does not meet the highest hierarchical level of evidence proposed by Jefferson *et al.* (2021) to document fecal presence as an important exposure source of SARS-CoV-2.

Infectious virus concentrations in the few reported virus-positive stool (fecal) samples in the literature have not been reported or quantified (Zapor 2020; Heneghan *et al.* 2021b; Jefferson *et al.* 2021). A systematic review by Heneghan *et al.* (2021b) found only 6 of 77 primary studies that attempted to culture SARS-CoV-2 in feces (Jeong *et al.* 2020; Kim *et al.*

2020; Wang *et al.* 2020; Wölfel *et al.* 2020; Xiao *et al.* 2020; Zhang *et al.* 2020a, 2020b). Of these studies, only three reported evidence of SARS-CoV-2 by cell culture infectivity in some but not all fecal samples tested; the other three studies detected no infectious virus by cell culture. Only one study observed viral CPE in cell culture, and none reported or attempted serial viral culture positivity as confirmatory evidence.

To further elaborate on these reviewed primary studies, Wang *et al.* (2020) reported fecal sample positivity for SARS-CoV-2 infectivity in cell culture in two out of four samples, with visual confirmation only by electron microscopy. In the report by Xiao *et al.* (2020), the stool of a single COVID-19 patient was cell culture-positive, with virus presence further supported by visible cytopathic effects after second-round cell culture passage. Cell culture supernatant contained coronavirus-like particles by electron microscopy as well as full-length SARS-CoV-2 RNA detected by sequencing. In the same study, fecal samples from two additional patients were also positive for infectious virus by cell culture analysis. Zhang *et al.* (2020a) reported that stools of only one patient from among an unreported number of patients was positive for infectious virus. The presence of SARS-CoV-2 was further confirmed by electron microscopic observation of coronavirus particles and by full length genome sequencing of viral RNA. Although Jeong *et al.* (2020) were unable to directly infect cell cultures with a fecal sample, they successfully infected ferrets by nasal inoculation, which resulted in signs and symptoms of illness, as well as evidence of virus infection in nasal washes collected 2–6 days post-infection based on increasing viral RNA concentrations and then successful cell culture detection of SARS-CoV-2 from these nasal washes.

The rarity of detecting infectious, replication-capable SARS-CoV-2 despite high levels of viral RNA in shed feces has a plausible explanation, which is the rapid inactivation of virus infectivity in the colon (lower intestines). Zang *et al.* (2020) observed SARS-CoV-2 replication in the small intestine of humans. However, they also reported using a recombinant SARS-CoV-2 mNeonGreen reporter virus, to document experimentally that most of the viruses (99%) are inactivated in 1 h by simulated colonic fluid. This evidence helps to explain the low frequency of infectious, replication-capable SARS-CoV-2 detection in fecal samples.

Other studies for the presence of SARS-COV-2 in feces of COVID-19-infected people also lack evidence for infectious, replication-capable virus. Two such studies report histological and immunofluorescence evidence of SARS-CoV-2 virus antigen presence in biopsied intestinal tissues. However, no confirmation of the presence of infectious, replication-competent virus in cell culture was done (Qian *et al.* 2020; Xiao *et al.* 2020). Xiao *et al.* (2020) reported evidence of SARS-CoV-2 replication in intestinal tissue only by histologic and immunofluorescent staining, using SARS-CoV-2 nucleoprotein antibodies for detection by microscopic analyses. Qian *et al.* (2020) reported evidence of SARS-CoV-2 in rectal tissues by positivity for SARS-CoV-2 RNA using RT-PCR. They also reported the apparent observation of coronavirus particles in rectal tissues by electron microscopy and the presence of SARS-CoV-2 nucleoprotein antigen in rectal tissue specimens using immunofluorescence microscopy.

Despite the rare and unquantified presence of infectious, replication-capable SARS-CoV-2 in fecal wastes of people infected with COVID-19, the possibility of such virus presence has generated interest and concern by some for its presence in environmental fecal wastes, such as wastewater, biosolids, fecal sludge, and environmental waters and wastes used beneficially by humans. The focus has been on possible virus presence in drinking, recreational and irrigation waters and in wastewater and fecal solids used in food crop agriculture.

Interest in the potential presence of infectious SAR-CoV-2 in environmental waters and fecal wastes has been motivated by the documented presence of SARS-CoV-2 RNA in feces, sewage, biosolids, latrine samples and fecally contaminated waters. This is because COVID-19 infections in community members result in viral RNA presence in shed bodily wastes, such as saliva, sputum, feces, urine and ocular discharges of the population that occur in community wastewaters and fecal sludges. This finding has led to several reports speculating on fecal-associated and water-related environmental exposures to infectious, replication-capable SARS-CoV-2 (Kang *et al.* 2020; Kitajima *et al.* 2020; La Rosa *et al.* 2020; Abdelodun *et al.* 2021; Del Brutto *et al.* 2021; Giacobbo *et al.* 2021; Gwenzi 2021; Mohan *et al.* 2021; Thakur *et al.* 2021; Tran *et al.* 2020). However, no infectious, replication-capable SARS-CoV-2 has yet to be reported, quantified and further confirmed from such environmental exposure sources.

Although rare and unlikely, it is important to recognize that if infectious, replication SARS-COV-2 was shed fecally or was otherwise present in shed human wastes, it would be expected to decline relatively rapidly and more rapidly than viral RNA levels in the environment (Bivins *et al.* 2020b) This is because wastewater, biosolids and fecal sludge treatment processes, and various environmental antagonists, including elevated temperatures, sunlight, chemical antagonists such as surfactants, metals, enzymes (proteases, nucleases amylases and lipases) and disinfectants, as well as microbial predation, will all

contribute to relatively rapid loss of infectivity of infectious SARS-CoV-2 virions released into the aquatic environment (Bosch *et al.* 2006; Pinon & Vialette 2018; Paul *et al.* 2021). Indeed, enveloped viruses such as SARS-CoV-2 are considered the least resistant of all microbes, compared to non-enveloped viruses, vegetative bacteria, protozoan parasites, mycotic agents and bacteria spores. The infectivity of these viruses would decline relatively rapidly (Brisolara *et al.* 2021; Maal-Bared *et al.* 2021). Antiviral environmental stressors are a likely contributing reason why all reported efforts to detect and quantify infectious, replication-capable SARS-CoV-2 in fecal wastes and water have been unsuccessful (Huraimel *et al.* 2020; Rimoldi *et al.* 2020; Tran *et al.* 2020; Gwenzi 2021; Kumar *et al.* 2021; Westhaus *et al.* 2021). Because such evidence of virus inactivation has been well documented in previous reviews, it will not be covered in further detail here (Maal-Barad *et al.* 2021; Brisolara *et al.* 2021; Nassri *et al.* 2021; Chen *et al.* 2021a).

COVID-19 INFECTION, DISEASE AND HEALTH RISKS: HEALTH EFFECTS ASSESSMENT

COVID-19 infection and disease occur mainly in the respiratory tract, with abundant virus presence in cells and tissues of the lungs, trachea, nose, throat and mouth, often resulting in cellular and tissue damage and inflammation. The disease occurs in both the upper and lower respiratory tract, with pneumonia in the lower respiratory tract as the more severe health effect. The adverse effects of pneumonia are greater in more susceptible hosts, such as immune-deficient people and those with other existing health conditions (e.g., obesity, heart disease and chronic lung disease) (Bar-on *et al.* 2020; Yang *et al.* 2020). The virus can also be present in other organs and tissues, including the intestinal tract (the gut), liver, gallbladder and pancreas, the eye, the genito-urinary system and skin. The respiratory tract is the main site of infection and illness and the major source of virus release in saliva and mucus that becomes airborne as droplets and smaller particles (aerosols). Such released virus then results in virus exposures to others to cause further transmission by infection of other susceptible human hosts.

COVID-19 infection can produce human disease of differing severity (Rod *et al.* 2020). It is also noteworthy that some COVID-19-infected people, between 4 and 94% depending on geographic location and population demographics (e.g., age and preexisting health conditions), remain asymptomatic but still shed the virus and its RNA as a source of virus exposure to others (Heneghan *et al.* 2020; Lauer *et al.* 2020; Lee *et al.* 2020; WHO 2020a; Gao *et al.* 2021; Tan *et al.* 2021; Wilmes *et al.* 2021). For asymptomatic COVID-19 patients that are positive for SARS-CoV-2 by RNA analysis, the time period to when some developed symptoms of illness averaged 15 days in one reported study (Lee *et al.* 2020). Other studies report typical presymptomatic durations of only few days, but up to 14 days in some cases (Lauer *et al.* 2020; WHO 2020a; Tan *et al.* 2021). In the study by Lee *et al.* 2020, both symptomatic and asymptomatic patients had comparable RNA levels in respiratory specimens. Based on viral RNA, viral loads of asymptomatic patients decreased more slowly than those of symptomatic (including presymptomatic) patients. In the same study, viral RNA Ct values from upper and lower respiratory tract specimens of asymptomatic and symptomatic (including presymptomatic) patients were not different. However, some recent studies indicate lower risks of transmission by asymptomatic than by symptomatic patients (Miller 2021; Widders *et al.* 2020). Yet other evidence suggests that asymptomatic cases pose high risks of transmission because both they and others who are susceptible to infection are unaware that they are contagious, thus unwittingly resulting in more risky interactions between them. Overall, the extent to which symptomatic and asymptomatic people with COVID-19 contribute to further transmission to other people still remains uncertain.

COVID-19 disease can range from mild to moderate illness resembling seasonal influenza and other common respiratory illnesses (a 'cold'). However, when the disease progresses to more serious illness as severe pneumonia, it requires urgent medical care and often can be life-threatening. Those with pneumonia have difficulty breathing, chest pain, shortness of breath, hemodynamic instability and sometimes loss of taste or smell, nausea or vomiting and diarrhea; they typically require oxygenation or mechanical ventilation therapy to survive.

Virus and viral nucleic acid (RNA) shed in respiratory releases of COVID-19-infected people by actions, such as coughing, sneezing, spitting, talking and singing, begins a few days (typically 2–5 days) after initial infection. Shed viral RNA can be detected for up to 2 months post-infection and sometimes even longer in some high-risk COVID-19 hosts (Zapor 2020). However, infectious virus is detectable in respiratory secretions only for up to 1.5–2 weeks post-infection (van Kampen *et al.* 2021). As was previously noted above, viruses released from the respiratory tract can also be deposited on surfaces or other fomites, such as tissues, tables, doorknobs and handles, cell phones and outer garments. These deposited viruses can potentially be transferred to others who touch or otherwise come in contact with such materials and surfaces and then transfer the viruses to their mouth, nose or eyes to initiate infection at these sites.

COVID-19 infections in many (from 30 to 60%) but not all infected people also occur extensively in the gastrointestinal tract, sometimes producing diarrhea and related enteric symptoms. The duration and magnitude of virus RNA shedding from the gut and presence in feces is often somewhat longer than in respiratory secretions. Only rarely (in about 5% of infections) is the virus and its RNA found in urine, as another but less common source of virus shedding (Jones *et al.* 2020; Zapor 2020). The estimated concentrations of viral RNA as gene copies (GC) per ml are about 10^5 – 10^{11} in respiratory fluids, 10^2 – 10^7 in feces and 10^2 – 10^5 in urine. These data further document respiratory secretions as the major transmission source of virus shed from infected people (Jones *et al.* 2020; Zapor 2020); Chen *et al.* 2021a.

SARS-CoV-2 TRANSMISSION, EPIDEMIOLOGY AND HUMAN INFECTIVITY FOR RISK CHARACTERIZATION

Based on the extensive clinical and epidemiological evidence gathered so far, COVID-19 transmission can be described by the established process known as the chain of infection and its six elements (Ahmad *et al.* 2020). The first element is the infectious agent, SARS-CoV-2, which causes the disease COVID-19. The second element is the reservoir or the place where the infectious agent, SARS-CoV-2, is present, which is primarily COVID-19-infected human hosts. The third element is how the agent exits the infected COVID-19 host, which occurs primarily through the mouth and nose when a person coughs or sneezes to release SARS-CoV-2 in respiratory secretions. The fourth element is the mode of transmission, or how SARS-CoV-2 gets from one person to another. For those with COVID-19 infection or disease, SARS-CoV-2 release is as respiratory secretions from direct contact (e.g., kissing), or as airborne particles (coughs, sneezes, etc.), or by indirect contact such as deposition on fomites that another person can touch to acquire the virus. The fifth element in the chain of COVID-19 infection is the portal of entry, or where SARS-CoV-2 enters another person's body. This is essentially the same as the portal of exit. Virus entry into susceptible human hosts is via the mouth, nose or eye and is the sixth element in the chain. The clinical and epidemiological evidence in support of this infection chain for COVID-19 transmission is well documented for the respiratory transmission scenario. However, there is no conclusive virological or epidemiological evidence documenting other transmission routes or pathways for SARS-CoV-2 virus transmission that result in COVID-19 infection or disease, such as from environmental fecal wasters and waters.

Like other viruses as well as other pathogens, the ability of a person infected with SARS-CoV-2 to transmit the virus to others is quantified as the number of people they successfully infect with COVID-19. This is referred to as its reproduction number or R_0 value. The estimated R_0 for COVID-19 ranges from 1.9 to 6.5 based on 20 reported studies, with 13 of these studies in the range of 2–3 (Spencer *et al.* 2020). This R_0 value is typical of many respiratory viruses, except for the measles virus, for which the R_0 is estimated to be >10 . It is noteworthy that the R_0 value of SARS-CoV-2 differs among the several known variants of the virus. The recent so-called Delta variant, which has become prevalent globally, has a higher R_0 value and observed greater transmissibility than earlier variants (Liu & Rocklöv 2021).

The transmission of COVID-19 through direct human-to-human contact is most commonly reported among health-care employees and primary caregivers of diseased patients who must come in direct contact with them. However, the human transmission of COVID-19 is also considered to occur from sources such as respiratory secretions released as airborne droplets and smaller airborne particles and possibly from contact with fomites that results in observed clusters or outbreaks of COVID-19 among people in other settings. These settings include households, nursing homes, colleges and schools, crowded bars and restaurants, and other commercial and public settings such as food production facilities and retail commercial enterprises. Such indoor locations can have large numbers of people in poorly ventilated and often crowded spaces (Chen *et al.* 2021b).

To reduce COVID-19 transmission risks, public health agencies and other concerned stakeholders invoke and promote public health and social measures of prevention and control. These measures include: (1) keeping physical distance from other people (usually 1–2 m), (2) encouraging frequent hand hygiene, (3) rapidly identifying people with COVID-19 and encouraging or requiring their isolation, (4) encouraging or requiring timely quarantining (confinement) of people who have been in recent contact with others who have COVID-19, infection or illness, (5) encouraging or requiring the wearing of face masks or respirators by both those with COVID-19 infection or illness (source control) and others who may become exposed to them (susceptibles) and (6) disinfecting various surfaces and objects to inactivate any SARS-CoV-2 on them (Ahmad *et al.* 2020). In some communities and countries, preventing or reducing COVID-19 transmission is further addressed by encouraging or requiring people to stay home and not venture out, except for essential reasons (e.g., buying

groceries); this is referred to as 'lockdown'. Some countries restrict the entrance of people from other countries having high levels of COVID-19 disease.

COVID-19 transmission by environmental fecal wastes and waters has been raised as a possibility, and there is no credible virological or epidemiological evidence documenting this transmission source or chain of infection. The World Health Organization and other health agencies such as the US Centers for Disease Control and Prevention (CDC) have developed formal and rigorous categorical criteria to determine if an exposure medium such as water or wastewater is a plausible and documentable cause of infectious disease transmission by a pathogen considered wastewater- and water-borne (Tillett *et al.* 1998; Eisenberg *et al.* 2001; Bartram & Hunter 2015; CDC 2019). The quality or strength of evidence is based on documenting the presence of the pathogen in the exposure medium at the time of human exposure using culture-based and molecular methods and then temporally linking the estimated exposure to ill people who ingested such contaminated water or fecal wastes. The volume of water ingested is also a key criterion to establish and quantify risk, because the more water consumed the higher the potential risk of becoming infected and ill (i.e., a dose-response relationship).

While there are no virological or epidemiological data documenting infectious, replication-capable SARS-CoV-2 presence or epidemiologically documented COVID-19 infection and disease attributable water- and fecal waste-related exposures and transmission routes, there is one reported COVID-19 disease incident tentatively and only weakly linked to fecal matter. A cluster of nine COVID-19 cases among three families was speculated, based on only circumstantial evidence, to have been caused by airborne exposure to fecal droplets from plumbing in a high-rise building in Guangzhou, China (Kang *et al.* 2020). However, no infectious, replication-capable virus was found in this fecal waste or in environmental fomite samples, although some environmental samples in bathrooms were positive for viral RNA. Furthermore, other possible routes of exposure for transmission such as fomites could not be ruled out. This report is similar to an earlier outbreak of another coronavirus disease, SARS, in Hong Kong in 2004. That outbreak was tentatively attributed to entrained fecal droplets as the airborne exposure medium for SARS disease transmission in a high-rise apartment complex (Yu *et al.* 2004). However, the epidemiological evidence was only circumstantial, and no infectious, replication-capable virus or viral RNA was looked for in the suspected human fecal waste source (faulty toilets) or in other environmental samples.

The extent to which infectious, replication-capable SARS-CoV-2 would pose a risk of human infection from environmental exposures, such as by ingestion of fecally contaminated drinking or recreational water or wastewater-irrigated produce, is also dependent on the magnitude and duration of exposure. That is, an environmental exposure would have to be high enough and long enough for there to be a high probability of resulting in infection in a susceptible human host. A sufficient number or quantity of infectious, replication-capable SARS-CoV-2 would need to enter a target site in the body, such as the mouth, nose, throat or intestinal tract, to cause infection. It is also noteworthy that, to this author's knowledge, no documented epidemiological evidence of infection, illness or death has been reported for people who are occupationally exposed regularly to environmental fecal wastes, such as those in contact with wastewater and biosolids at wastewater treatment and management facilities.

The presence of readily detectable SARS-CoV-2 RNA in wastewater by RT-PCR has led to the development and implementation of monitoring and surveillance efforts for COVID-19 based on SARS-CoV-2 RNA detection in wastewater samples. Such surveillance is becoming increasingly used as an alert and tracking system for the presence of COVID-19 infections in communities and more specific settings, such as college campuses, hospitals and other workplaces (Bivins *et al.* 2020a; Hill *et al.* 2021; Lundy *et al.* 2021; Mackul'ak *et al.* 2021; Panchal *et al.* 2021). Such wastewater-based epidemiology (WBE) has become a complementary approach to inform public health systems, programs and resources of SARS-CoV-2 presence. This wastewater-based epidemiological approach complements conventional public health surveillance based on identifying COVID-19-infected people by testing individual community members for evidence of infection. Such community-based testing typically uses RT-PCR analysis of nasopharyngeal clinical specimens for the presence of SARS-CoV-2 RNA genome targets to identify COVID-19-infected individuals. In some countries, regions and communities, WBE has become a supporting approach to (1) monitor and track the magnitude of presence of COVID-19 infections in communities and other specific locations, (2) identify 'hotspots' of high infection prevalence in geographic areas and their populations that require more effective COVID-19 vaccination coverage and (3) confirm virus absence, presence and magnitude of presence in specific locations (Bivins *et al.* 2020a; Fuschi *et al.* 2021; Smith *et al.* 2021).

It is also noteworthy that reported concentrations of SARS-CoV-2 RNA in wastewater samples are typically relatively low based on estimated genome copies or CT values, compared to their concentrations in saliva, sputum and fresh feces of infected humans. Hence, the typical concentrations of SARS-CoV-2 RNA in wastewaters and fecally contaminated waters

are usually much too low and variable to be indicative of the presence and concentrations of infectious and replication-capable SARS-CoV-2.

An important data gap in estimating the potential transmission risks of COVID-19 infection, illness and death from exposure to environmental fecal, wastewater and water contamination is the absence of a documented human infectivity dose–response relationship for infectious, replication-capable SARS-CoV-2. There are no reported studies in which human volunteers were dosed with different known quantities of infectious SARS-CoV-2 by a specific exposure route to develop dose–response relationships (Karimzadeh *et al.* 2021). In addition, there are no documented epidemiological data for COVID-19 infection, illness or death from human exposure to environmental fecal wastes or waters in which infectious, replication-capable was detected and quantified.

There are limited and poorly quantified data on the infectivity of SARS-CoV-2 in experimental animal models that are susceptible to infection, such as ferrets, Syrian hamsters, minks and genetically modified mice (Karimzadeh *et al.* 2021). However, these few animal studies rarely used a range of infectious SARS-CoV-2-graded doses, most doses administered were very high and an insufficient number of replicate animals were challenged per dose to develop reliable quantitative dose–response relationships. The one exception is the study by Rosenke *et al.* (2020) who challenged Syrian hamsters with infectious SARS-CoV-2 at graded doses from 1 to 100,000 TCID₅₀. They estimated the 50% infectious dose of SARS-CoV-2 to be about five infectious virions. However, the infectivity dose–response data were not presented in the published paper, and therefore, it is impossible to know how this dose–response relationship result was determined and if it is accurate. Furthermore, whether such animal infectivity dose–response data can be assumed to represent human infectivity dose–response relationships is unknown, highly uncertain and cannot be verified directly until human infectivity dose–response studies are done. It is noteworthy that a human volunteer dose–response study was approved and recently began in the United Kingdom (Akst 2021).

Despite the absence of quantitative human infectivity dose–response data for SARS-CoV-2, there have been unverifiable assumptions about what the infectivity dose–response relationship might be (Karimzadeh *et al.* 2021). Such attempts have been based on the use of human infectivity dose–response data for other viruses in quantitative microbial risk assessments (QMRA) (Zaneti *et al.* 2020; Karimzadeh *et al.* 2021). Such an analysis was done to quantify the risks from a sewage drainage system in multi-unit apartment buildings as a transmission route of SARS-CoV-2 (Shi *et al.* 2020). Such estimated COVID-19 infection risks not based on human infectivity dose–response data for infectious SARS-CoV-2 have been based on either (1) concentrations of viral RNA and unverifiable assumptions of the ratio RNA GC to infectious viruses (Kumar *et al.* 2021) or (2) they have been based on the use of human infectivity dose–response relationships for other viruses such as SARS (Zaneti *et al.* 2020). Therefore, the validity of these QMRAs as being representative of COVID-19 infection risks from infectious SARS-CoV-2 is uncertain and highly questionable (Haas *et al.* 2021).

In summary, there is an absence of documented evidence for the presence and concentrations of infectious, replication-capable SARS-CoV-2 in environmental fecal wastes and waters. There is also an absence of documented epidemiological evidence that environmental fecal wastes and waters have ever caused human cases, clusters or outbreaks of COVID-19 infection, illness or death. For these reasons, there is no basis for establishing a chain of infection or providing evidence of human health risk for COVID-19 transmission from environmental fecal waste and water exposures. To address this absence of evidence, greater and better-coordinated efforts are recommended to (1) determine if infectious, replication-capable SARS-CoV-2 is present in environmental fecal wastes and waters and at what concentrations using state-of-the-science detection methods and (2) improve and increase rigorous epidemiological efforts to determine if COVID-19 infection, illness and death can be attributed to such environmental fecal waste and water exposures.

CONCLUSIONS

Based on the information reviewed and analyzed in this study as well as reviews and analyses by expert authorities such as the World Health Organization, the US Centers for Disease Control and Prevention, Water Environment Federation and their many expert scientific and technical staff and advisers, as well as recent reviews by others, there is no credible evidence for the presence of infectious, replication-capable SARS-COV-2 in fecal wastes and waters such as those used for recreation, agricultural irrigation or drinking. Furthermore, there is no documented epidemiological evidence of human infections, clusters of cases or outbreaks due to such environmental exposure sources of fecal wastes and waters (CDC 2020; WHO 2020b; WEF 2021). These conclusions are also supported by other investigators, such as Jones *et al.* (2020), who stated that ‘... the

likelihood of infection due to contact with sewage-contaminated water (e.g., swimming, surfing, angling) or food (e.g., salads, shellfish) is extremely low or negligible based on very low predicted abundances and limited environmental survival of SARS-CoV-2, and that ‘... exposure to feces or wastewater has never been implicated as a transmission vector’. Overall, it is concluded that there is no documented evidence for the presence of infectious, replication-capable SARS-CoV-2 in fecally related environmental media or of epidemiologically documented COVID-19 human health risks from environmental water and waste exposures or that COVID-19 disease has resulted from such exposure.

Greater and better-coordinated investigation is recommended to determine if infectious, replication-capable SARS-CoV-2 is present in environmental fecal wastes and waters by the use of state-of-the-science methods for recovery, detection and quantitative analysis and to determine if such presence results in epidemiologically documented COVID-19 infection, illness or death.

DATA AVAILABILITY STATEMENT

All relevant data are available from an online repository or repositories.

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