



Review

Occurrence of various viruses and recent evidence of SARS-CoV-2 in wastewater systems

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ABSTRACT

Viruses are omnipresent and persistent in wastewater, which poses a risk to human health. In this review, we summarise the different qualitative and quantitative methods for virus analysis in wastewater and systematically discuss the spatial distribution and temporal patterns of various viruses (i.e., enteric viruses, Caliciviridae (Noroviruses (NoVs)), Picornaviridae (Enteroviruses (EVs)), Hepatitis A virus (HAV)), and Adenoviridae (Adenoviruses (AdVs))) in wastewater systems. Then we critically review recent SARS-CoV-2 studies to understand the ongoing COVID-19 pandemic through wastewater surveillance. SARS-CoV-2 genetic material has been detected in wastewater from France, the Netherlands, Australia, Italy, Japan, Spain, Turkey, India, Pakistan, China, and the USA. We then discuss the utility of wastewater-based epidemiology (WBE) to estimate the occurrence, distribution, and genetic diversity of these viruses and generate human health risk assessment. Finally, we not only promote the prevention of viral infectious disease transmission through wastewater but also highlight the potential use of WBE as an early warning system for public health assessment.

1. Introduction

Viruses are omnipresent and persistent in raw and treated wastewater, which is a concern because they can pose risks to human health (Corpus et al., 2020). A significant source of viruses in wastewater is human faecal matter, mainly from known infected persons with or without indications (Haramoto et al., 2018). Water-borne viruses, such as Noroviruses (NoVs), enteroviruses (EVs), Hepatitis A virus (HAV), and Adenoviruses (AdVs), are widely distributed in wastewater (La Rosa et al., 2020). Another example, SARS-CoV-2, is responsible for the COVID-19 pandemic that is currently affecting the world (Casanova et al., 2009; Peng et al., 2020). SARS-CoV-2 has also been detected in the faeces of infected patients (Holshue et al., 2020) and in wastewater (Holshue et al., 2020; Mallapaty, 2020). SARS-CoV-2 RNA is shed in

body fluids, such as faeces, saliva, and sputum, and these fluids are often disposed of via wastewater systems (Holshue et al., 2020; Mallapaty, 2020). To date, studies have identified SARS-CoV-2 in the wastewater systems of different countries, including Australia (Ahmed et al., 2020), France (Wurtzer et al., 2020), Italy (La Rosa et al., 2020), the Netherlands (Medema et al., 2020), Spain (Randazzo et al., 2020), the Czech Republic (Mlejnkova et al., 2020), Japan (Haramoto et al., 2020), Turkey (Kocamemi et al., 2020), Israel (Or et al., 2020), India (Kumar et al., 2020), Pakistan, (Sharif; Yaqub et al., 2020), China (Chen et al., 2020; Zhang et al., 2020) and the USA (Wu et al., 2020). Based on these studies, clear evidence of the presence of SARS-CoV-2 in sewage is available, and this transmission pathway increases the risk of human exposure to the virus.

To prevent wastewater transmission and decrease the threat to

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human health, there is a need to better understand the critical role of wastewater as a potential source of viruses, including SARS-CoV-2. In the current review, we first briefly introduce multiple human viruses, including HAV, EVs, NoVs, AVs, AdVs, and SARS-CoV-2, whose RNA has been detected in wastewater in recent studies. Moreover, methods for qualitative and quantitative analysis of these viruses are also discussed. We summarise the main human viruses found in wastewater and demonstrate the spatial and temporal distribution of viruses in the wastewater of various countries. We further highlight the key future approaches required to strengthen our knowledge and understanding of the existence, persistence, and possible human health risks associated with the presence of EV, NoV, AV, AdV, and SARS-CoV-2 RNA in wastewater systems. We also describe the presence of SARS-CoV-2 in wastewater and critically discuss current knowledge regarding wastewater surveillance to develop a better understanding of the epidemiology of several human viruses, including SARS-CoV-2, which causes COVID-19. The occurrence of SARS-CoV-2 in wastewater suggests wastewater analysis as a potential tool for investigating the invasion, occurrence, molecular epidemiology, and possible eradication of human viruses in human populations. Finally, our findings indicate that in addition to providing information about the transmission of infectious diseases through wastewater, wastewater analyses can also provide information that could be used to monitor viral circulation in communities and serve as a warning of potential outbreaks of contagious diseases. The abbreviations used in the current review are presented in Table S1.

2. Viruses: genomes, classification, and infection symptoms

In this section, we introduce various human viruses, such as EVs, NoVs, AVs, AdVs, and SARS-CoV-2, and detailed information about their genomes, classification, and health symptoms are listed in Table S2. Human enteric viruses are significant causes of severe acute water-borne diseases in both developed and developing countries (Prevost et al., 2015). Due to their long-term persistence in environmental water systems and their strong resistance to decontamination rather than disinfection, these enteric viruses can cause severe illness. Most respiratory viruses appear to be as infective in humans as in tissue culture. Doses < 1 TCID₅₀ of influenza virus, AdVs, and rhinovirus were reported to infect 50% of the tested population (Yezli and Otter, 2011). Likewise, low doses of the enteric viruses, HAV, NoV, poliovirus rotavirus, and echovirus, also caused infection in at least some of the volunteers tested (Yezli and Otter, 2011).

Human diseases caused by enteric viruses are frequently asymptomatic or paucisymptomatic; nonetheless, they can also induce numerous respiratory, intestinal, and conjunctival symptoms or hepatic infection (Kitajima et al., 2020). Human enteric viruses pose a risk of severe disease and mortality in high-risk populations, including children, older adults, and immunocompromised patients (Pavlinac et al., 2014). Enteric viruses are mainly produced inside human host cells and are substantially transmitted through the faecal-oral route (Bhunja, 2018).

The most crucial characteristic of enteric viruses is the ease with which they can be transferred from person to person; they can cause disease at low transfer doses of less than 20 particles (Warriner, 2005). Human enteric viruses are more stably resistant to environmental alterations than bacteriological endospores (Warriner, 2005). These human enteric viruses multiply inside the digestive tracts of their hosts, who then excrete them in faeces at significant quantities of up to 10¹¹ viral particles/g stool for periods ranging from days to months. Accordingly, wastewater is likely to contain a considerable abundance of enteric viruses (Prevost et al., 2015).

These effluents are generally treated at wastewater treatment plants (WWTPs) that are not explicitly designed to remove human enteric viruses (Prado et al., 2011). Generally, treated wastewater from WWTP sewage flows directly in a riverine system that is used for diverse purposes, such as shellfish farming, agriculture, recreation, market

gardening, and catchment to recharge the groundwater system and produce drinking water (Prevost et al., 2015). There are four major groups of human gastroenteritis viruses: AsVs, AdVs, Rotaviruses (RoVs), and calicivirus, which includes NoV and SaV. These viruses can be transmitted not only through the faecal-oral route but also by consumption of contaminated water and food.

AsVs are the main source of human acute gastroenteritis worldwide (Bosch et al., 2014). These non-enveloped viruses have a positive-sense single-stranded RNA, and their genomes consist primarily of open reading frames (ORFs and ORF2) that encode the capsid protein predecessor; this permits discrimination of eight different AsV genotypes, AsV-1 to AsV-8 (Prevost et al., 2015). The eight distinct serotypes of AsVs can infect individuals of all ages, including elderly people, adults, and young children; after a 3–4-day incubation period, they cause mild gastroenteritis and a wide variety of other symptoms, such as nausea, dehydration, vomiting, and diarrhoea. A considerable number of AsV viral particles are eliminated in the faeces of infected persons and circulate in wastewater systems (Prevost et al., 2015). These viruses have been found in wastewater in France (Kaas et al., 2019), Uruguay (Lizasoain et al., 2018), Spain (López-Gálvez et al., 2018), Canada (Qiu et al., 2016), Japan (Kobayashi et al., 2017), New Zealand (Gyawali et al., 2018), Egypt (Shaheen and Elmahdy, 2019), and India (Prevost et al., 2015).

AdVs with a high occurrence in water systems have been recommended as index organisms for viral pathogens since they fit most of the requirements of an ideal indicator (Kundu et al., 2013). Several studies have estimated that more than 90% of the human population worldwide is seropositive for one or more of the AdV serotypes. Human AdVs occur at significantly higher frequencies in sewage systems than other enteric viruses, and they are excreted by infected persons at higher concentrations of up to 10¹¹ particles/g stool (Fong et al., 2010).

Outbreaks of human AdV infections primarily occur in day-care centres, hospitals, swimming pools, military quarters, and similar facilities. Although more than 51 human AdVs have been identified, only one-third of the AdV types cause human infections. These human AdV serotypes are well defined and represent six species (A-F) within the AdV genus of the family Adenoviridae.

Several AdV serotypes have been reported to have significant clinical consequences in terms of specific infections; for example, serotypes 40 and 41 of type F have been shown to be present in most cases of AdV-linked gastroenteritis in children, whereas serotypes 1, 2, and 5 of variety C cause childhood respiratory infections (Kuo et al., 2010).

In addition to replication in the respiratory tract, replication in the urinary bladder has been observed. These viruses are spread via the faecal-oral route. AdVs are among the most common viruses in untreated sewage, and their concentrations can be ten times greater than those of EVs. AdVs have been widely found in wastewater in France (Kaas et al., 2019), Uruguay (Lizasoain et al., 2018), Tunisia (Ibrahim et al., 2018), the USA (Gerba et al., 2018; Jahne et al., 2020), Brazil (Assis et al., 2018), Canada (Qiu et al., 2018), Greece (Kaliakatsos et al., 2019), and Japan (Tandukar et al., 2020).

EVs are transported in the environment to a significant degree via groundwater, seawater, river aerosols typically released from WWTPs, estuarine water, inadequately treated water, private wells, and drinking water that directly or indirectly receives either treated or untreated wastewater (Tiwari and Dhole, 2018). These EVs are frequently transmitted through the faecal-oral route and infect and replicate inside the intestinal tract of the infected host to a significant degree (Van Der Sanden et al., 2018).

The EVs include poliovirus, coxsackievirus (CB) groups A and B, and echoviruses (E) (Tseng et al., 2007). To date, more than 100 enterovirus serotypes have been identified, including more than 70 serotypes that have been discovered in humans (Tiwari and Dhole, 2018). Enteroviruses cause symptoms in humans that vary from asymptomatic contagion to severe gastroenteritis and include aseptic meningitis and myocarditis. Human coxsackievirus and echovirus show a varying

pattern in terms of the serotypes most commonly found in wastewater, and clinical segregates such as echovirus 3, 6, and 19 and coxsackievirus A9, B4, and B5 have consecutively been identified as the most widespread serotypes (Tiwari and Dhole, 2018). In the environment, EVs can survive for long periods at pH values ranging from acidic to alkaline (3–10), mainly at low temperatures (Kocwa-Haluch, 2001). EVs such as coxsackievirus serotypes B-3, B-4, and B-5 and E1, E7, and E11 were significantly quantified in wastewater (Tiwari and Dhole, 2018).

Overall, EVs show vast potential for use as a water quality indicator to quantify the risk of infectious EV transmission and assess the primary source of faecal pollution in water systems (Kitajima et al., 2014). At least 100 human EV types have been identified. These viruses have been found in wastewater in Iran (Moazeni et al., 2017), the USA (Brinkman et al., 2017; Gerba et al., 2018), Italy (Pennino et al., 2018), Canada (Qiu et al., 2018) (Simhon et al., 2019), Greece (Kaliakatsos et al., 2019), and Japan (Tandukar et al., 2020). Most EVs are transmitted through the faecal-oral route, and these viruses are most frequently detected in wastewater-contaminated water.

HAV is one of the most important water-borne viruses, causes human enteric hepatitis, and is mainly transmitted through the faecal-oral route (Ouwardani et al., 2016). HAV is morphologically indistinguishable from other members of its family. Nevertheless, HAV easily contaminates wastewater due to the vast number of viral particles eliminated by diseased individuals who may be symptomatic or asymptomatic; these particles pass in significant amounts through ineffective sewage treatment plants and can quickly spread to water systems, such as lakes, rivers, and oceans (Ouwardani et al., 2016).

Because it is a non-enveloped virus, HAV is stable in the environment for an extended period and is resistant to wastewater treatment processes (La Rosa et al., 2010). Once excreted in faeces, HAV remains alive and can be disseminated through consumption of contaminated water and food (Vohra, 2010). HAV infection is associated with a wide range of common symptoms, including jaundice, vomiting, nausea, pale stools, fatigue, abdominal pain, and dark urine (Kaya et al., 2013). However, in some cases, mainly in teenagers and children under the age of six years, HAV can be asymptomatic (Ciocca, 2000). HAV has been detected and reported in wastewater in various countries, including South Africa (Adefisoye et al., 2016), Tunisia (Ouwardani et al., 2016), France (Kaas et al., 2019), and other industrialised countries (Fenaux et al., 2019).

Hepatitis E virus (HEV) is the principal aetiological mediator of enteric transmission through drinking water polluted with faecal matter and non-A hepatitis worldwide. HEV is the only member of the family Hepeviridae type Herpesvirus. The genome of HEV, which is approximately 7.2 kb in length, includes three open reading frames (ORF1, ORF2, and ORF3) (Long et al., 2017). ORF1 encodes non-structural proteins that are mainly involved in HEV replication, ORF2 encodes the main capsid proteins, and ORF3 encodes a small protein that may be involved in HEV–host interaction as well as in virion morphogenesis (Meng, 2010).

HEV infection can lead to acute viral hepatitis in young and middle-aged people (15–40 years old) in developing countries and areas. HEV presents an incubation period ranging from 2 to 9 weeks and is clinically indistinguishable from hepatitis A. This virus has been detected in wastewater in France (Miura et al., 2016; Courault et al., 2017; Kaas et al., 2019), China (Li et al., 2017), Colombia (Baez et al., 2017), Portugal (Matos et al., 2018), Italy (Di Profio et al., 2019), Germany (Beyer et al., 2020), and some other industrialised countries (Fenaux et al., 2019).

RoV-induced gastroenteritis is a self-limiting disease that can be mild to severe. Although person-to-person transmission is an important route, the main RoV transmission route is the faecal-oral route. More than 1000 RoV particles can be present in one gram of faeces, and these viruses have been detected at significant levels in wastewater in many countries, such as South Africa (Adefisoye et al., 2016; Osuolale and Okoh, 2017), Japan (Yasui et al., 2016), Canada (Qiu et al., 2016), the USA (Schmitz et al., 2016; Gerba et al., 2018), Tunisia (Ibrahim et al.,

2016), Italy (Mezzanotte et al., 2016), France (Kaas et al., 2019), Japan (Kobayashi et al., 2017), Uruguay (Lizasoain et al., 2018), Egypt (Rizk et al., 2019), the UK (Adriaenssens et al., 2018), Brazil (Assis et al., 2018), New Zealand (Gyawali et al., 2018), Canada (Qiu et al., 2018), New Zealand (Clemens et al., 2020), and Tunisia (Ibrahim et al., 2020).

SARS-CoV-2 is a single-stranded positive-sense enveloped RNA virus that belongs to a group of SARS-related coronavirus species in the sub-genus *Sarbecovirus* of the family Coronaviridae (Kitajima et al., 2020). SARS-CoV-2 contains four structural proteins: S (spike), M (membrane), E (envelope), and N (nucleocapsid); N encloses the RNA genome, and S, E, and M compose the viral envelope (Bosch et al., 2014; Fox, 2020). SARS-CoV-2 is also indistinctly linked to “classical” human CoV strains, such as HKU1, OC43, 229E, NL63, in the genus *Alphacoronavirus* or *Betacoronavirus*; these strains were initially differentiated in the 1960s and have been recognised to cause approximately 15–30% of common cold cases worldwide (Mesel-Lemoine et al., 2012; Kitajima et al., 2020).

SARS-CoV-2 caused the recent outbreak of the zoonotic disease that is now widely known as coronavirus disease 2019 (COVID-19), a pneumonia-like sickness caused by a hitherto uncharacterised aetiological agent (Arora et al., 2020). The coronavirus research group of the International Committee on Taxonomy of Viruses recently classified that zoonotic virus as SARS-CoV-2, a member of the Coronaviridae family, based mainly on its genetic structure, the crown- or halo-like structure of its envelope glycoprotein, its typical chemical composition, and its method of replication (Arora et al., 2020). SARS-CoV-2 virus, the aetiological agent of COVID-19, is mainly spread through respiratory droplets and human-to-human interactions.

The common symptoms of infection with SARS-CoV-2, the coronavirus responsible for COVID-19, include headache, loss of taste, sore throat, congestion, runny nose, nausea, vomiting, diarrhoea, fever, chills, cough, shortness of breath, fatigue, and body aches (Chen et al., 2020; Kitajima et al., 2020; Wurtzer et al., 2020). Detection of SARS-CoV-2 in wastewater systems represents an advantageous approach to assessing the COVID-19 epidemic in different communities. A method of detecting SARS-CoV-2 in wastewater systems was initially implemented in the Netherlands. The study reported that in the three weeks before the first COVID-19 case was reported in the Netherlands, SARS-CoV-2 viral RNA was not detected in the wastewater system; however, the amount of viral RNA began to increase over time, and the number of COVID-19 cases also increased (Medema et al., 2020).

3. Virus detection methods in wastewater systems

Several studies have been performed on virus detection in the wastewater system. The virus detection accuracy significantly relies on the sample volume, the nucleic acid extraction yield (nucleic acid-base methods), and the purity (Corpuz et al., 2020). At the same time, sample processing methods can equally influence the efficacy of the subsequent detection method. Therefore, the choice of a precise method for quantification or detection also substantially relies on the ease of cultivation of the viruses under laboratory circumstances. The viruses' particular characteristics affect the genome copy (GC) concentration and detection methods used for the samples being analysed (Ibrahim et al., 2017; Corpuz et al., 2020). For instance, recent research conducted by Hjelmsø et al. (2017) indicated that the concentration of nucleic acids and the nature of the methods applied for extraction substantially influence viral metagenomic assay findings, especially those of viral community composition, viral specificity, and viral pathogen detection. Hence, the methods for viral concentration, nucleic acid extraction, and virus detection must be chosen appropriately. For further detail regarding the concentration methods used for wastewater samples, the sample processing methods used for sludge samples, and nucleic acid extraction methods, see Corpuz et al. (2020).

Additionally, this review summarises different studies in which various human viruses (i.e., HAV, EVs, NoVs, AVs, and AdVs) have been

detected at a variety of sampling points, and recent evidence of SARS-CoV-2 in wastewater systems in the analysed WWTPs has been reported worldwide in these studies, which are outlined in [Tables 1 and 2](#). Some individual studies assessed the fate of various human viruses and SARS-CoV-2 within WWTPs by analysing wastewater samples collected from secondary treatment steps ([Ibrahim et al., 2017](#); [Balboa et al., 2020](#); [Haramoto et al., 2020](#); [Randazzo et al., 2020](#)). Other studies collected samples before and after treatment to evaluate virus removal efficiency ([Hamza et al., 2017](#); [Hamza and Hamza, 2018](#); [Balboa et al., 2020](#)). Aside from viruses in the wastewater, the occurrence of viruses in sludges generated in the examined WWTP operations has also been investigated ([El-Senousy et al., 2018](#); [Elmahdy et al., 2019](#)). The sludge and wastewater samples contain both types of RNA and DNA (enveloped and non-enveloped) viruses. The encapsulation of their nucleic acids by capsid proteins characterises non-enveloped viruses. Enveloped viruses contain an additional lipid bilayer membrane surrounded by capsid proteins ([Corpuz et al., 2020](#)). During sample processing, lipid bilayer disruption can decrease recovery and affect the subsequent detection ([Ye et al., 2016](#); [Corpuz et al., 2020](#)). This is of particular significance for investigations associated with coronaviruses (CoVs) ([Corpuz et al., 2020](#)). The diverse methods employed to detect and quantify viruses in the wastewater system include pulsed-field gel electrophoresis, epifluorescence microscopy, immunofluorescence assays, electronic transmission microscopy, flow cytometry, traditional cell culture, and molecular methods ([Wu and Liu, 2009](#); [Corpuz et al., 2020](#)). These methods mainly provide distinct information about the presence of viruses (qualitative data and quantitative data) in both sludge and wastewater samples. The molecular methods primarily applied for quantifying a virus are based on determining the number of selected segments of the virus's genetic material. The virus can be detected through this method even if inactivated, i.e., the viral capsid or envelope compromised and even when the viral genetic material is incoherent. In contrast, immunological methods and cell culture-based methods are mainly used to analyse the viability of viruses. Furthermore, a description of each method type and its advantages and disadvantages are summarised in [Table S3](#).

4. Occurrence of viruses worldwide in wastewater

4.1. Spatial distribution of human viruses in wastewater systems

Here, we discuss the detection of various viruses in wastewater in multiple countries worldwide. According to the collected data, wastewater on all inhabited continents contains detectable viruses. Detailed information on each virus found in wastewater in each country is presented in this section (see [Fig. 1](#)). Further details showing the diversity of human virus continent distributions, quantification methods used, concentration/pre-treatment method, total sample analysed, positive samples, and virus concentrations (range GC/L) in wastewater systems worldwide are summarised in [Table 1](#). We also describe in detail the viruses in wastewater in different areas in Part 1 of Supporting information.

4.2. Temporal patterns of human viruses in wastewater systems

Although most studies have indicated that changes in the levels of viral pathogens in sewage over time are not governed by predictable rules ([Farkas et al., 2018](#)), some studies have found that the levels of specific viral pathogens in sewage follow a pattern of imperceptible daily changes and obvious seasonal changes ([Abe et al., 2016](#); [Kitajima et al., 2018](#)). [Farkas et al.](#) detected diurnal patterns during sampling periods; however, obvious changes in viral titres were not found, apart from slight fluctuations in raw wastewater ([Farkas et al., 2018](#)).

These results showed that the viral level in sewage is not affected by daily chemical fluctuations. Therefore, one sample taken during the day may be adequate for enumerating the viral load of treated wastewater

within an order of magnitude, while collection of four samples per day is recommended for testing wastewater influent samples. Regarding seasonal changes, [Farkas et al.](#) also found that AdV titres were high and relatively constant in different seasons. In contrast, high concentrations of NoV GI and GII and SaV GI titres were demonstrated in winter and fall, and low counts were observed in summer ([Farkas et al., 2018](#)).

AiV-1 was also frequently detected in the colder months of the year in Fray Bentos, Bella Unión, Paysandú, and Salto, Uruguay ([Burutarán et al., 2016](#)). Similarly, [Ouwardani et al.](#) demonstrated that HAV circulates throughout the year in Tunisia, with high concentrations in winter and autumn. In contrast, in coastal areas, the highest rates occur in summer and fall ([Ouwardani et al., 2016](#)). [Ibrahim et al. \(2017\)](#) and [Abe et al. \(2016\)](#) demonstrated a clear difference in the monthly and seasonal distributions, respectively, of viruses in wastewater.

[Brinkman et al. \(2017\)](#) collected monthly municipal wastewater samples for 1 year and quantified EVs in wastewater. Sequence analysis and principal component analysis demonstrated that EV A and EV B were present, with EV A comprising over 45% of detections in the spring and EV B accounting for more than 80% of detections during the summer and autumn. EV C was detected throughout the year, while EV D was observed occasionally. The analysis of human EV in wastewater has provided novel insights into seasonal trends in EVs at the community level and could help elucidate the EV disease burden.

[Nguyen et al. \(2018\)](#) demonstrated NoV contamination in oysters from Hue city, Vietnam. The concentration of NoV GII was lower in the dry season than in the flood season, suggesting that seasonal flooding and sewage cause NoV contamination of oysters. However, the temporal patterns of SARS-CoV-2 in wastewater are still unclear and require more research. Nonetheless, a recent study conducted by [Vallejo et al.](#) applied a wastewater detection approach in the urban area of Coruña (Spain), and investigative sampling, analysis, and monitoring were conducted on April 15th. The initial results indicated that a substantial level of SARS-CoV-2 viral RNA was present in the wastewater system of the Bens WWTP. In addition, starting on April 19th, 24 h composite wastewater samples were continually analysed until early June, and surveillance will continue at the Bens WWTP until the viral genetic material disappears. The results of this study identified a decrease in COVID-19 incidence and further confirmed that the time course of the quantifiable discovery of SARS-CoV-2 in wastewater was inversely correlated with the number of confirmed COVID-19 cases ([Vallejo et al., 2020](#)).

4.3. Respiratory viruses in wastewater systems and associated human risks

In the field of ecological virology, studies of water-borne transmission have focused mainly on EVs. Respiratory viruses such as AdVs, CoVs, and SARS-CoV-2 have been reported in wastewater systems ([Ahmed et al., 2020](#); [Kitajima et al., 2020](#)). According to early descriptions, these human viruses cause severe diarrhoea as well as respiratory illness.

4.4. Conventional respiratory viruses

Respiratory diseases are the most common human diseases worldwide, and most of them are caused by viruses such as the influenza virus, coronaviruses, rhinoviruses, AdV, and respiratory syncytial virus ([Bruning et al., 2017](#); [Corpuz et al., 2020](#); [Zhang et al., 2020](#)). These viruses can infect the upper and lower respiratory tract, leading to acute viral rhinitis and pharyngitis, bronchitis, and pneumonia. Respiratory infection usually leads to self-inoculation because virus-contaminated fingers or hands rub the eyes or cause viral transmission through the nose or mouth. Inhalation of contaminated aerosols is another important route of transmission. Although faecal-oral transmission is not the main route of transmission of respiratory infectious diseases, some viruses that cause contagious respiratory diseases are extensively detected in faeces and wastewater.

Table 1

Reported diversity of human virus distribution, detection/quantification and nucleic acid extraction methods used, number of positive samples, and concentrations in wastewater systems in different countries.

Country	Virus types	Viral detection/quantification and nucleic acid extraction method used	Concentration/Pre-treatment method	Total sample analysed, positive samples %	Concentration (range) GC/L	References
Africa						
Continent						
Eastern Cape, South Africa	HAdV, RV, HAV	HAdV DNA extracted from 200 µL of wastewater samples using DNA extraction kits (Quick gDNA™ Mini-Prep; Zymo Research, USA), RNA extraction of HAV and RV using RNA purification kits (Quick-RNA™ Mini-Prep; Zymo Research, Irvine, USA), and detection using TaqMan Probe-Based qPCR quantitative assays.	Pre-filtered using glass fibre (Millipore, Ireland), AlCl ₃ pass through HA filter, & elution followed with Tris-EDTA (TE).	Total of 48 samples analysed. HAdV 16/48 (33.3%). HAV 3/48 (6.25%). RV (ND).	HAdV (8.4×10^1 to 1.3×10^5) HAV < 1	(Adefisoye et al., 2016)
Eastern Cape, South Africa	AiV-1	Sewage samples analysed for the presence of AiV-1 using RT-PCR. Amplification and sequencing of 3CD and VP1 genomic regions, followed by a phylogenetic study of selected genome sequences, revealed the occurrence of AiV-1, genotype B.	Elution with glycine	Total of 12 samples analysed. AiV-1 10/12 (83.3%).	NA	(Onosi et al., 2019)
Eastern Cape, South Africa	RoVs, HEVs	RNA extracted from 100 µL of wastewater samples using a Z. R. Viral RNA Kit™ (Zymo Research Corporation, 17062 Murphy Ave., Irvine, CA 92614, USA). HEVs detected with singleplex RT-PCR assays.	Adsorption-elution-method (Al-method & Mg-method) Adsorption-elution using electronegative membrane	Total of 70 samples analysed. RoVs (41.7%). HEVs (ND).	RoVs (1.9×10^3 to 1.2×10^5)	(Osuolale and Okoh, 2017)
Tunis, Tunisia	AiV	RNA extracted from (800 µL) wastewater samples using an automatic extractor NucliSENS ^R Easy Mag™ platform (BioMerieux, Marcy L'Etoile, France). AiV genome detected and quantified via RT-PCR using primer sets Ai6261 and Ai6779 to amplify a 519-bp fragment at the 3CD junction.	Beef extract & AlCl ₃ method followed by -polyethylene glycol (PEG)	Total of 102 samples analysed. 51/102 (50%).	NA	(Ibrahim et al., 2017)
Tunisia	HAV	tRNA extracted from concentrates (150 µL) of wastewater samples using a Nucleo Spin RNA Virus Kit (Macherey-Nagel, Germany). Genotype of HAV strains detected; semi-nested RT-PCR was performed to amplify a 222-bp fragment at the VP3/VP1 junction.	Beef extract & AlCl ₃ method followed by PEG	Total of 271 samples of wastewater analysed, 146/271 (53.9%).	(6.7×10^1 to 5.6×10^7)	(Quardani et al., 2016)
Tunis, Tunisia	RVA	RNA extracted from 800 µL of wastewater samples using an automatic extractor NucliSENS ^R Easy Mag™ platform (BioMerieux, Marcy L'Etoile, France). The RVA genome was detected and quantified by RT-PCR using various primers (Vp2-F1 to Vp2-F5, Vp2-R1, Vp2-R2) and a Vp2-P probe.	Beef extract & AlCl ₃ method followed by PEG	Total of 102 wastewater samples analysed, 51/102 (50%).	(3.9×10^1 to 2.8×10^3)	(Ibrahim et al., 2016)
Greater Cairo, Egypt	HBoV	Viral nucleic acids extracted from 200 µL of wastewater suspensions using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). TaqMan probe assay used for quantification of HBoV-1, HBoV-2, 3, and 4; SYBR green	Beef extract & AlCl ₃ method followed by PEG	Total of 66 treated wastewater samples processed, 38/66 (57.5%).	(6.0×10^3 to 4.9×10^4)	(Hamza et al., 2017)

(continued on next page)

Table 1 (continued)

Country	Virus types	Viral detection/quantification and nucleic acid extraction method used	Concentration/Pre-treatment method	Total sample analysed, positive samples %	Concentration (range) GC/L	References
Egypt	HPV, HPyV	qPCR assay conducted; real-time qPCR performed. Viral nucleic acids extracted from 200 μ L of wastewater suspensions using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Nested and semi-nested PCR targeting the L1 coding region employed to quantify a broad spectrum of cutaneous and mucosal HPV genotypes. Quantitative SYBR green qPCR assays performed using the primers GP5+/GP6+, which target a partial sequence of L1 genes. HPyV detected and quantified through real-time PCR targeting the VP1 capsid protein-encoding gene.	Elution with beef extract-glycine	Total of 66 treated wastewater samples processed, H HPV (30.5%), HPyV (82.4%).	HPV median (3.9×10^3) HPyV (5.1×10^{02} to 4.72×10^{03})	(Hamza and Hamza, 2018)
Tunis, Tunisia	HAdV	DNA extracted from 800 μ L of hospital wastewater samples using an automatic extractor platform (NucliSENS ^R Easy Mag TM , BioMerieux, Marcy L'Etoile, France). Nested PCR performed using two primer pairs (Adv-Hex1DEG/Adv-Hex2DEG and Adv-Hex3DEG/Adv-Hex4DEG) to amplify the gene segment coding for HAdV.	Beef extract & AlCl ₃ method followed by PEG	Total of 102 hospital wastewater samples analysed, (64%).	NA	(Ibrahim et al., 2018)
South Africa	NoV	Viral nucleic acid recovered from 100 μ L of wastewater suspensions using a NucliSENS ^R Easy Mag TM platform (BioMerieux, Marcy L'Etoile, France). Norovirus RNA-dependent RNA polymerase (RdRp) (region A, 326 bp) amplified from selected NoV GI- and GII-positive samples through conventional PCR using two pairs of primers (JV12Y and JV13D).	Elution with beef extract-glycine	Total of 108 wastewater samples analysed, NoV 78/108 (72.2%)	NoV (1.02×10^2 to 3.41×10^6) NoV GI and GII (5.00×10^3 to 1.31×10^6)	(Mabasa et al., 2018)
Egypt	AiV and HBoV	Nucleic acids recovered from (240 μ L) of sewage eluate using QIAamp Viral RNA and DNA kits (Qiagen, Inc., Valencia, CA, USA). The viral RNA was reverse transcribed via random primers in the presence of AiV-1 and detected through semi-nested PCR. HBoV was identified by nested PCR targeting the VP1/VP2 region to identify HBoV-2/3/4 species. PCR to amplify a 543-bp fragment was performed using the primers 234F1 and 234R1.	Adsorption-elution using electronegative membrane, method followed by PEG	Total of 24 wastewater samples analysed (12 untreated and 12 treated). AiV-1 2/12 (16.6%) untreated and 1/12 (8.3%) treated. HBoV 5/12 (41.6%) untreated and 3/12 (25%) treated.	NA	(Shaheen et al., 2019)
Egypt	Pepper mild mottle virus (PMMoV) and HAdV	Nucleic acids recovered from (60 μ L) of wastewater influent and effluent samples using a QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). A viral nucleic acid. DNA standard for PMMoV was prepared using the primers HaPMMV2 and PM1602, targeting ~ 319 bp of the genome. The DNA standard for HAdV was produced by	Virus adsorption & elution (VIRADEL)	Total of 66 wastewater influent and effluent samples analysed. PMMoV (94%) of influent and (78%) of effluent. HAdV (88%) of influent and (78%) of effluent.	PMMoV (3.9×10^4 to 3.3×10^5) influent and (3.9×10^4 to 1.2×10^7) effluent HAdV (1.5×10^4 to 1.5×10^7) influent and (2.6×10^4 to 4.4×10^6) effluent	(Hamza et al., 2019)

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Table 1 (continued)

Country	Virus types	Viral detection/quantification and nucleic acid extraction method used	Concentration/Pre-treatment method	Total sample analysed, positive samples %	Concentration (range) GC/L	References
Egypt	HAdV	cloning through real-time qRT-PCR. Viral nucleic acid primarily extracted from (300 µL) wastewater samples using a DNA extraction kit (Patho Gene-spin™, Korea). Real-time PCR for HAdV was performed using SYBR GREEN (Applied Biosystems Step One™ Real-time PCR system).	Sewage sample elution with beef extract- glycine. Sludge samples ultracentrifugation followed beef extract elution	Total of 96 samples, including 32 raw sewage, 32 treated sewage, and 32 sewage sludge samples, analysed. HAdV was found in 17/32, 27/32, 16/32, and 25/32 (28.3%, 84%, 50%, and 78%) of the raw sewage, treated sewage, and sewage sludge samples, respectively.	(2.02×10^6 to 7.23×10^6) stool, (8.7×10^5 to 4.3×10^5) raw sewage, (1.22×10^4 to 3.7×10^6) treated sewage, and (1.48×10^6 to 1.77×10^7) sludge	(Elmahdy et al., 2019)
Giza, Egypt	Coxsackievirus, EVs	Viral RNA recovered from concentrated (100 µL) clinical specimens of water and wastewater samples using BIOZOL Total RNA Extraction reagent (BIOFLUX—Japan). Nested RT-PCR was performed using primers in 1 st . and 2 nd . round PCR to amplify a 138-bp fragment.	Elution with glycine. Eluted viruses were re-concentrated by PEG	Total of 48 samples (12 raw Nile water, 12 drinking water, 12 raw sewage, and 12 treated sewage samples) analysed. EVs were found in 33%, 25%, 25%, and 8.3% of these samples, respectively.	(9×10^1 to 7×10^5) the raw Nile, (9×10^1 to 2×10^2) drinking water, (9×10^2 to 8×10^7) raw sewage, and (9×10^1 to 7×10^3) treated sewage	(El-Senousy et al., 2018)
Tunisia	SaV	RNA recovered from (800 µL) of wastewater using an automatic extractor (NucliSENS® Easy Mag™ platform, BioMérieux, Marcy L'Etoile, France). SaV quantification performed through real-time RT-PCR using SaV124F and SaV1245R primers and a SaV124TP probe to amplify the segment of the gene that encodes the polymerase.	Beef extract & AlCl ₃ method followed by PEG	Total of 102 wastewater samples analysed, SaV 30/102 (29.4%).	NA	(Ibrahim et al., 2019)
Tunisia	SaV	RNA extracted from (150 µL) of concentrated wastewater samples using the Nucleo Spin RNA Virus Kit (Macherey-Nagel; Germany). SaV quantification performed through real-time RT-qPCR using SaV124F and SaV1245R primers and the SaV124TP probe, which mainly target the polymerase-capsid junction in ORF-1.	Beef extract & AlCl ₃ method followed by PEG	Total of 218 wastewater samples analysed, SaV 87/218 (39.9%)	(4.3×10^3 to 5.3×10^8)	(Varela et al., 2018)
North America Continent						
Arizona, USA	GIV NoV	RNA extracted from a concentrated (360 µL) wastewater sample spiked with murine norovirus (MNV, S7-PP3 strain) using the ZR Viral DNA/RNA Kit (Zymo Research, Irvine, CA). GIV NoV quantification performed by semi-nested PCR using COG4F, G4SKF, and G4SKR primers to amplify a 340-bp region of the GIV NoV partial capsid gene.	Ultrafiltration by using electronegative filter	Total of 50 wastewater samples analysed, 13/50 (26%).	NA	(Kitajima et al., 2016)
USA	ReoV	ReoV RNA recovered from cell culture homogenous lysates through the study of dsRNA segment patterns. Primers that mainly target conserved regions of the ReoV L1, L3, and S2 genes were developed and used in the molecular	Adsorption-elution	Total of 30 wastewater samples analysed, 9/30 (30%).	NA	(Betancourt and Gerba, 2016)

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Table 1 (continued)

Country	Virus types	Viral detection/quantification and nucleic acid extraction method used	Concentration/Pre-treatment method	Total sample analysed, positive samples %	Concentration (range) GC/L	References
Calgary, Canada	RV, AdV	detection of ReoV RNA in water. These assays were performed using RT-PCR. RNA extracted from a concentrated (200 µL) wastewater sample. Virus mix containing NoV GII and AdV was expected from clinical stool samples and confirmed through in-house RT-qPCR assays.	VIRADEL	Total of 12 wastewater samples analysed, RV 6/6 (50%). Adv 6/12 (50%)	RV (6.6 log ₁₀) Adv (NA)	(Qiu et al., 2016)
Canada	NoV	Viral nucleic acid primarily recovered from concentrated (327 µL) wastewater samples by cell culture. Virus mixture containing NoV from clinical stool samples using real-time quantitative PCR (RT-qPCR) and confirmed by in-house RT-qPCR.	VIRADEL	Total of 12 wastewater samples analysed.	NoV most numerous (6.6 log ₁₀)	(Qiu et al., 2016)
Ohio, USA	EVs	Nucleic acids extracted from (10 mL) concentrated wastewater samples using a QIAamp DNA Blood Maxi Extraction kit (Qiagen, Valencia, CA). Quantitative reverse transcriptase PCR used to detect EVs. EVs counted in each sample using RT-qPCR.	Filtration and elution with beef extract-Celite	Total of 12 wastewater samples analysed.	(7.05 × 10 ³ to 8.3 × 10 ⁵)	(Brinkman et al., 2017)
Arizona, USA	AiV 1, SaV	Viral RNA extracted from concentrated (650 µL) wastewater samples using the ZR Viral DNA/RNA Kit (Zymo Research, Irvine, CA). Quantification of AiV 1 genotype-specific qPCR performed by real-time PCR using forward and reverse primers (AiV-AB-F and AiV-AB-R) and probes (AiV-A-TP and AiV-B-TP).	Filtration by using electronegative filter & elution followed with Tris-EDTA (TE)	Total of 26 wastewater (13 influent and 13 effluent) samples analysed SaV 8/13(62%) influent and 1/13 (8%) effluent. AiV 7/13 (54%) influent and 7/13 (54%) effluent	NA	(Kitajima et al., 2018)
South America Continent						
Rio De Janeiro, Brazil	NoV	Viral nucleic acid recovered from concentrated (140 µL) wastewater samples using the QIAamp Viral RNATM Mini Kit (QIAGEN, Valencia, CA, USA). NoV quantification by semi-nested PCR targeting the 50-end ORF2 region.	NA	Total of 156 wastewater samples analysed (52%).	(10 ⁴ to 10 ⁶)	(Fioretti et al., 2018)
Rio de Janeiro, Brazil	NoV	Viral RNA recovered from concentrated (140 µL) wastewater samples using the QIAamp Viral RNA Mini Kit (QIAGEN, CA, USA) qPCR) method.	Adsorption of viruses to pre-flocculated skimmed-milk proteins followed centrifugation	Total of 156 wastewater samples analysed, NoV GI and GII 38.5% and 96.1%, respectively.	NoV GI and GII (4 to 6.2 log ₁₀) and (4.4 to 7.3 log ₁₀), respectively	(Fumian et al., 2019)
Rio De Janeiro, Brazil	HSaV	Viral RNA recovered from concentrated (140 µL) wastewater and faecal samples using the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA, USA). HSaV quantitative detection performed using the TaqMan™-based real-time PCR through the polymerase-capsid junction localised in ORF1 of HSaV (GI, GII, GIV, and GV) in a single reaction.	Adsorption of viruses to pre-flocculated skimmed-milk proteins followed centrifugation	Total of 156 wastewater samples analysed, 51/156 (33.0%).	(10 ⁴ to 10 ⁶)	(Fioretti et al., 2016)
Antioquia, Colombia	HEV	Viral RNA extracted from concentrated wastewater samples using a commercial kit (QIAamp Viral RNA Mini,	Filtration & tangential ultrafiltration	Total of 30 wastewater and drinking water samples analysed,	NA	(Baez et al., 2017)

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Table 1 (continued)

Country	Virus types	Viral detection/quantification and nucleic acid extraction method used	Concentration/Pre-treatment method	Total sample analysed, positive samples %	Concentration (range) GC/L	References
Uruguay	AIV-1	QIAgen, Netherlands). RT-nested PCR method used to target the ORF2/3 region (nt 5258–5394) to detect the HEV genome. Nested PCR method used for amplification of the 3CD junction section by Taq DNA polymerase (5 U/II) with primers 6261/6779, which amplifies a 519-bp region, in the 1 st -round PCR and primers C94b/246 k to amplify a 266-bp region in the 2 nd -round PCR.	Ultracentrifugation	Wastewater 5/30 (16.7%). Drinking water 7/30 (23.3%).	Total of 96 wastewater samples analysed, 54/96 (56%).	NA
(Burutarán et al., 2016) Santiago, Chile	JCPyV	Viral nucleic acid obtained from concentrated wastewater samples using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Germany). JCPyV detection and quantification performed through real-time qPCR. An 89-bp fragment of the large T antigen (LTag) coding region of JCPyV (positions 4251 to 4339 in reference sequence NC_001699.1) was amplified using forward and reverse JE3 (Mad-1) primers and a 6-FAM/BHQ1 JE3 (Mad1) probe.	Ultracentrifugation	Total of 72 (36 influents and 36 effluents) wastewater samples analysed, JCPyV 29/36 (80.56%) influent and 18/36 (50%) effluent.	NA	(Levicán et al., 2019)
Germany	HEV	Viral nucleic acid obtained from concentrated (5 mL) wastewater samples using NucliSENS easy MAG (BioMérieux, Germany). Nested RT-PCR used to amplify a 332-bp product from HEV (ORF1).	Ultracentrifugation followed PEG	Total of 184 (111 influent and 83 effluent) wastewater samples analysed, 93/111 (84%) influents and 26/83 (31%) effluents.	Median 3×10^3 (influent) and 1×10^3 (effluent)	(Beyer et al., 2020)
Mexico	Poliovirus	Viral nucleic acid obtained from concentrated wastewater samples. Poliovirus serotype detected by qRT-PCR. Sabin isolates screened through real-time PCR assay for VDPVs by sequencing in VP1.	Elution with beef extract-glycine	Total of 125 wastewater samples analysed, poliovirus 37/125 (29.6%).	NA	(Estívariz et al., 2019)
Asia Continent						
India	EVs (polio)	EVs (polio) found in wastewater from clinically isolated samples using pan-EV primers (CDC, Atlanta, GA). Tissue culture used for EV isolation, and serotype confirmed by EV neutralisation tests.	Centrifugation followed PEG	Total of 109 wastewater samples analysed, (50.0%)	NA	(Tiwari and Dhole, 2018)
Pakistan	EVs (polio)	EV RNA content of wastewater concentrates estimated by real-time RT-PCR using the qScript XLT qPCR Toughmix system (Quantabio) in a Rotor-Gene Q instrument (Qiagen). Nucleotide sequence of the VP1 coding region of EV strains analysed using the Sanger method.	Separation-inoculated on rhabdomyosarcoma cell culture flasks.	NA	(7.0 to 7.5 log ₁₀)	(Majumdar et al., 2018)
Pakistan	HEV	RNA extracted from the virus in concentrated wastewater using the QIAamp RNA extraction kit (Qiagen, Hamburg, Germany).	Centrifugation followed PEG	Total of 86 wastewater samples analysed 35/86 (40.7%)	NA	(Ahmad et al., 2010)

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Table 1 (continued)

Country	Virus types	Viral detection/quantification and nucleic acid extraction method used	Concentration/Pre-treatment method	Total sample analysed, positive samples %	Concentration (range) GC/L	References
Japan	NoV	RNA extracted from the virus in concentrated wastewater using the QIAamp Viral RNA Mini QIAcube Kit (Qiagen, Hilden, Germany) and QIAcube (Qiagen, Hilden, Germany). Genotypes and variants quantified through amplification of the partial capsid protein (VP1) and RNA-dependent RNA polymerase genes of NoV GI and GII by single-round PCR, nested PCR, and sequencing using the primers p290, COG1F, COG2F, G1SKR, and G2SKR.	Centrifugation followed PEG	Total of 147 (70 wastewater and 77 stool) samples analysed, NOV GII (77%) stool. NOV GII (81%)	NA	(Masago et al., 2016)
Miyagi, Japan	HPeV	Viral RNA recovered from concentrated (140 µL) clinical wastewater samples using the QIAamp Viral RNA Mini Kit (Qiagen) and complementary DNA (cDNA) synthesised via RT using superscript II-RT. HPeV directly verified by PCR targeting the VP1 region and purified PCR products of the VP1 region.	Centrifugation followed PEG	Total of 79 wastewater samples analysed, HPeV 14/79 (18%),	NA	(Abe et al., 2016)
Japan	NoV GI	RNA recovered from concentrated (140 µL) wastewater samples using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) with QIAcube (Qiagen) and cDNA produced using the iScript Advanced cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). NoV GI quantified through qPCR using the CFX96 real-time PCR quantification system with primers and probes COG1F, COG1R, RING1(a)-TP, and RING1(b)-TP for GI and COG2F, COG2R, and RING2AL-TP for GII.	Centrifugation followed PEG	Total of 17 wastewater samples analysed, NoV GI 17/17(100%).	Up to (8.7×10^4)	(Kazama et al., 2016)
Japan	NoV GII	Viral RNA recovered from concentrate (100 µL) using NucliSENS® miniMAG® (BioMerieux, Tokyo, Japan). Matching DNA (cDNA) prepared using the Prime Script RT reagent kit (Takara Bio, Japan). TaqMan qPCR assays used in MF-qPCR for quantification of NoV GI, NoV GII, NoV GIV, MgV, MNV, and IAC plasmid DNA.	Centrifugation followed PEG	Total of 26 wastewater samples analysed, 12/26 (46%).	NA	(Ito et al., 2017)
Shen Zhen, China	HEV	RNA recovered from concentrated wastewater samples using the UltraPure™ RNA Kit (CWBI, Beijing, China). Recovered RNA further used for matching DNA (cDNA) synthesis using the HiFiScript 1 st strand cDNA Synthesis Kit (CWBI, Beijing, China). Nested PCR used to amplify a fragment of ORF2 (nt 5,983-6,349) of the HEV genome.	Centrifugation followed PEG	Total of 152 wastewater samples analysed. 2/152(1.32%).	NA	(Li et al., 2017)
Karaj, Iran	SAFV	RNA extracted from concentrated (100 µL) river and wastewater samples using TRIzol reagent (Thermo Fisher	Centrifugation followed Dextran-PEG	Total of 50 (28 river, 12 treated, and 10 untreated) wastewater samples	$(2 \times 10^6$ to $6.4 \times 10^6)$ river	(Aminipour et al., 2020)

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Table 1 (continued)

Country	Virus types	Viral detection/quantification and nucleic acid extraction method used	Concentration/Pre-treatment method	Total sample analysed, positive samples %	Concentration (range) GC/L	References
		Scientific, Waltham, MA, USA). RT-PCR and RT-qPCR method used a gold standard to quantify SAFV through targeting the 5'UTR region of the genome.		analysed, 10/28 (35.7%) river, 4/12 (33.3%) treated, 4/10 (40%) untreated.	$(1.2 \times 10^6$ to 5.2×10^6) treated 2.4×10^6 to 6.8×10^6 untreated	
Tehran, Iran	TTV	Viral nucleic acids recovered from concentrated (140 μ L) final wastewater elute samples using the QIAamp RNA Mini Kit (Qiagen, Germany). Nested PCR used to detect the presence of TTV using 1 st -round primers (NG054 and NG147) and 2 nd -round amplification using (NG132 and NG133).	Elution with beef extract-glycine	Total of 13 wastewater samples analysed, (76.9%).	NA	(Tavakoli Nick et al., 2019)
Vietnam	NoV	Viral RNA recovered from concentrate (300 μ L) from wastewater samples using the Direct-zol RNA MiniPrep kit (Zymo Research, Irvine, CA, USA). cDNA obtained via RT using the iScript Advanced cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). qPCR assays performed to quantitatively detect NoV GI and GII using SsoFast Probes Supermix with primers COG1F and COG1R and probes RING1-TP(a) and RING1-TP(b) for NoV GI and primers COG2F and ALPF and probes COG2R and RING2AL-TP for NoV GII quantification.	Filtration with mixed cellulose ester membrane	Total of 39 wastewater samples analysed, positive rates of NoV GI and GII were 87% and 95%, respectively.	NoV GI maximum (5.6×10^2) NoV GII (1.3×10^1 to 3.1×10^3)	(Nguyen et al., 2018)
Europe Continent						
Italy	HSaV	Nucleic acid recovered from concentrated (100 μ L) wastewater samples and purified using the NucliSens extraction kit (BioMerieux, Paris, France). Nested RT-PCR assay targeting the capsid region (VP1) that detects all HSAV genotypes was performed using three forward primers (SaV124F, SaV1F, and SaV5F) and two reverse primers (SV-R13 and SV-R14) in the 1 st round of PCR and primers, 1245Rfwd and SV-R2, in the nested PCR.	Polyethylene glycol-dextran separation	Total of 166 wastewater samples analysed, 56/166 (33.7%).	NA	(Mancini et al., 2019)
Portugal	HEV	Nucleic acid recovered from concentrated (140 μ L) wastewater samples using the QIAmp® Viral RNA Mini Kit (QIAGEN, Hilden, Germany). HEV quantification was done using an RT-qPCR TaqMan probe assay targeting the open reading frame (ORF) 2 region of the HEV.	Ultracentrifugation	Total of 60 wastewater samples analysed, 2/60 (3.3%)	NA	(Matos et al., 2018)
French Polynesia	HEVs, NOV, SaV, RoV, HAdV, HPyV	Viral nucleic acids recovered from concentrated (200 μ L) wastewater samples using the High Pure Viral Nucleic Acid Kit (Roche Molecular Biochemicals Ltd, Mannheim, Germany). Viral RNA genomes (NoV GI and GII, AsV, SaV, EV, HAV, HEVs, HPyV, and RoV)	Flocculation with PEG	Total of 6 wastewater samples analysed. NoV GI 2/6(33.3%) and GII 5/6 (83.3%). HAdV 5/6 (83.3%). HEVs 4/6 (66.7%). RoV and AsV 3/6 (50%).	HEVs average (2.3×10^5) and SaV (8.3×10^6). average (7.2×10^2) and GII (1.7×10^7) HAdV average (1.7×10^5). HEV (LOD) RoV and AsV average (3.3×10^4 to 2.6×10^3)	(Kaas et al., 2019)

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Table 1 (continued)

Country	Virus types	Viral detection/quantification and nucleic acid extraction method used	Concentration/Pre-treatment method	Total sample analysed, positive samples %	Concentration (range) GC/L	References
Gothenburg, Sweden	NoV	detected using RT-qPCR or qPCR assays. RNA recovered from concentrated (300 µL) wastewater samples using Biorobot EZ1 (Qiagen) with the EZ1 Virus Mini Kit v.2.0 (Qiagen). Real-time qPCR method used as a duplex PCR for NoV GGI and GGII with primers (NV-G1-fwd1b, NV-G1-rev, NV-G2fwd, and COG2R) at regions of the ORF1-ORF2 junction for NoV GGI and GGII quantification.	Fluctuations in concentration	HAV (ND). HPyV 6/6 (100%) Total of 160 wastewater samples analysed, NoV GI 22/26 (84.6%) (warm) and 25/28 (89.2%) (cold) untreated wastewater. NoV GII 15/26 (57.69%) (warm) and 26/28 (92.85%) (cold) untreated wastewater. NoV GI 16/26 (61.5%) warm, 17/28 (60.7%) cold and NoV GII 15/26(57.69%) warm, 26/28 (92.85%) cold in treated water.	HPyV average (3.4×10^7). Average NoV GI and GII (6.2 and $6.8 \log_{10}$) genome equivalents (g.e.)/L cold (5.3 and $5.9 \log_{10}$) warm g.e./L untreated Treated detection limit (BDL) warm to average ($3.8 \log_{10}$ g.e./L cold	(Dienus et al., 2016)
Western France	HEV	Viral RNA extracted from concentrated wastewater using a NucliSENS kit (BioMerieux, Lyon, France). HEV quantification using an Ultrasens QRT-PCR kit (Invitrogen, France). RNA assessed based on a standard curve obtained from in vitro transcription of a plasmid comprising a fragment of the HEV genotype 3 f strain.	Centrifugation followed PEG	Total of 32 (18 influent and 14 effluent) samples analysed, collected from 4 WWTP (A, B, C, and D) sites HEV (10%, 11%, 13%, and 12% influent) and (8%, 5%, 9%, and 13% effluent) WWTP A, B, C and D positive, respectively.	NA	(Miura et al., 2016)
Southern Italy	HEV	RNA extracted from concentrated wastewater using the TRIzol LS (Invitrogen, Ltd., Paisley, UK) method and studied through HEV-specific RT-qPCR, focusing on a conserved 68-nucleotide region of the ORF3 genome.	Elution with phosphate buffered saline	Total of 56 wastewater samples analysed, 13/56 (23.2%).	(6.1×10^2 to 5.8×10^5) GC/mL	(Di Profio et al., 2019)
Southern Italy	HEVs	RNA extracted from concentrated wastewater. HEV quantified through PCR, and virus typing performed by seroneutralization. The presence of EVs in the CPE-positive samples was measured through RT-nested PCR using primers focused on VP1 and AN89.	Two-phase separation method by Dextran-PEG	Total of 731 wastewater samples analysed, 161/731 (22.0%).	NA	(Pennino et al., 2018)
North Wales, UK	EVs	RNA obtained from concentrated wastewater. RNA viruses (NoVGI and GII, SaVGI), along with potential EVs (HAdVs and PyV strains BK and JC), were measured by RT-qPCR.	Beef extract elution followed PEG	Total of 91 (52 influent and 39 effluent) wastewater samples analysed. AdV and JCV (100%). NoVGI, GII and SaVGI (35%, 62% and 27%) influent and (38%, 49% and 10%), respectively.	AdV and JCV (10^4 & 6×10^3) NoVGI, GII and SaVGI (NA)	(Farkas et al., 2018)
Italy	HBoVs	Viral nucleic acids recovered from concentrated (10 mL) chloroform-treated wastewater samples using NucliSENS easyMAG (BioMerieux, Marcy L'Etoile, France). Viral DNA was used as a template for nested PCR using a broad-range pair of	Centrifugation	Total of 134 wastewater samples analysed, 106 (79.1%).	($5.51E+03$ to $1.84E+05$)	(Iaconelli et al., 2016)

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Table 1 (continued)

Country	Virus types	Viral detection/quantification and nucleic acid extraction method used	Concentration/Pre-treatment method	Total sample analysed, positive samples %	Concentration (range) GC/L	References
Catalonia, Spain	HAdV, NoV	primers targeting the VP1/VP2 region of HBoV. Viral nucleic acids extracted from concentrated wastewater samples using the QIAmp Viral RNA kit (Qiagen, Inc., Valencia, CA). HAdV and NoV GII quantitated using real-time qPCR and RT-qPCR.	Skimmed milk flocculation	Total of 12 wastewater samples analysed, HAdV and NoV GII 12/12 (100%) samples.	HAdV and NoV GII (1.98×10^5) and (5.17×10^6), respectively	(Gonzales-Gustavson et al., 2019)
Australia Sydney and Melbourne, Australia	HAdVs	Viral DNA obtained from concentrated wastewater samples using the QIAamp Viral Mini Kit (Qiagen, Hilden, Germany). HAdV quantification was performed using qPCR. The standard for quantification was obtained as a 301-bp fragment in 1 st -round PCR through the product of the hexon gene cloned into pET26b (Gen Script) and then measured by spectrophotometry.	Ultracentrifugation	Total of 68 wastewater samples analysed.	Average (1.8×10^7)	(Lun et al., 2019)
Queensland, Australia	HPyV	qPCR amplification method used for EC H8 and HF183 and qPCR amplification in a (20 μ L) reaction mixture using (10 μ L) of SsoFast EvaGreen supermix (Bio-Rad Laboratories, CA, USA), 400 nM each primer (EC H8 assay), 300 nM each primer (HF183 assay), and 3 μ L of template DNA.	Filtration by using electronegative filter followed Tenfold serial dilutions	NA	HPyV average (2.56×10^5) GC/mL	(Hughes et al., 2017)

Not Detected = (ND); Not Available = (NA); Low Detection = (LOD).

For instance, rhinoviruses have been identified in sewage in the USA (Brinkman et al., 2017). According to Fong et al. (2010), AdVs (40 and 41 type F), both respiratory AdVs (2 and 3 types C and B) and AdVs (12 types) that cause meningoencephalitis with early replication in the digestive or respiratory tract, are found in wastewater systems and in drain overflows and waterways that receive these releases (Fong et al., 2010). While it is possible that swimming in sewage-polluted waters is also linked to respiratory illness, aetiological agents associated with respiratory diseases are not frequently detected in swimming areas (Wade et al., 2006; Kitajima et al., 2020).

4.5. SARS-CoV-2 in wastewater systems

According to previous studies, the occurrence of CoVs in wastewater systems is limited; however, few ecological research studies have focused on CoVs. It has been assumed but not well confirmed that enveloped CoVs are primarily spread by human-to-human contact rather than by the faecal-oral route; therefore, the occurrence of CoVs in faeces requires further nuanced clarification (Kitajima et al., 2020). Although few studies have used the appearance of CoV genetic RNA in wastewater systems as a main disease surveillance tool, use of the method for this purpose is gaining traction (Ahmed et al., 2020; Kitajima et al., 2020). During the SARS epidemic in China in 2004, SARS RNA was first detected in 10/10 (100%) untreated and 3/10 (30%) disinfected hospital wastewater samples collected in Beijing, China, which was used to identify the presence of SARS patients (Wang et al., 2005).

In December 2019, cases of SARS-CoV-2, the aetiological agent of the ongoing COVID-19 pandemic, were first reported (Haramoto et al., 2020). To date, the available studies on the surrogate SARS-CoV-2 viruses recommend assuming that the strain that causes COVID-19 might be less persistent in wastewater systems, primarily due to the occurrence of either carbon-based matter or matrix autochthonous flora in such systems; such influences are certainly able to trigger metabolic pathways that hasten the die-off of viruses (Carraturo et al., 2020). However, recent cases of SARS-CoV-2 have been accompanied by insistent shedding of RNA viruses in faecal samples in 27–89% of patients at concentrations ranging from 0.8 to 7.5 log¹⁰ genome copies/g (Ahmed et al., 2020; Wölfel et al., 2020; Zhang et al., 2020). Thus, it is clear that SARS-CoV-2 is also present in wastewater systems (Lodder and de Roda Husman, 2020).

Actually, SARS-CoV-2 has been detected in wastewater systems in several countries, and this could be very informative for risk measurement. Respiratory symptoms are frequently reported in patients with COVID-19, and several ongoing studies have revealed that the intestinal tract can also be affected by SARS-CoV-2 (Wong et al., 2020). A meta-analysis confirmed that approximately 15% of COVID-19 patients experienced mostly intestinal symptoms and that approximately 10% of patients had intestinal symptoms but not respiratory symptoms (Ali et al., 2020). Correspondingly, SARS-CoV-2 RNA was frequently detected in the faeces of COVID-19 patients who did not experience intestinal symptoms (Vallejo et al., 2020; Yeo et al., 2020). Several studies reported that approximately 53.9% of patients were positive for faecal

Table 2

The reported SARS-CoV-2 distribution, nucleic acid extraction and detection/quantification methods used, concentration/pre-treatment methods used, target genes, number of positive samples, and concentrations in wastewater systems in different countries.

Country & Location	Nucleic acid extraction & Detection/quantification method used	Concentration/Pre-treatment method	Target genes	Total sample analysed & water type	Positive samples	Concentration range (GC/L)	References
France Paris	Viral particles and genomes extracted from concentrated (11 mL) ultracentrifugation wastewater samples using an optimised protocol (Power Faecal Pro kit in a QIA symphony extractor, QIAGEN). SARS-CoV2 quantitative analysis done by RT-qPCR.	Ultracentrifugation	RNA-dependent RNA polymerase gene (RdRp)	Total of 31 wastewater samples analysed (23 untreated wastewater, 8 treated wastewater).	23/23 (100%) untreated & 6/8 (75%) treated samples detected positive	Max: $> 10^{6.5}$ untreated Max: $\sim 10^5$ treated	(Wurtzer et al., 2020)
Netherlands	Viral RNA genome recovered from concentrated sewage samples using the RNeasy Power Microbiome Kit (Qiagen, Hilden, Germany). SARS-CoV2 quantitative analysis done by real-time RT-PCR.	Centrifugation	Envelope protein gene (E)	Total of 24 untreated wastewater samples analysed.	14/24 (58%) samples detected positive	NA	(Medema et al., 2020)
Australia Queensland	Viral RNA genome recovered from concentrated wastewater samples using a combination of two kits (RNeasy PowerWater Kit and RNeasy PowerMicrobiome Kit; Qiagen, Hilden, Germany). SARS-CoV2 quantitative analysis done by RT-qPCR.	Filtration (0.4 μ m pore size)	Nucleocapsid gene (N)	Total of 9 untreated wastewater samples analysed.	2/9 (22%) samples detected positive	Max: 1.2×10^2	(Ahmed et al., 2020)
ItalyRome	Viral nucleic acids recovered from concentrated sewage samples using the NucliSENS miniMAG semi-automated extraction system. SARS-CoV2 quantitative analysis done by nested RT-PCR	Pasteurization (57 °C, 30 min)	Open reading frame 1ab (ORF1ab)	Total of 12 treated wastewater samples analysed	6/12 (50%) samples detected positive	NA	(La Rosa et al., 2020)
USA Massachusetts	Viral RNA genome recovered from concentrated wastewater samples using polyethylene glycol 8000 (PEG) & by reverse transcriptase NEB & qPCR (TaqMan fast advanced master mix, Thermo Fisher). SARS-CoV2 quantitative analysis done by RT-qPCR.	Pasteurisation (60 °C, 90 min) & Filtration (0.2 μ m pore size)	Spike protein gene (S)	Total of 14 untreated wastewater samples analysed.	10/14 (71%) samples detected positive	Max: $> 2 \times 10^4$	(Wu et al., 2020)
USA Bozeman	Viral RNA genome recovered from concentrated wastewater samples. SARS-CoV2 quantitative analysis done by RT-qPCR.	Filtration (5 μ m & 0.4 μ m pore size)	Nucleocapsid gene (N)	Total of 7 untreated wastewater samples analysed.	7/7 (100%) samples detected positive	Max: $> 3 \times 10^5$	(Nemudryi et al., 2020)
USA New Haven	Viral RNA genome recovered from 2.5 mL of primary well mixed sludge using the RNeasy Power Soil Total RNA kit (Qiagen). SARS-CoV2 quantitative analysis done by one-step qRT-PCR.	NA	Nucleocapsid gene (N)	Total of 44 untreated primary sewage samples analysed.	44/44 (100%) samples detected positive	1.7×10^6 to 4.6×10^8	(Peccia et al., 2020)
USA Southeast Virginia	Viral RNA genome recovered from wastewater samples using (NucliSENS easyMag, bioMerieux, Inc., Durham, NC, USA). SARS-CoV2 quantitative analysis done by reverse transcription droplet digital PCR (RT-ddPCR).	Centrifugation & filtration	Nucleocapsid gene (N)	Total of 198 raw wastewater samples analysed	98/198 (49.5%) samples detected positive	10^1 to 10^4	(Gonzalez et al., 2020)
Spain Murcia	Viral RNA genome recovered from concentrated (150 μ L) wastewater samples using the NucleoSpin RNA virus kit (Macherey-Nagel GmbH and Co., Düren, Germany). SARS-CoV2 quantitative analysis done by RT-qPCR.	pH adjustment at 6;	Nucleocapsid gene (N)	Total of 72 samples analysed 42 influent samples, 18 secondary treated & 12 tertiary treated wastewater	35/42 (83%) influent & 2/18 (11%) secondary treated samples detected positive.	NA	(Randazzo et al., 2020)
Spain Valencia	Viral RNA genome recovered from concentrates (150 μ L) wastewater samples using the NucleoSpin RNA virus kit (Macherey-Nagel GmbH and Co., Düren, Germany). SARS-CoV-2 RNA was detected using the One Step RT-PCR Kit & by RT-qPCR.	pH adjustment at 6;	Nucleocapsid gene (N)	Total of 24 wastewater samples analysed (15 untreated & 9 treated)	Only 12/15 (80%) untreated samples detected positive	10^5 to 10^6 untreated	(Randazzo et al., 2020)
Spain Ourense	Viral RNA genome recovered from concentrated wastewater samples by (Seegene, Seoul, South Korea) SARS-CoV-2 RNA quantified by one-step multiplex RT-qPCR.	Ultrafiltration	E, N, ORF1ab, RdRp & S	Total 35 samples analysed 5 untreated wastewaters 25 treated (secondary 18 & tertiary 12)	Only 2/18 (11.1%) secondary & 5/5 (100%) untreated samples detected positive	$< 2.5 \times 10^5$ secondary & untreated NA	(Balboa et al., 2020)
Czech Republic			NA			NA	(Mlejnkova et al., 2020)

(continued on next page)

Table 2 (continued)

Country & Location	Nucleic acid extraction & Detection/quantification method used	Concentration/Pre-treatment method	Target gens	Total sample analysed & water type	Positive samples	Concentration range (GC/L)	References
Israel	Viral RNA genome isolated from concentrated wastewater samples using the NucliSENSfi miniMAGfi system (BioMérieux, Marcy l'Etoile, France). SARS-CoV2 quantitative analysis done by RT-qPCR.	Direct flocculation using beef extract-glycine & centrifugation		Total 112 untreated wastewater samples analysed	13/112 (11.6%) samples detected positive		
Various locations	Viral RNA recovered from concentrated wastewater samples using the RNeasy mini kit (QIAGEN) & Easy MAG (bioMérieux, France). SARS-CoV2 quantitative analysis done by RT-qPCR.	Centrifugation	Envelope protein gene (E)	Total 26 untreated wastewater samples analysed	10/26 (38.5%) samples detected positive	NA	(Or et al., 2020)
Turkey	RNA genome extracted from concentrated wastewater samples using the QIAamp Cador Pathogen Mini Kit (Qiagen, Hilden, Germany). SARS-CoV2 quantitative analysis done by RT-qPCR	Centrifugation; filtration (0.45 µm & 0.2 µm pore size); pH adjustment at 7.0 to 7.2	NA	Total 7 untreated wastewater samples analysed	5/7 (71.4%) samples detected positive	ND to 9.33×10^4	(Kocameci et al., 2020)
Istanbul							
Japan	Viral RNA genome recovered from concentrated (140 µL) sewage samples using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). SARS-CoV2 quantitative analysis done by RT-qPCR.	NA	Nucleocapsid gene (N)	Total of 13 samples analysed 5 secondary-treated wastewater samples 5 influents samples 3 river water samples	Only 1/5 (20%) secondary-treated samples detected positive	2.4×10^3	(Haramoto et al., 2020)
Yamanashi							
India	Viral RNA genome recovered from concentrated wastewater samples using the Allplex™ 2019-nCoV Assay kit (RP10244Y RP10243X). SARS-CoV2 quantitative analysis done by RT-qPCR.	Filtration (0.45 µm) & PEG centrifugation (4 °C for 30 minutes)	Spike protein gene (S)	Total 6 untreated wastewater samples analysed	2/6 (33.3%) samples detected positive	NA	(Arora et al., 2020)
Jaipur							
India	Viral RNA genome recovered from concentrated wastewater samples using the NucleoSpin® RNA Virus Kit (Macherey-Nagel GmbH & Co. KG, Germany). SARS-CoV2 quantitative analysis done by RT-qPCR.	Filtration (0.22 µm) & PEG centrifugation	ORF1ab, N & S	20 untreated & treated wastewater samples analysed	20/20 (100%) untreated 20/20 (100%) treated samples detected positive	5.6×10 to 3.5×10^2 untreated	(Kumar et al., 2020)
Ahmedabad							
Pakistan	Viral RNA genome recovered from concentrated wastewater samples using the Spin star viral nucleic acid kit 1.0 (ADT Biotech, Phileo Damansara 1, Petaling Jaya Part No.811803). SARS-CoV2 quantitative analysis done by RT-qPCR	Filtration & PEG centrifugation	ORF1ab	Total of 78 untreated wastewater samples analysed	21/78 (26.9%) samples detected positive	NA	(Sharif)
Various locations							
Pakistan	Viral RNA genome recovered from concentrated wastewater samples using BSL-3 of IM, UVAS. SARS-CoV2 quantitative analysis done by RT-qPCR	Centrifugation (4 °C for 15 minutes)	ORF1ab	Total of 28 untreated wastewater samples analysed	22/28 (78.6%) samples detected positive	2.67×10^2 to 3.60×10^4	(Yaqub et al., 2020)
Lahore							
China	SARS-CoV2 quantitative analysis done by RT-qPCR	NA	NA	Total of 42 untreated stool samples analysed	28/42 (66.67%) samples detected positive	NA	(Chen et al., 2020)
Wuhan							
China	SARS-CoV2 quantitative analysis done by RT-qPCR	Centrifugation (56 °C for 30 minutes inactivation)	ORF1ab	Total of 15 untreated stool samples analysed	4/15 (26.7%) samples detected positive	0.05 to 1.87×10^5	(Zhang et al., 2020)
Wuhan							

Not Detected = (ND); Not Available = (NA).

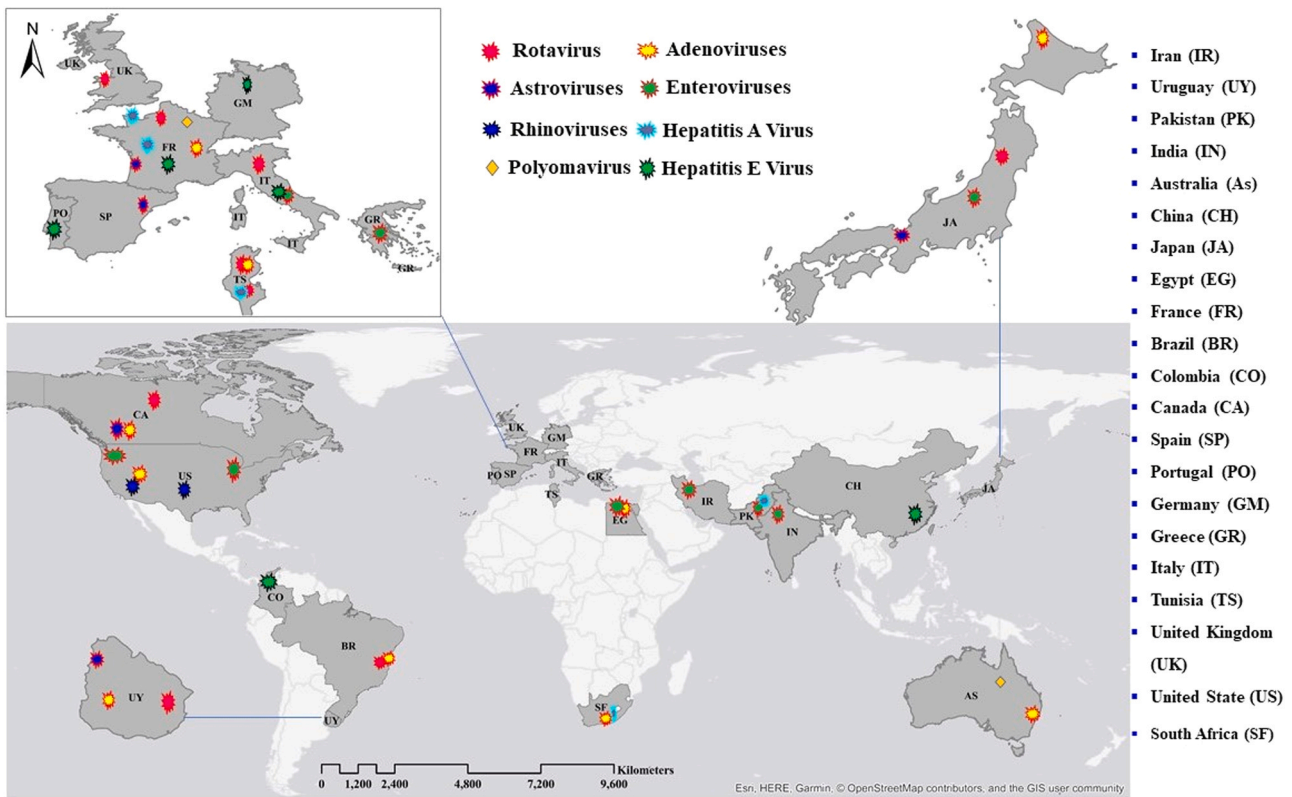


Fig. 1. Spatial patterns and diversity of human viruses in wastewater systems worldwide (Points on the map do not correspond to precise geographical locations).

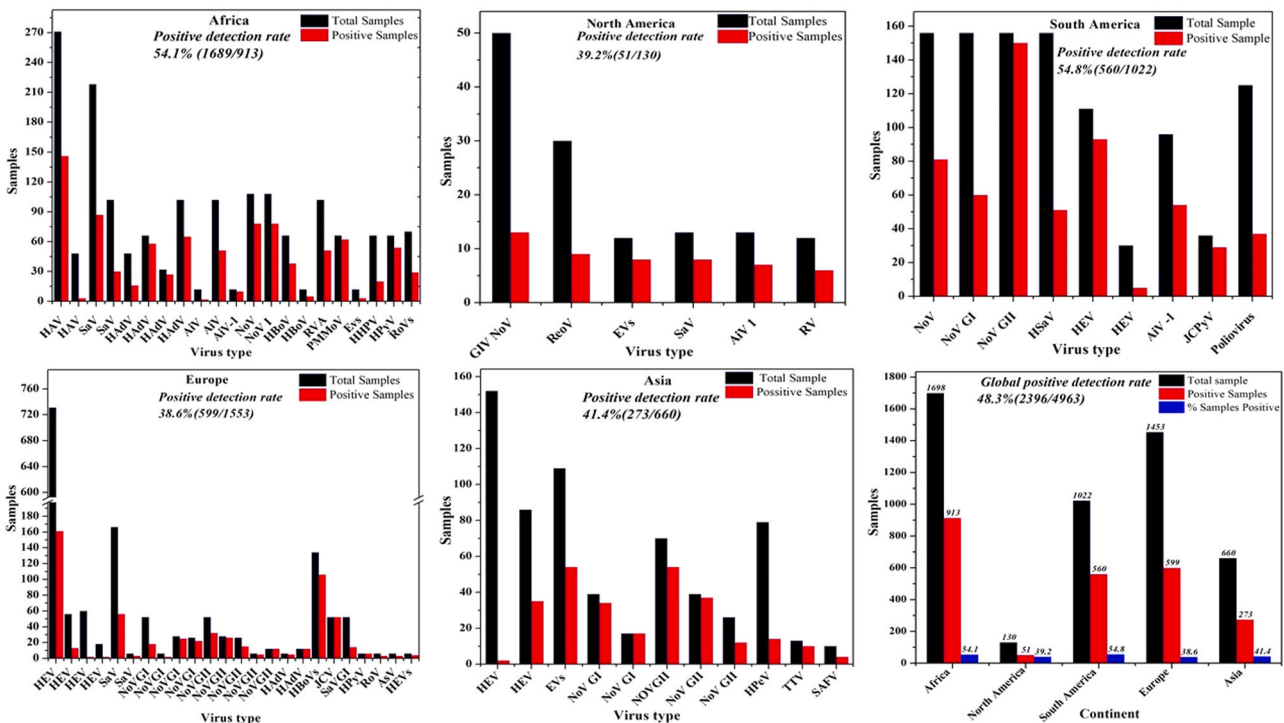


Fig. 2. Positive detection rates of different human viruses in effluent wastewater systems worldwide.

viral RNA (Vallejo et al., 2020), and some studies indicated that viruses were excreted in significant amounts in the patients' stools for an extended period of time (Chen et al., 2020); moreover, in a few cases, the individual was confirmed to be negative based on respiratory samples after a month or more (Vallejo et al., 2020; Wölfel et al., 2020). In

addition, the virus can significantly infect enterocytes in the human small intestine (Lamers et al., 2020). The occurrence of the infectious virus in human faeces highlights the potential for viral replication in the intestinal epithelium of infected individuals (Zhou et al., 2020).

As shown in Fig. 3, recent studies have identified SARS-CoV-2 in

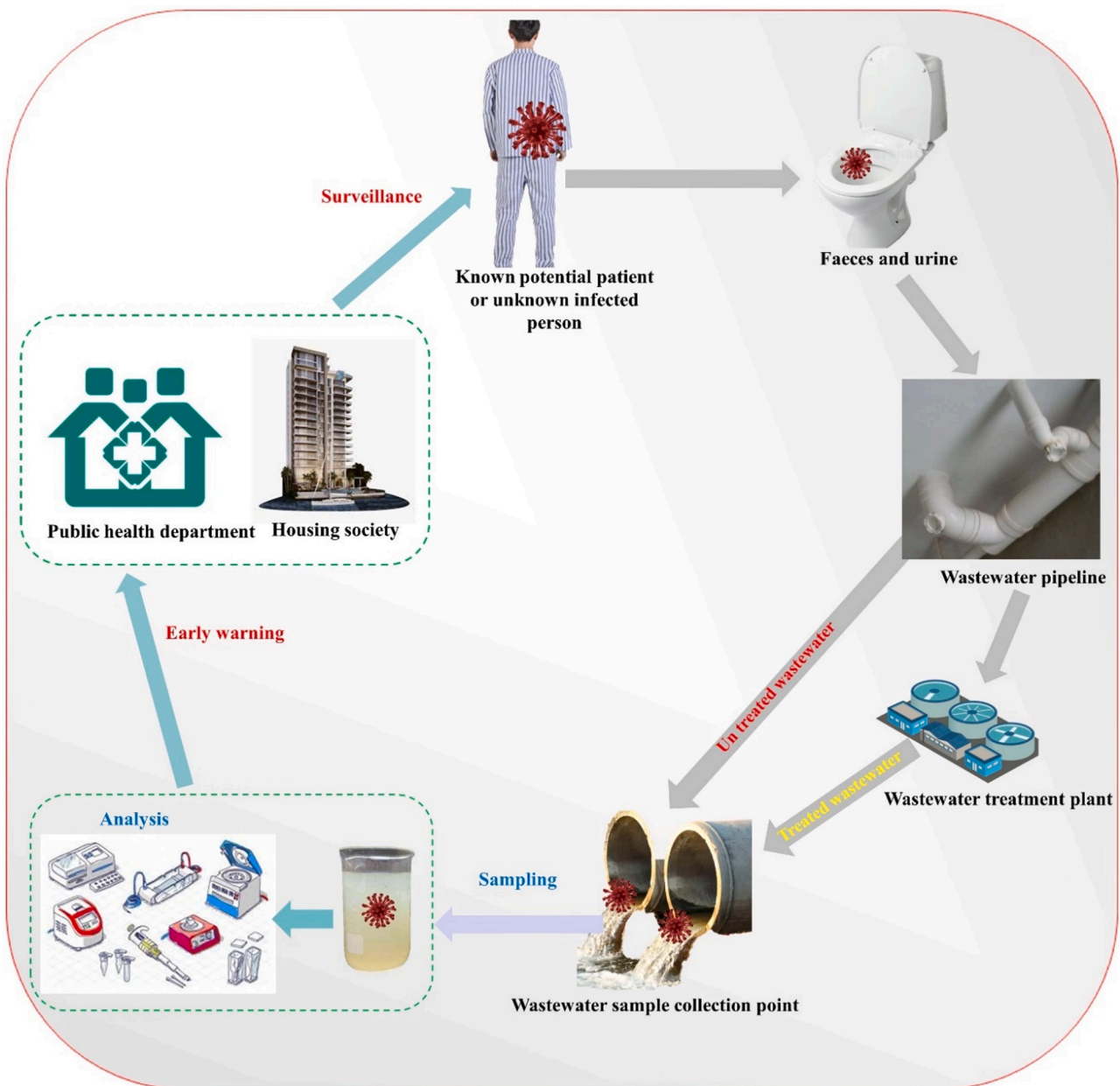


Fig. 3. Conceptual model of the wastewater-based epidemiology (WBE) surveillance and early warning system for infectious disease epidemics caused by SARS-CoV-2 pathogens (Mao et al., 2020; Mao et al., 2020).

wastewater systems in many countries on different continents (Europe, Australia, Asia, and America), including the Netherlands in a sample collected in February 2020. Of the samples, 14/24 (58%) that were positive (Medema et al., 2020) were from Rome, Italy, collected between February 3 and April 2, 2020. There were 6/12 (50%) positive (La Rosa et al., 2020) samples from Yamanashi, Japan collected between March 17 and May 7, 2020. We found that 1/5 (20%) secondarily treated positive samples (Haramoto et al., 2020) were from Israel, collected from various locations at the end of March to April 2020. Additionally, 10/26 (38.5%) of the samples (Or et al., 2020) from Istanbul, Turkey, collected on April 21, 2020 were positive; 5/7 (71.4%) (Kocamemi et al., 2020) samples from Paris, France, collected from March 5 to April 23, 2020 were positive; 23/23 (100%) samples (Wurtzer et al., 2020) from Murcia, Spain, and Valencia collected from March 12 to April 14, 2020 were positive, of which 35/42 (83%) influent samples were positive, 2/18 (11%) secondarily treated samples were positive (Randazzo et al., 2020), and 12/15 (80%) were positive (Randazzo et al., 2020). Ourense

samples were collected twice a week from April 6–21 2020. The 2/18 (11.1%) secondary and 5/5 (100%) untreated positive samples (Balboa et al., 2020) from the Czech Republic were collected from April to June 2020. Among the 13/112 (11.6%) positive samples (Mlejnkova et al., 2020) from Queensland, Australia, 2/9 (22%) were positive (Ahmed et al., 2020). Among the Jaipur and Ahmedabad samples collected from India between May 3 and June 14, 2020 (Arora et al., 2020) and May 8 and 27, 2020 (Kumar et al., 2020), 2/6 (33.3%) and 20/20 (100%) were positive, respectively. From March 20 to April 9, 2020, among samples collected from various locations in 17 districts, 21/78 (26.9%) were positive (Sharif), and Lahore samples were collected on alternate days between July 13–25, 2020. We found that 22/28 (78.6%) of the positive samples (Yaqub et al., 2020) from Wuhan Pulmonary Hospital, China, were collected after the initial identification of 2019-nCoV. Moreover, 4/15 (26.7%) (Zhang et al., 2020) samples collected from Zhongnan Hospital of Wuhan University from January 20 to February 9, 2020, were positive. We found that 28/42 (66.67%) (Chen et al., 2020)

samples from the USA were positive. The samples were collected from Massachusetts (Wu et al., 2020), Bozeman (Nemudryi et al., 2020), New Haven (Peccia et al., 2020), and Southeast Virginia (Gonzalez et al., 2020) from March 18–25, 2020, March 27, 2020, March 19 to May 1, 2020, and March 11 to July 27, 2020, respectively, and 10/14 (71%), 7/7 (100%), 44/44 (100%), and 98/198 (49.5%) samples were positive, respectively. (Fig. 4).

Medema et al. (2020) first reported detection of SARS-CoV-2 in wastewater system samples collected from WWTPs in the Netherlands; 14/24 (58%) untreated wastewater samples were positive. In this first study, we used two different commercial systems, the RNeasy Power Microbiome Kit and the Nuclisens kit, in combination with the semi-automated KingFisher mL purification system to extract RNA from SARS-CoV-2 viral particles in wastewater samples. For identification, the one-step RT-qPCR method was used with primers specific for three regions (N1-N3) of the nucleocapsid protein gene (N) and envelope protein gene (E). A quantitative culture assay for F-specific RNA phage was additionally performed in this study to indirectly evaluate the efficacy of SARS-CoV-2 recovery through the purification and concentration treatment steps, and the effect of these sample processing steps on the viability of the viruses was examined.

Wurtzer et al. (2020) investigated 23/23 (100%) untreated wastewater samples that were found to be positive; the samples were collected from three main WWTPs in Paris and analysed using RT-qPCR with primers mainly targeting the RNA-dependent RNA polymerase gene (RdRP) and envelope protein gene (E). Following concentration via ultracentrifugation, the viral genome was extracted using an optimized protocol from the commercial PowerFecal Pro kit on a QIA Symphony extractor (Qiagen).

Through RT-PCR, La Rosa et al. (2020) assessed 6/12 (50%) untreated wastewater samples from an Italian WWTP that were SARS-CoV-2 positive. This study used a group of primers for SARS-CoV-2: a newly built set specifically targeting ORF1ab and an available set mainly intended for the pharyngeal swab, specific for the spike protein gene (S). In this study, wide-range primers, which were primarily developed before quantifying the new coronavirus strain, were also examined,

amplifying a conserved region of the ORF1ab of Coronaviridae members. In contrast, these specific primers did not give signals due to nucleotide variations in this region discovered with subsequent sequencing of the recent SARS-CoV-2 strain.

Using RT-qPCR, Ahmed et al. (2020) found that 2/9 (22%), with a maximum concentration of 1.2×10^2 GC/L, wastewater samples were SARS-CoV-2 positive in Australia, Queensland. In this study, RT-qPCR was used with the primers N_Sarbeco and NIID_2019-nCOV_N specific for the nucleocapsid protein gene (N). A one-step kit RT-qPCR was adopted, and reverse transcription and qPCR occurred properly in the identical reaction.

Based on RT-qPCR analysis, Kumaret et al. (2020) reported that 20/20 (22%) wastewater samples were positive for SARS-CoV-2, with a concentration ranging from 5.6×10^2 – 3.5×10^2 GC/L, in Ahmedabad, India. In this study, the viral RNA genome was recovered using a NucleoSpin® RNA Virus Kit (Macherey-Nagel GmbH and Co. KG, Germany). RT-qPCR was employed with primers targeting the Spike protein gene (S), nucleocapsid protein gene (N), and ORF1ab.

Yaqub et al. (2020) reported that 21/78 (26.9%) wastewater samples from Lahore, Pakistan, were positive for SARS-CoV-2 at concentrations ranging from 2.67×10^2 to 3.60×10^4 GC/L, and the quantitative analysis was performed using RT-qPCR. The viral RNA genome was recovered in BSL-3 of IM, UVAS, using RT-qPCR with primers targeting the ORF1ab.

Or et al. (2020) reported 10/26 (38.5%) wastewater samples collected from various SARS-CoV-2-positive samples detected in Israel, and the quantitative analysis was conducted using RT-qPCR. This study examined the viral RNA genome isolated using a Spin star viral nucleic acid kit 1.0 (ADT Biotech, Phileo Damansara 1, Petaling Jaya Part No.811803). RT-qPCR with primers targeting the Envelope protein gene (E) was also employed.

Using RT-qPCR, Nemudryi et al. (2020) found that 7/7 (22%) wastewater samples from Bozeman, USA, were positive for SARS-CoV-2, with a maximum concentration $> 3 \times 10^5$ GC/L. In this study, Nemudryi et al. (2020) also assessed the phylogenetic relationship of the isolated SARS-CoV-2 genome with the other global sequences by using

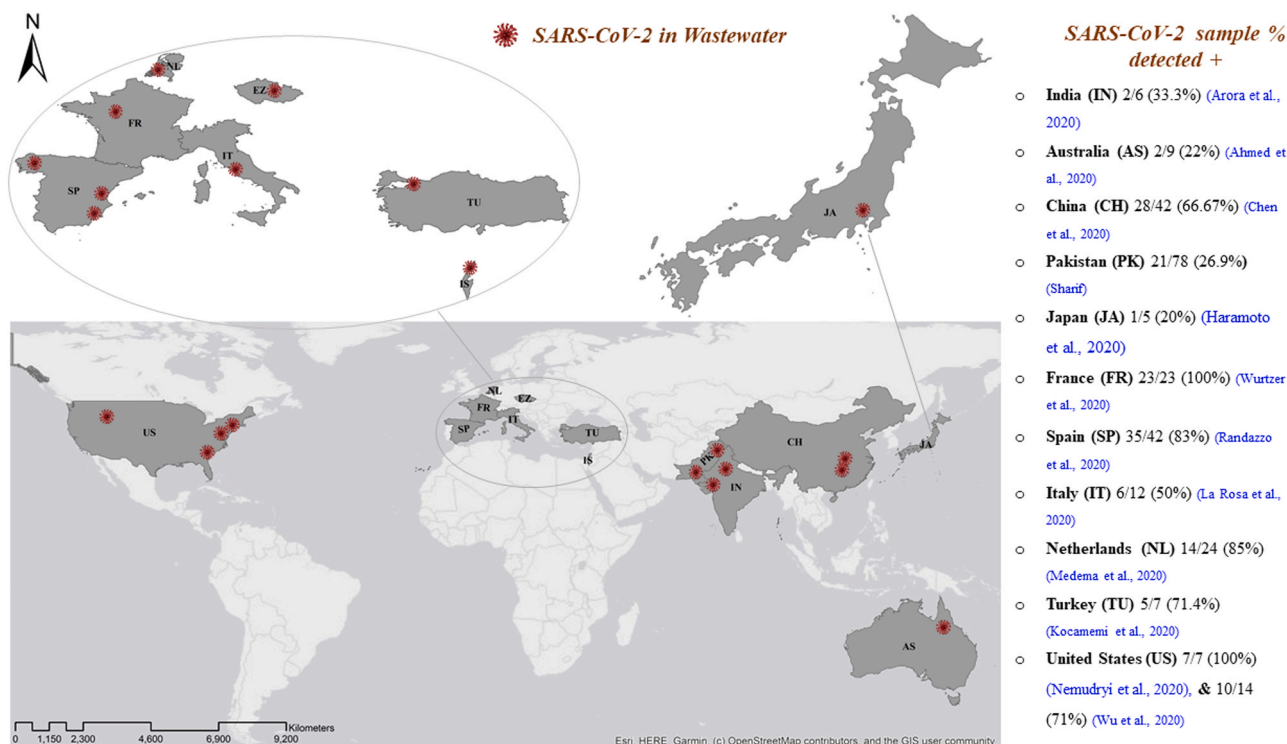


Fig. 4. Spatial distribution of SARS-CoV-2 in wastewater systems worldwide.

ten available primer pairs for amplification together with non-quantitative RT-PCR and sequenced several polymorphous regions diffused in the genome. In contrast, Wu et al. (2020) assessed 7/7 (22%) samples with a maximum concentration $> 2 \times 10^4$ GC/L, Peccia et al. (2020) assessed 44/44 (100%) with a concentration ranging from 1.7×10^6 to 4.6×10^8 GC/L, and Gonzalez et al. (2020) evaluated 98/198 (49.5%) samples with a concentration ranging from 10^1 to 10^4 GC/L

among SARS-CoV-2-positive wastewater samples detected in Massachusetts, New Haven, and Southeast Virginia, USA, respectively.

Similarly, Randazzo et al. (2020a) evaluated 35/42 (83%), Randazzo et al. (2020b) reported 12/15 (80%) samples with concentrations ranging from 10^5 to 10^6 GC/L, and Balboa et al. (2020) assessed 2/18 (11.1%) secondary and 5/5 (100%) untreated wastewater samples that were SARS-CoV-2-positive in Murcia, Valencia, and Ourense, Spain,

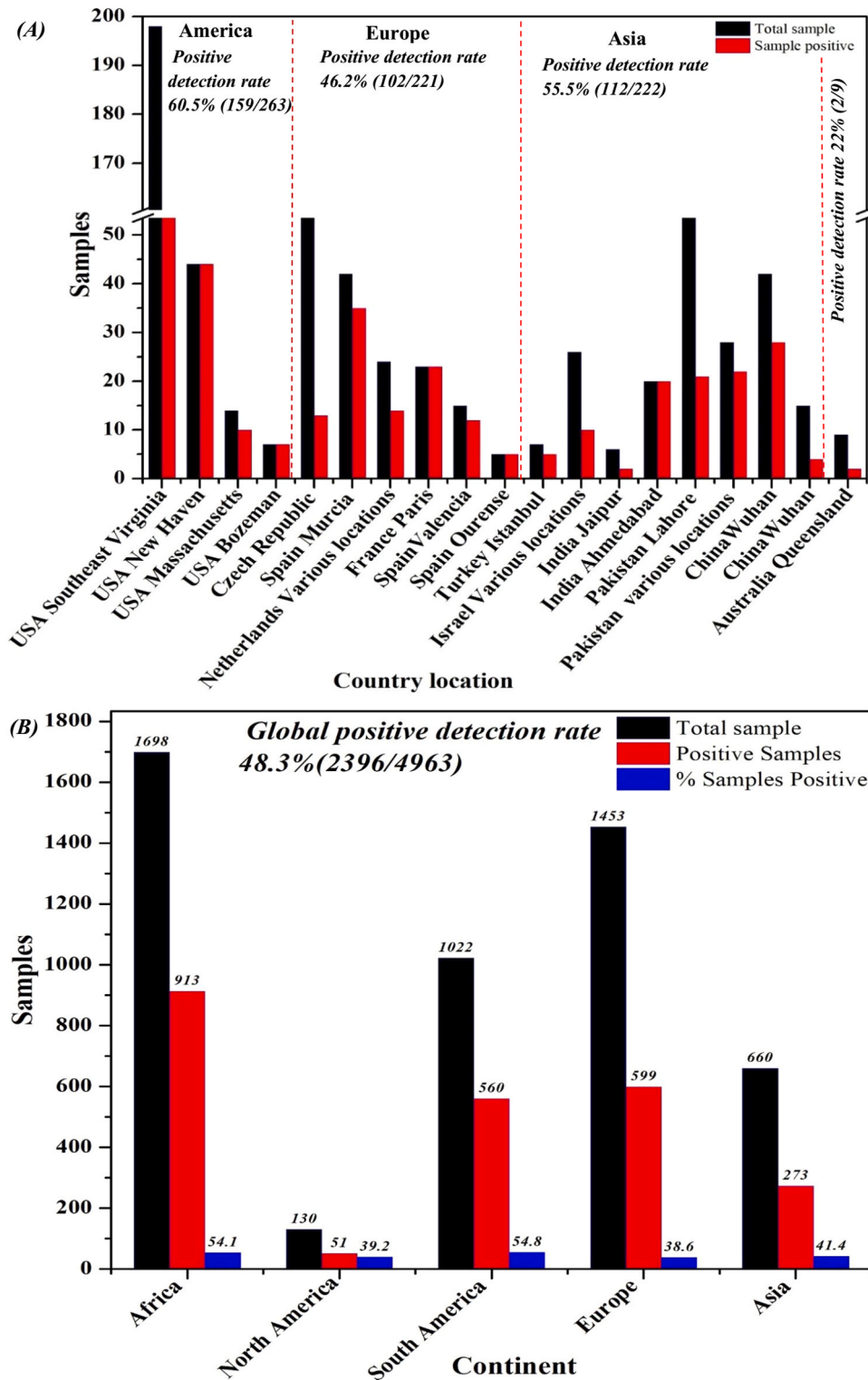


Fig. 5. SARS-CoV-2 detected in effluent wastewater systems in different countries located on the American, Asian, and European continents and the positive detection rates (A). Global positive detection rate (B).

respectively. Furthermore, a study conducted by Vallejo et al. applied a wastewater detection approach in the urban area of A Coruña (Spain) with the key objective of developing novel statistical regression models that could be used to assess the dynamics of the COVID-19 epidemic among the population of 369,098. The results of the regression models suggested that the real number of individuals infected with COVID-19 was determined with a reliability of approximately 90% (Vallejo et al., 2020).

A study in Wuhan, China, conducted by Zhang et al. (2020) revealed that SARS-CoV-2 genomes were not detected in the influent of a hospital septic tank (after a primary disinfection tank). Nonetheless, 4/15 (26.7%) in effluent samples were found to be positive, with concentrations ranging from 0.50×10^3 to 1.87×10^5 GC/L, after disinfection with sodium hypochlorite. The SARS-CoV-2 viral genomes in the septic tank effluent were detected after the free chlorine concentration had dropped to a non-detectable level. The presence in the influent and absence in the effluent indicated the release of embedded SARS-CoV-2 in human stool particles, which provided protection from the treatment process. Though a viability analysis was not performed in the latter research, the results suggested the need for more research to verify that the wastewater or sewage treatment systems are not SARS-CoV-2-spreading pathways.

Additionally, details regarding the distribution of these reports, the nucleic acid extraction and detection/quantification methods used, the concentration/pre-treatment methods used, the target genes, the total number of samples analysed, the water type, the number of positive samples, and the concentration range (GC/L) of SARS-CoV-2 in wastewater systems worldwide are presented in Table 2.

Overall, our results showed that the total positive SARS-CoV-2 detection rate in effluent wastewater systems worldwide is approximately 52.5% (375/715). The positive detection rates measured in America, Asia, Europe, and Australia were 60.5% (159/263), > 55.5% (112/222), > 46.2% (102/221) and > 22% (2/9), respectively (Fig. 5A and B). These results indicate that the incidence of SARS-CoV-2 in effluent wastewater systems poses a severe epidemiological health threat in America, Asia, and Europe.

Nevertheless, despite various studies showing that transmission of the virus can occur via the faecal-oral axis (Ding and Liang, 2020; Vallejo et al., 2020), inadequate evidence is available to confirm this route of transmission (Vallejo et al., 2020; Ronchi et al.). Moreover, no evidence is available to demonstrate infection via wastewater, which might lead to uncertainty regarding the presence of SARS-CoV-2 in wastewater systems (Cha et al., 2020; Chin et al., 2020; Yeo et al., 2020). Considering that several respiratory viruses other than EV, including SARS-CoV-2, have been identified in wastewater systems, other respiratory infectious disease-causing viruses in sewage still require attention to reduce the risk of environmental transmission through avenues such as aerosol inhalation.

5. Understanding the ongoing COVID-19 pandemic via wastewater surveillance

With respect to understanding ongoing infectious diseases via wastewater surveillance, WBE is an important approach that uses wastewater to identify the transmission of viruses to the public, and it provides an opportunity to assess the occurrence, distribution, and genetic diversity of viruses (Choi et al., 2018; Kitajima et al., 2020). In comparison to patient testing, WBE has advantages; for instance, it is a cost-efficient method of acquiring extensive population data and of early detection (Hart and Halden, 2020). WBE can now be used to detect and manage SARS-CoV-2, which is associated with severe infectious disease transmission among communities (Bivins et al., 2020). A conceptual model of a WBE and early warning system for epidemics of infectious diseases caused by SARS-CoV-2 pathogens is presented in Fig. 2 (Mao et al., 2020; Mao et al., 2020).

One study has shown that SARS-CoV-2 viral RNA is detectable in

human faeces from days to a week prior to the start of symptoms (Chen et al., 2020). Similarly, another study suggested that monitoring SARS-CoV-2 RNA concentrations in wastewater might predict COVID-19 epidemics seven days before they are revealed by individual patient testing and three days before they are revealed by hospital admission (Peccia et al., 2020). Thus, these studies conclude that the presence of viral DNA in wastewater is a significant indicator that can be used to detect hotspots in local community regions. Therefore, while the tool has a comparatively low cost, it offers an early warning system that can be used to identify new epidemics, trends in existing outbreaks, and the occurrence of contagions.

SARS-CoV-2 is known to primarily cause asymptomatic/pauci-symptomatic infections (Prezioso et al., 2020), and it is difficult to assess the actual degree of viral spread/circulation among the public and make comparisons among diverse countries that have differing clinical analytic testing competencies and may even use different diagnostic approaches or assays (Kitajima et al., 2020). Several uncertainties associated with the use of WBE have been noted; these include changes in the rate of viral excretion by infected persons (Choi et al., 2018), temporal delays, spatial inconsistencies due to travel and time, precipitation dilution, inactivation during sample transport, infrequent clinical testing, the stability of the viral genome in wastewater, sampling variability, and the lack of sensitive detection assays that can detect low virus loads, and these uncertainties limit the technique's ability to detect and quantify viruses (Kitajima et al., 2020). Therefore, in the future, additional work should be performed in this research field, including efficient standardisation of sampling, improvements in quality control, and development of analysis methods. For example, Bivins et al. called for a global collaborative to maximise contributions in the fight against COVID-19 using wastewater analysis (Bivins et al., 2020).

Despite these limitations, several efforts to develop wastewater surveillance methods for SARS-CoV-2 are ongoing (Hart and Halden, 2020; Daughton, 2020). As mentioned above, there are several preliminary reports on the molecular detection of SARS-CoV-2 viral RNA in wastewater in the Netherlands, Turkey, India, Pakistan, Australia, France, and the USA. The findings of these reports and other ongoing efforts worldwide might contribute to epidemiological modelling of the occurrence of SARS-CoV-2 in communities and might serve as an indicator of its threat to communities endeavouring to slow the spread of the contagion. To increase human acceptance of wastewater scrutiny, an agenda highlighting the moral issues associated with primary access to hygiene, privacy, and privileges might be required (Kitajima et al., 2020). WBE has the advantage of providing epidemiological knowledge regarding public infection/disease occurrence without identifying the affected individuals, which occurs when the outcomes of medical diagnoses during current COVID-19 epidemics are tabulated (Hirano and Murakami, 2020).

6. Conclusions and future outlook

A variety of viral pathogens are frequently reported in sewage in different countries on diverse continents, including Africa, North America, Asia, Europe, South America, and Australia. Based on individual studies reporting the number of samples positive for viruses, our results showed that the total positive detection rate for various human viruses such as NoVs, EVs, HAV, and AdVs in effluent wastewater systems worldwide is approximately 48.3% (2396/4963). Detection rates of 54.8% (560/1022), 54.1% (1689/913), 41.4% (273/660), 39.2% (51/130), and 38.6% (599/1553) were measured in South America, Africa, Asia, North America, and Europe, respectively.

Although some viral pathogens change seasonally, most are irregularly detected. In South Africa, Tunisia, and Egypt, the effluents of some sewage treatment plants do not meet standards and represented a potential threat to public health through environmental transmission when HAdV entered the aqueous environment. Moreover, municipal wastewater effluents that contain a variety of human viruses can circulate in

the environment when the water is used for agricultural irrigation or for wastewater reclamation and reuse. SARS-CoV-2 has been detected in wastewater from France, the Netherlands, the Czech Republic, Australia, Italy, Israel, Japan, Spain, Turkey, India, Pakistan, China, and the USA. Worldwide, the positive detection rate for SARS-CoV-2 in effluent wastewater systems is approximately 52.5% (375/715); this is obviously linked to the current pandemic. The rank order of SARS-CoV-2 positive detection rates is America > Asia > Europe > Australia. Based on our findings, the levels of SARS-CoV-2 and other viruses in effluent wastewater systems in America are comparatively higher than those in the rest of the world. The high incidence of SARS-CoV-2, the causative agent of the COVID-19 epidemic, and other viruses in American wastewater systems indicates that adverse human health effects may be higher in America than on other continents. Wastewater may also pose a risk to environmental and public health, which indicates that the fate of SARS-CoV-2 and its transfer in the environment must be better understood.

The foregoing considerations show that it is essential to take adequate measures to monitor the risk of sewage-mediated transmission. Although viral pathogens in sewage represent a risk, sewage can also provide efficient information and an early warning of the presence of infectious viral diseases, and this information can be used to improve public security in certain areas, such as in the community. In recent years, the use of WBE to assess drug abuse in community populations has produced initial results.

Some experts have attempted to use sewage analysis to screen for potential viral carriers and asymptomatic patients, and this approach has developed into an early warning system. Several efforts to develop WBE surveillance methods for SARS-CoV-2 via wastewater are ongoing. WBE provides an efficient, low-cost surveillance tool and an early warning system for identification of new epidemics, evaluation of trends in existing outbreaks and prediction of the occurrence of contagions.

These global reports might contribute to epidemiological modelling of the occurrence of SARS-CoV-2 in communities and help investigators determine how to use wastewater information as a threat indicator for communities endeavouring to slow the spread of the contagion.

Knowledge gaps remain regarding the possible role of wastewater systems in the transmission of SARS-CoV-2 viral RNA. The presence of SARS-CoV-2 viral RNA in different environmental matrices, including wastewater systems, is unknown.

Ongoing studies have suggested that the stability of SARS-CoV-2 viral RNA in water is similar to its stability in aerosols and on surfaces. In future research, similar tools should be used to measure the stability of SARS-CoV-2 viral RNA in different water systems.

At present, RT-qPCR assays are mainly designed for medical specimen testing and are being used to detect SARS-CoV-2 viral RNA in wastewater samples. The application of different assays to different water matrices might produce conflicting results. Current WBE surveillance methods must focus more attention on improving the sensitivity of SARS-CoV-2 viral RNA detection in wastewater systems.

In the future, individuals belonging to communities in some countries may face some degree of health risk from various viruses present in wastewater systems. On the one hand, adequate measures must be taken to prevent the risk of wastewater transmission; on the other hand, the presence of viruses in wastewater can be used to provide new perspectives. For example, we can make full use of the information available from sewage to evaluate public health and to further prevent the outbreak of large-scale infectious diseases while preventing the spread of viral pathogens in wastewater systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2021.125439](https://doi.org/10.1016/j.jhazmat.2021.125439).

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