Journal of Hazardous Materials, Volume 414, July 2021, Article number 125439
DOI:10.1016/j.jhazmat.2021.125439

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

Waqar Ali[a], Hua Zhang[a*], Zhenglu Wang[b], Chuanyu Chang[a], Asif Javed[c], Kamran Ali[d], Wei Du[e], Nabeel Khan Niazi[f], Kang Mao[a*], and Zhugen Yang[g]

[a] *State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, 550081, China*

[b] *Key Laboratory of Marine Hazards Forecasting, Ministry of Natural Resources, College of Oceanography, Hohai University, Nanjing, 210098, PR China*

[c] *Department of Earth and Environmental Sciences, Bahria University Islamabad*

[d] *Institute of Environmental Sciences and Engineering (IESE), School of Civil and Environmental Engineering (SCEE), National University of Science and Technology (NUST), Islamabad 44000, Pakistan*

[e] *Key Laboratory of Geographic Information Science of the Ministry of Education, School of Geographic Sciences, East China Normal University, Shanghai 200241, PR China*

[f] *Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad - 38040, Pakistan*

[g] *Cranfield Water Science Institute, Cranfield University, Cranfield, MK43 0AL, United Kingdom*

*Corresponding author

Email: zhanghua@mail.gyig.ac.cn (Prof. Hua Zhang)

Contents

Abstract

Viruses are omnipresent and persistent in wastewater, which poses a risk to human health. In this review, we summarize 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.

Keywords: Viruses; SARS-CoV-2; Spatial distribution; Wastewater; Human health hazards.

1. Introduction

Viruses are omnipresent and persistent in raw and treated wastewater, which is a concern because they can pose risks to human health [1]. A significant source of viruses in wastewater is human faecal matter, mainly from known infected persons with or without indications [2]. Water-borne viruses, such as Noroviruses (NoVs), enteroviruses (EVs), Hepatitis A virus (HAV), and Adenoviruses (AdVs), are widely distributed in wastewater [3]. Another example, SARS-CoV-2, is responsible for the COVID-19 pandemic that is currently affecting the world [4, 5]. SARS-CoV-2 has also been detected in the faeces of infected patients [6] and in wastewater [6, 7]. 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 [6, 7]. To date, studies have identified SARS-CoV-2 in the wastewater systems of different countries, including Australia [8], France [9], Italy [10], the Netherlands [11], Spain [12], the Czech Republic [13], Japan [14], Turkey [15], Israel [16], India [17], Pakistan, [18, 19], China [20, 21] and the USA [22]. 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 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 summarize 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 [23].

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 TCID 50 of influenza virus, AdVs, and rhinovirus were reported to infect 50% of the tested population [24]. Likewise, low doses of the enteric viruses, HAV, NoV, poliovirus rotavirus, and echovirus, also caused infection in at least some of the volunteers tested [24].

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 [25]. Human enteric viruses pose a risk of severe disease and mortality in high-risk populations, including children, older adults, and immunocompromised patients [26]. Enteric viruses are mainly produced inside human host cells and are substantially transmitted through the faecal-oral route [27].

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 [28]. Human enteric viruses are more stably resistant to environmental alterations than bacteriological endospores [28]. These human enteric viruses multiply inside the digestive tracts of their hosts, who then excrete them in faeces at significant quantities of up to 10^{11} viral particles/g stool for periods ranging from days to months. Accordingly, wastewater is likely to contain a considerable abundance of enteric viruses [23].

These effluents are generally treated at wastewater treatment plants (WWTPs) that

are not explicitly designed to remove human enteric viruses [29]. 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 [23]. 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 [30]. 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 [31]. 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 [31]. These viruses have been found in wastewater in France [32], Uruguay [33], Spain [34], Canada [35], Japan [36], New Zealand [37], Egypt [38], and India [31].

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 [39]. 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 10^{11} particles/g stool [40].

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 [41].

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 [32], Uruguay [33], Tunisia [42], the USA [43, 44], Brazil [45], Canada [46], Greece [47], and Japan [48].

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 [49]. 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 [50].

The EVs include poliovirus, coxsackievirus (CB) groups A and B, and echoviruses (E) [51]. To date, more than 100 enterovirus serotypes have been identified, including more than 70 serotypes that have been discovered in humans [52]. 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 [49]. In the environment, EVs can survive for long periods at pH values ranging from acidic to alkaline (3 to 10), mainly at low temperatures [53]. EVs such as coxsackievirus serotypes B-3, B-4, and B-5 and E1, E7, and E11 were significantly quantified in wastewater [49].

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 [54]. At least 100 human EV types have been identified. These viruses have been found in wastewater in Iran [55], the USA [43, 56], Italy [57], Canada [46] [58], Greece [47], and Japan [48]. 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 [59]. 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 [59].

Because it is a non-enveloped virus, HAV is stable in the environment for an extended period and is resistant to wastewater treatment processes [60]. Once excreted in faeces, HAV remains alive and can be disseminated through consumption of contaminated water and food [61]. HAV infection is associated with a wide range of common symptoms, including jaundice, vomiting, nausea, pale stools, fatigue, abdominal pain, and dark urine [62]. However, in some cases, mainly in teenagers and children under the age of six years, HAV can be asymptomatic [63]. HAV has been detected and reported in wastewater in various countries, including South Africa [64], Tunisia [59], France [32], and other industrialized countries [65].

Hepatitis E virus (HEV) is the principal aetiologic 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) [66]. ORF1 encodes non-structural proteins that are mainly involved in HEV replication, ORF2 encrypts the main capsid proteins, and ORF3 encrypts a small protein that may be involved in HEV–host interaction as well as in

virion morphogenesis [67].

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 [32, 68, 69], China [70], Colombia [71], Portugal [72], Italy [73], Germany [74], and some other industrialized countries [65].

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 [64, 75], Japan [76], Canada [35], the USA [43, 77], Tunisia [78], Italy [79], France [32], Japan [36], Uruguay [33], Egypt [80], the UK [81], Brazil [45], New Zealand [37], Canada [46], New Zealand [82], and Tunisia [83].

SARS-CoV-2 is a single-stranded positive-sense enveloped RNA virus that belongs to a group of SARS-related coronavirus species in the subgenus *Sarbecovirus* of the family Coronaviridae [25]. 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 [30, 84]. SARS-CoV-2 is also indistinctly linked to "classical" human CoV strains, such as HKU1, OC43, 229E, OC43, and NL63, in the genus *Alphacoronavirus* or *Betacoronavirus*; these strains were initially

differentiated in the 1960s and have been recognized to cause approximately 15 to 30% of common cold cases worldwide [25, 85].

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 uncharacterized aetiologic agent [86]. 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 [86]. 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 [9, 20, 25]. 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 [11].

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 [1]. 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 [1, 87]. 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 specificness, and viral pathogen detection [88]. 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 Corpuzet al. (2020) [1].

Additionally, this review summarizes 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 [14, 87, 89, 90]. Other studies collected samples before and after treatment to evaluate virus removal efficiency [90-92]. Aside from viruses in the wastewater, the occurrence of viruses in sludges generated in the examined WWTP operations has also been investigated [93, 94]. 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 characterizes non-enveloped viruses. Enveloped viruses contain an additional lipid bilayer membrane surrounded by capsid proteins [1]. During sample processing, lipid bilayer disruption can decrease recovery and affect the subsequent detection [1, 95]. This is of particular significance for investigations associated with coronaviruses (CoVs) [1]. 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 [1, 96]. 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 summarized 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 summarized in Table 1**.** We also describe in detail the viruses in wastewater in different areas in Part 1 of Supporting information.

Fig. 1.

Fig. 2.

Table 1.

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 [97], some studies have found that the levels of specific viral pathogens in sewage follow a pattern of imperceptible daily changes and obvious seasonal changes [98, 99]. 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 [100].

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 [100].

AiV-1 was also frequently detected in the colder months of the year in Fray Bentos, Bella Unión, Paysandú, and Salto, Uruguay [101]. Similarly, Ouardani 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 [59]. Ibrahim et al. [87] and Abe et al. [98] 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 [56]. 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 [102]. 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-hour 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 [103].

5.1. 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 [8, 25]. According to early descriptions, these human viruses cause severe diarrhoea as well as respiratory illness.

5.1. 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 [1, 104, 105]. 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.

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 [106]. 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 [25, 107].

5.2. 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 [25]. 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 [8, 25]. 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 [108].

In December 2019, cases of SARS-COV-2, the aetiological agent of the ongoing COVID-19 pandemic, were first reported [14]. 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 [109]. However, recent cases of SARS-CoV-2 have been accompanied by insistent shedding of RNA viruses in faecal samples in 27% to 89% of patients at concentrations ranging from 0.8 to 7.5 $\log^{10}$ genome copies/g [8, 21, 110]. Thus, it is clear that SARS-CoV-2 is also present in wastewater systems [111].

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 [112]. 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 [113]. Correspondingly, SARS-CoV-2 RNA was frequently detected in the faeces of COVID-19 patients who did not experience intestinal symptoms [103, 114]. Several studies reported that approximately 53.9% of patients were positive for faecal viral RNA [103], and some studies indicated that viruses were excreted in significant amounts in the patients' stools for an extended period of time [20]; moreover, in a few cases, the individual was confirmed to be negative based on respiratory samples after a month or more [103, 110]. In addition, the virus can significantly infect enterocytes in the human small intestine [115]. The occurrence of the infectious virus in human faeces highlights the potential for viral replication in the intestinal epithelium of infected individuals [116].

As shown in Fig. 3, recent studies have identified SARS-CoV-2 in 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 [11] were from Rome, Italy, collected between February 3 and April 2, 2020. There were 6/12 (50%) positive [10] samples from Yamanashi, Japan collected between March 17 and May 7, 2020. We found that 1/5 (20%) secondarily treated positive samples [14] were from Israel, collected from various locations at the end of March to April 2020. Additionally, 10/26 (38.5%) of the samples [16] from Istanbul, Turkey, collected on April 21, 2020 were positive; 5/7 (71.4%) [15] samples from Paris, France, collected from March 5 to April 23, 2020

were positive; 23/23 (100%) samples [9] 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 [89], and 12/15 (80%) were positive [12]. Ourense samples were collected twice a week from April 6 to 21 2020. The 2/18 (11.1%) secondary and 5/5 (100%) untreated positive samples [117] from the Czech Republic were collected from April to June 2020. Among the 13/112 (11.6%) positive samples [13] from Queensland, Australia, 2/9 (22%) were positive [8]. Among the Jaipur and Ahmedabad samples collected from India between May 3 and June 14, 2020 [86] and May 8 and 27, 2020 [17], 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 [18], and Lahore samples were collected on alternate days between July 13 to 25, 2020. We found that 22/28 (78.6%) of the positive samples [19] from Wuhan Pulmonary Hospital, China, were collected after the initial identification of 2019-NCoV. Moreover, 4/15 (26.7%) [21] samples collected from Zhongnan Hospital of Wuhan University from January 20 to February 9, 2020, were positive. We found that 28/42 (66.67%) [20] samples from the USA were positive. The samples were collected from Massachusetts [22], Bozeman [118], New Haven [119], and Southeast Virginia [120] from March 18 to 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.

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 QIAsymphony 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 to 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 > 3×10^5 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 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 > 2×10^4 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, 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% [103].

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 SARSCoV-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.

Fig. 3.

Fig. 4.

Fig. 5.

Table 2.

Nevertheless, despite various studies showing that transmission of the virus can occur via the faecal-oral axis [103, 121], inadequate evidence is available to confirm this route of transmission [103, 122]. 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 [114, 123, 124]. 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.

6. 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 [25, 125]. 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 [126]. WBE can now be used to detect and manage SARS-CoV-2, which is associated with severe infectious disease transmission among communities [127]. 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 [128, 129].

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 [20]. 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 [119]. 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 ~~WBE surveillance~~ 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/paucisymptomatic infections [130], 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 [25]. Several uncertainties associated with the use of WBE have been noted;

these include changes in the rate of viral excretion by infected persons [125], 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 [25]. Therefore, in the future, additional work should be performed in this research field, including efficient standardization of sampling, improvements in quality control, and development of analysis methods. For example, Bivins et al. called for a global collaborative to maximize contributions in the fight against COVID-19 using wastewater analysis [127].

Despite these limitations, several efforts to develop wastewater surveillance methods for SARS-CoV-2 are ongoing [126, 131]. 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 [25]. 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 [132].

7. 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.

Acknowledgements

This work was funded by the Science and Technology Program of Guizhou Province (Qiankehe Zhicheng [2020]4Y190), STS of CAS (KFJ-STS-QYZD-185); Chinese Academy of Sciences, President's International Fellowship Initiative (CAS, PIFI) for 2021 (2021PC0058); ZY thnaks UK NERC Fellowship grant (NE/R013349/2). Dr. Nabeel Khan Niazi is thankful to the University of Agriculture in Faisalabad, Pakistan. The authors would also like to thank all the anonymous reviewers for their constructive comments that significantly improved the quality of the manuscript.

Notes

The authors declare no competing financial interests.

References

[1] M.V.A. Corpuz, A. Buonerba, G. Vigliotta, T. Zarra, F. Ballesteros Jr, P. Campiglia, V. Belgiorno, G. Korshin, V. Naddeo, Viruses in wastewater: occurrence, abundance and detection methods, Science of the Total Environment, 745 (2020) 140910.

[2] E. Haramoto, M. Kitajima, A. Hata, J.R. Torrey, Y. Masago, D. Sano, H. Katayama, A review on recent progress in the detection methods and prevalence of human enteric viruses in water, Water research, 135 (2018) 168-186.

[3] G. La Rosa, L. Bonadonna, L. Lucentini, S. Kenmoe, E. Suffredini, Coronavirus in water environments: Occurrence, persistence and concentration methods-A scoping review, Water research, (2020) 115899.

[4] X. Peng, X. Xu, Y. Li, L. Cheng, X. Zhou, B. Ren, Transmission routes of 2019-nCoV and controls in dental practice, International Journal of Oral Science, 12

(2020) 1-6.

[5] L. Casanova, W.A. Rutala, D.J. Weber, M.D. Sobsey, Survival of surrogate coronaviruses in water, Water Research, 43 (2009) 1893-1898.

[6] M.L. Holshue, C. DeBolt, S. Lindquist, K.H. Lofy, J. Wiesman, H. Bruce, C. Spitters, K. Ericson, S. Wilkerson, A. Tural, G. Diaz, A. Cohn, L. Fox, A. Patel, S.I. Gerber, L. Kim, S. Tong, X. Lu, S. Lindstrom, M.A. Pallansch, W.C. Weldon, H.M. Biggs, T.M. Uyeki, S.K. Pillai, V.C.I. Washington State-nCo, First Case of 2019 Novel Coronavirus in the United States, New England Journal of Medicine, 382 (2020) 929-936.

[7] S. Mallapaty, How sewage could reveal true scale of coronavirus outbreak, Nature, (2020).

[8] W. Ahmed, N. Angel, J. Edson, K. Bibby, A. Bivins, J.W. O'Brien, P.M. Choi, M. Kitajima, S.L. Simpson, J. Li, First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community, Science of the Total Environment, (2020) 138764.

[9] S. Wurtzer, V. Marechal, J.-M. Mouchel, L. Moulin, Time course quantitative detection of SARS-CoV-2 in Parisian wastewaters correlates with COVID-19 confirmed cases, MedRxiv, (2020).

[10] G. La Rosa, M. Iaconelli, P. Mancini, G.B. Ferraro, C. Veneri, L. Bonadonna, L. Lucentini, E. Suffredini, First detection of SARS-CoV-2 in untreated wastewaters in Italy, Science of the Total Environment, (2020) 139652.

[11] G. Medema, L. Heijnen, G. Elsinga, R. Italiaander, A. Brouwer, Presence of SARS-Coronavirus-2 in sewage, MedRxiv, (2020).

[12] W. Randazzo, P. Truchado, E.C. Ferrando, P. Simon, A. Allende, G. Sanchez, SARS-CoV-2 RNA titers in wastewater anticipated COVID-19 occurrence in a low prevalence area, medRxiv, (2020).

[13] H. Mlejnkova, K. Sovova, P. Vasickova, V. Ocenaskova, L. Jasikova, E. Juranova, Preliminary Study of Sars-Cov-2 Occurrence in Wastewater in the Czech Republic, International Journal of Environmental Research and Public Health, 17 (2020) 5508.

[14] E. Haramoto, B. Malla, O. Thakali, M. Kitajima, First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan, medRxiv, (2020).

[15] B.A. Kocamemi, H. Kurt, S. Hacioglu, C. Yarali, A.M. Saatci, B. Pakdemirli, First Data-Set on SARS-CoV-2 Detection for Istanbul Wastewaters in Turkey, MedRxiv, (2020).

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

[17] M. Kumar, A.K. Patel, A.V. Shah, J. Raval, N. Rajpara, M. Joshi, C.G. Joshi, First proof of the capability of wastewater surveillance for COVID-19 in India through detection of genetic material of SARS-CoV-2, Science of the Total Environment,

(2020) 141326.

[18] S. Sharif, Detection of SARs-CoV-2 in wastewater, using the existing environmental surveillance network: An epidemiological gateway to an early warning for COVID-19 in communities.

[19] T. Yaqub, M. Nawaz, M.Z. Shabbir, M.A. Ali, I. Altaf, S. Raza, M.A.B. Shabbir, M.A. Ashraf, S.Z. Aziz, S.Q. Cheema, A longitudinal survey for genome-based identification of SARS-CoV-2 in sewage water in selected lockdown areas of Lahore city, Pakistan; a potential approach for future smart lockdown strategy, medRxiv, (2020).

[20] Y. Chen, L. Chen, Q. Deng, G. Zhang, K. Wu, L. Ni, Y. Yang, B. Liu, W. Wang, C. Wei, The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients, Journal of medical virology, (2020).

[21] W. Zhang, R.-H. Du, B. Li, X.-S. Zheng, X.-L. Yang, B. Hu, Y.-Y. Wang, G.-F. Xiao, B. Yan, Z.-L. Shi, P. Zhou, Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes, Emerging microbes & infections, 9 (2020) 386-389.

[22] F. Wu, A. Xiao, J. Zhang, X. Gu, W.L. Lee, K. Kauffman, W. Hanage, M. Matus, N. Ghaeli, N. Endo, SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases, MedRxiv, (2020).

[23] B. Prevost, F. Lucas, A. Goncalves, F. Richard, L. Moulin, S. Wurtzer, Large scale survey of enteric viruses in river and waste water underlines the health status of the local population, Environment international, 79 (2015) 42-50.

[24] S. Yezli, J.A. Otter, Minimum infective dose of the major human respiratory and enteric viruses transmitted through food and the environment, Food and Environmental Virology, 3 (2011) 1-30.

[25] M. Kitajima, W. Ahmed, K. Bibby, A. Carducci, C.P. Gerba, K.A. Hamilton, E. Haramoto, J.B. Rose, SARS-CoV-2 in wastewater: State of the knowledge and research needs, Science of the Total Environment, (2020) 139076.

[26] P.B. Pavlinac, G.C. John-Stewart, J.M. Naulikha, F.M. Onchiri, D.M. Denno, E.A. Odundo, B.O. Singa, B.A. Richardson, J.L. Walson, High-risk enteric pathogens associated with HIV-infection and HIV-exposure in Kenyan children with acute diarrhea, AIDS (London, England), 28 (2014) 2287.

[27] A.K. Bhunia, Biology of Microbial Pathogens, in: Foodborne Microbial Pathogens, Springer, 2018, pp. 25-42.

[28] K. Warriner, Pathogens in vegetables, in: Improving the safety of fresh fruit and vegetables, Elsevier, 2005, pp. 3-43.

[29] T. Prado, D.M. Silva, W.C. Guilayn, T.L. Rose, A.M.C. Gaspar, M.P. Miagostovich, Quantification and molecular characterization of enteric viruses detected in effluents from two hospital wastewater treatment plants, Water research, 45 (2011) 1287-1297.

[30] A. Bosch, R.M. Pintó, S. Guix, Human astroviruses, Clinical microbiology reviews, 27 (2014) 1048-1074.

[31] B. Prevost, F.S. Lucas, K. Ambert-Balay, P. Pothier, L. Moulin, S. Wurtzer, Deciphering the diversities of astroviruses and noroviruses in wastewater

treatment plant effluents by a high-throughput sequencing method, Appl. Environ. Microbiol., 81 (2015) 7215-7222.

[32] L. Kaas, L. Ogorzaly, G. Lecellier, V. Berteaux-Lecellier, H.-M. Cauchie, J. Langlet, Detection of Human Enteric Viruses in French Polynesian Wastewaters, Environmental Waters and Giant Clams, Food and Environmental Virology, 11 (2019) 52-64.

[33] A. Lizasoain, L.F.L. Tort, M. García, L. Gillman, A. Alberti, J.P.G. Leite, M.P. Miagostovich, S.A. Pou, A. Cagiao, A. Razsap, J. Huertas, M. Berois, M. Victoria, R. Colina, Human enteric viruses in a wastewater treatment plant: evaluation of activated sludge combined with UV disinfection process reveals different removal performances for viruses with different features, Letters in Applied Microbiology, 66 (2018) 215-221.

[34] F. López-Gálvez, W. Randazzo, A. Vásquez, G. Sánchez, L.T. Decol, R. Aznar, M.I. Gil, A. Allende, Irrigating Lettuce with Wastewater Effluent: Does Disinfection with Chlorine Dioxide Inactivate Viruses?, Journal of Environmental Quality, 47 (2018) 1139-1145.

[35] Y. Qiu, B.E. Lee, N.J. Ruecker, N. Neumann, N. Ashbolt, X. Pang, A one-step centrifugal ultrafiltration method to concentrate enteric viruses from wastewater, Journal of Virological Methods, 237 (2016) 150-153.

[36] N. Kobayashi, M. Oshiki, T. Ito, T. Segawa, M. Hatamoto, T. Kato, T. Yamaguchi, K. Kubota, M. Takahashi, A. Iguchi, T. Tagawa, T. Okubo, S. Uemura, H. Harada, T. Motoyama, N. Araki, D. Sano, Removal of human pathogenic viruses in a

down-flow hanging sponge (DHS) reactor treating municipal wastewater and health risks associated with utilization of the effluent for agricultural irrigation, Water Research, 110 (2017) 389-398.

[37] P. Gyawali, D. Croucher, J. Hewitt, Preliminary evaluation of BioFire FilmArray® Gastrointestinal Panel for the detection of noroviruses and other enteric viruses from wastewater and shellfish, Environmental Science and Pollution Research, 25 (2018) 27657-27661.

[38] M.N. Shaheen, E.M. Elmahdy, Environmental monitoring of astrovirus and norovirus in the Rosetta branch of the River Nile and the El-Rahawy drain, Egypt, Water Supply, 19 (2019) 1381-1387.

[39] A. Kundu, G. McBride, S. Wuertz, Adenovirus-associated health risks for recreational activities in a multi-use coastal watershed based on site-specific quantitative microbial risk assessment, Water research, 47 (2013) 6309-6325.

[40] T.-T. Fong, M.S. Phanikumar, I. Xagoraraki, J.B. Rose, Quantitative detection of human adenoviruses in wastewater and combined sewer overflows influencing a Michigan river, Appl. Environ. Microbiol., 76 (2010) 715-723.

[41] D.H.-W. Kuo, F.J. Simmons, S. Blair, E. Hart, J.B. Rose, I. Xagoraraki, Assessment of human adenovirus removal in a full-scale membrane bioreactor treating municipal wastewater, Water research, 44 (2010) 1520-1530.

[42] C. Ibrahim, A. Hassen, P. Pothier, S. Mejri, S. Hammami, Molecular detection and genotypic characterization of enteric adenoviruses in a hospital wastewater, Environmental Science and Pollution Research, 25 (2018) 10977-10987.

[43] C.P. Gerba, W.Q. Betancourt, M. Kitajima, C.M. Rock, Reducing uncertainty in estimating virus reduction by advanced water treatment processes, Water Research, 133 (2018) 282-288.

[44] M.A. Jahne, N.E. Brinkman, S.P. Keely, B.D. Zimmerman, E.A. Wheaton, J.L. Garland, Droplet digital PCR quantification of norovirus and adenovirus in decentralized wastewater and graywater collections: Implications for onsite reuse, Water Research, 169 (2020) 115213.

[45] A.S.F. Assis, T.M. Fumian, M.P. Miagostovich, B.P. Drumond, M.L. da Rosa e Silva, Adenovirus and rotavirus recovery from a treated effluent through an optimized skimmed-milk flocculation method, Environmental Science and Pollution Research, 25 (2018) 17025-17032.

[46] Y. Qiu, Q. Li, B.E. Lee, N.J. Ruecker, N.F. Neumann, N.J. Ashbolt, X. Pang, UV inactivation of human infectious viruses at two full-scale wastewater treatment plants in Canada, Water Research, 147 (2018) 73-81.

[47] A. Kaliakatsos, N. Kalogerakis, T. Manios, D. Venieri, Efficiency of two constructed wetland systems for wastewater treatment: removal of bacterial indicators and enteric viruses, Journal of Chemical Technology & Biotechnology, 94 (2019) 2123-2130.

[48] S. Tandukar, S.P. Sherchan, E. Haramoto, Applicability of crAssphage, pepper mild mottle virus, and tobacco mosaic virus as indicators of reduction of enteric viruses during wastewater treatment, Scientific Reports, 10 (2020) 3616.

[49] S. Tiwari, T.N. Dhole, Assessment of enteroviruses from sewage water and

clinical samples during eradication phase of polio in North India, Virology journal, 15 (2018) 157.

[50] S.M. Van Der Sanden, N. Sachs, S.M. Koekkoek, G. Koen, D. Pajkrt, H. Clevers, K.C. Wolthers, Enterovirus 71 infection of human airway organoids reveals VP1-145 as a viral infectivity determinant, Emerging microbes & infections, 7 (2018) 1-9.

[51] F.C. Tseng, H.C. Huang, C.Y. Chi, T.L. Lin, C.C. Liu, J.W. Jian, L.C. Hsu, H.S. Wu, J.Y. Yang, Y.W. Chang, Epidemiological survey of enterovirus infections occurring in Taiwan between 2000 and 2005: analysis of sentinel physician surveillance data, Journal of medical virology, 79 (2007) 1850-1860.

[52] S. Tiwari, T.N. Dhole, Assessment of enteroviruses from sewage water and clinical samples during eradication phase of polio in North India, Virology journal, 15 (2018) 1-8.

[53] R. Kocwa-Haluch, Waterborne enteroviruses as a hazard for human health, Polish Journal of Environmental Studies, 10 (2001) 485-488.

[54] M. Kitajima, B.C. Iker, I.L. Pepper, C.P. Gerba, Relative abundance and treatment reduction of viruses during wastewater treatment processes—identification of potential viral indicators, Science of the Total Environment, 488 (2014) 290-296.

[55] M. Moazeni, M. Nikaeen, M. Hadi, S. Moghim, L. Mouhebat, M. Hatamzadeh, A. Hassanzadeh, Estimation of health risks caused by exposure to enteroviruses from agricultural application of wastewater effluents, Water Research, 125 (2017)

104-113.

[56] N.E. Brinkman, G.S. Fout, S.P. Keely, Retrospective Surveillance of Wastewater To Examine Seasonal Dynamics of Enterovirus Infections, mSphere, 2 (2017) e00099-00017.

[57] F. Pennino, A. Nardone, P. Montuori, S. Aurino, I. Torre, A. Battistone, R. Delogu, G. Buttinelli, S. Fiore, C. Amato, M. Triassi, Large-Scale Survey of Human Enteroviruses in Wastewater Treatment Plants of a Metropolitan Area of Southern Italy, Food and Environmental Virology, 10 (2018) 187-192.

[58] A. Simhon, V. Pileggi, C.A. Flemming, J.R. Bicudo, G. Lai, M. Manoharan, Enteric viruses in municipal wastewater effluent before and after disinfection with chlorine and ultraviolet light, Journal of Water and Health, 17 (2019) 670-682.

[59] I. Ouardani, S. Turki, M. Aouni, J.L. Romalde, Detection and Molecular Characterization of Hepatitis A Virus from Tunisian Wastewater Treatment Plants with Different Secondary Treatments, Applied and Environmental Microbiology, 82 (2016) 3834-3845.

[60] G. La Rosa, M. Pourshaban, M. Iaconelli, M. Muscillo, Quantitative real-time PCR of enteric viruses in influent and effluent samples from wastewater treatment plants in Italy, Annali dell'Istituto superiore di sanita, 46 (2010) 266-273.

[61] P. Vohra, HEPATITISA INFECTION, Tropical Hepato-Gastroenterology, (2010) 277.

[62] S. Kaya, A.E. Eskazan, N. Ay, B. Baysal, M.V. Bahadir, A. Onur, R. Duymus,

Acute acalculous cholecystitis due to viral hepatitis A, Case reports in infectious diseases, 2013 (2013).

[63] M. Ciocca, Clinical course and consequences of hepatitis A infection, Vaccine, 18 (2000) S71-S74.

[64] M.A. Adefisoye, U.U. Nwodo, E. Green, A.I. Okoh, Quantitative PCR Detection and Characterisation of Human Adenovirus, Rotavirus and Hepatitis A Virus in Discharged Effluents of Two Wastewater Treatment Facilities in the Eastern Cape, South Africa, Food and Environmental Virology, 8 (2016) 262-274.

[65] H. Fenaux, M. Chassaing, S. Berger, C. Gantzer, I. Bertrand, E. Schvoerer, Transmission of hepatitis E virus by water: An issue still pending in industrialized countries, Water Research, 151 (2019) 144-157.

[66] S.S. Long, C.G. Prober, M. Fischer, Principles and practice of pediatric infectious diseases E-Book, Elsevier Health Sciences, 2017.

[67] X. Meng, Hepatitis E virus: animal reservoirs and zoonotic risk, Veterinary microbiology, 140 (2010) 256-265.

[68] T. Miura, S. Lhomme, J.-C. Le Saux, P. Le Mehaute, Y. Guillois, E. Couturier, J. Izopet, F. Abranavel, F.S. Le Guyader, Detection of Hepatitis E Virus in Sewage After an Outbreak on a French Island, Food and Environmental Virology, 8 (2016) 194-199.

[69] D. Courault, I. Albert, S. Perelle, A. Fraisse, P. Renault, A. Salemkour, P. Amato, Assessment and risk modeling of airborne enteric viruses emitted from wastewater reused for irrigation, Science of The Total Environment, 592 (2017)

512-526.

[70] H. Li, W. Li, R. She, L. Yu, Q. Wu, J. Yang, F. Hu, M.H. Soomro, R. Shi, W. Hao, Y. Zhao, J. Mao, Hepatitis E Virus Genotype 4 Sequences Detected in Sewage from Treatment Plants of China, Food and Environmental Virology, 9 (2017) 230-233.

[71] P.A. Baez, M.C. Lopez, A. Duque-Jaramillo, D. Pelaez, F. Molina, M.-C. Navas, First evidence of the Hepatitis E virus in environmental waters in Colombia, PLoS One, 12 (2017) e0177525.

[72] A. Matos, J.R. Mesquita, D. Gonçalves, J. Abreu-Silva, C. Luxo, M.S. Nascimento, First detection and molecular characterization of hepatitis E virus in water from wastewater treatment plants in Portugal, Annals of Agricultural and Environmental Medicine, 25 (2018) 364-367.

[73] F. Di Profio, I. Melegari, A. Palombieri, V. Sarchese, A. Arbuatti, P. Fruci, F. Marsilio, V. Martella, B. Di Martino, High prevalence of hepatitis E virus in raw sewage in Southern Italy, Virus Research, 272 (2019) 197710.

[74] S. Beyer, R. Szewzyk, R. Gnirss, R. Johne, H.-C. Selinka, Detection and Characterization of Hepatitis E Virus Genotype 3 in Wastewater and Urban Surface Waters in Germany, Food and Environmental Virology, (2020).

[75] O. Osuolale, A. Okoh, Human enteric bacteria and viruses in five wastewater treatment plants in the Eastern Cape, South Africa, Journal of Infection and Public Health, 10 (2017) 541-547.

[76] N. Yasui, M. Suwa, K. Sakurai, Y. Suzuki, J. Tsumori, K. Kobayashi, H.

Takabatake, S.T. Lee, N. Yamashita, H. Tanaka, Removal characteristics and fluctuation of norovirus in a pilot-plant by an ultrafiltration membrane for the reclamation of treated sewage, Environmental Technology, 37 (2016) 2793-2801.

[77] B.W. Schmitz, M. Kitajima, M.E. Campillo, C.P. Gerba, I.L. Pepper, Virus Reduction during Advanced Bardenpho and Conventional Wastewater Treatment Processes, Environmental Science & Technology, 50 (2016) 9524-9532.

[78] C. Ibrahim, N. Cherif, S. Hammami, P. Pothier, A. Hassen, Quantification and Genotyping of Rotavirus A within Two Wastewater Treatment Processes, CLEAN – Soil, Air, Water, 44 (2016) 393-401.

[79] V. Mezzanotte, F. Marazzi, M. Bissa, S. Pacchioni, A. Binelli, M. Parolini, S. Magni, F.M. Ruggeri, C. De Giuli Morghen, C. Zanotto, A. Radaelli, Removal of enteric viruses and Escherichia coli from municipal treated effluent by zebra mussels, Science of The Total Environment, 539 (2016) 395-400.

[80] N. Rizk, A.A.-. Herrawy, M. Gad, M. Shaheen, E. Elmahdy, Existence and Removal of Rotaviruses Group A and Cryptosporidium Species in a Wastewater Treatment Plant, Polish Journal of Environmental Studies, 28 (2019) 4331-4339.

[81] E.M. Adriaenssens, K. Farkas, C. Harrison, D.L. Jones, H.E. Allison, A.J. McCarthy, Viromic Analysis of Wastewater Input to a River Catchment Reveals a Diverse Assemblage of RNA Viruses, mSystems, 3 (2018) e00025-00018.

[82] H. Clemens, L. Pang, L.K. Morgan, L. Weaver, Attenuation of rotavirus, MS2 bacteriophage and biomolecule-modified silica nanoparticles in undisturbed silt loam over gravels dosed with onsite wastewater, Water Research, 169 (2020)

115272.

[83] C. Ibrahim, S. Hammami, P. Pothier, N. Khelifi, A. Hassen, The performance of biological and tertiary wastewater treatment procedures for rotaviruses A removal, Environmental Science and Pollution Research, 27 (2020) 5718-5729.

[84] D. Fox, What you need to know about the Wuhan coronavirus, Nature, (2020).

[85] M. Mesel-Lemoine, J. Millet, P.-O. Vidalain, H. Law, A. Vabret, V. Lorin, N. Escriou, M.L. Albert, B. Nal, F. Tangy, A human coronavirus responsible for the common cold massively kills dendritic cells but not monocytes, Journal of virology, 86 (2012) 7577-7587.

[86] S. Arora, A. Nag, J. Sethi, J. Rajvanshi, S. Saxena, S.K. Shrivastava, A.B. Gupta, Sewage surveillance for the presence of SARS-CoV-2 genome as a useful wastewater based epidemiology (WBE) tracking tool in India, MedRxiv, (2020).

[87] C. Ibrahim, S. Hammami, S. Mejri, I. Mehri, P. Pothier, A. Hassen, Detection of Aichi virus genotype B in two lines of wastewater treatment processes, Microbial Pathogenesis, 109 (2017) 305-312.

[88] M.H. Hjelmsø, M. Hellmér, X. Fernandez-Cassi, N. Timoneda, O. Lukjancenko, M. Seidel, D. Elsässer, F.M. Aarestrup, C. Löfström, S. Bofill-Mas, Evaluation of methods for the concentration and extraction of viruses from sewage in the context of metagenomic sequencing, PloS one, 12 (2017) e0170199.

[89] W. Randazzo, P. Truchado, E. Cuevas-Ferrando, P. Simón, A. Allende, G. Sánchez, SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area, Water Research, (2020) 115942.

[90] S. Balboa, M. Mauricio-Iglesias, S. Rodríguez, L. Martínez-Lamas, F.J. Vasallo, B. Regueiro, J.M. Lema, The fate of SARS-CoV-2 in wastewater treatment plants points out the sludge line as a suitable spot for incidence monitoring, medRxiv, (2020).

[91] H. Hamza, M. Leifels, M. Wilhelm, I.A. Hamza, Relative abundance of human bocaviruses in urban sewage in Greater Cairo, Egypt, Food and environmental virology, 9 (2017) 304-313.

[92] H. Hamza, I.A. Hamza, Oncogenic papillomavirus and polyomavirus in urban sewage in Egypt, Science of The Total Environment, 610 (2018) 1413-1420.

[93] E.M. Elmahdy, N.I. Ahmed, M.N. Shaheen, E.-C.B. Mohamed, S.A. Loutfy, Molecular detection of human adenovirus in urban wastewater in Egypt and among children suffering from acute gastroenteritis, Journal of water and health, 17 (2019) 287-294.

[94] W.M. El-Senousy, A. Abdel-Moneim, M. Abdel-Latif, M.H. El-Hefnawy, R.G. Khalil, Coxsackievirus B4 as a Causative Agent of Diabetes Mellitus Type 1: Is There a Role of Inefficiently Treated Drinking Water and Sewage in Virus Spreading?, Food and Environmental Virology, 10 (2018) 89-98.

[95] Y. Ye, R.M. Ellenberg, K.E. Graham, K.R. Wigginton, Survivability, partitioning, and recovery of enveloped viruses in untreated municipal wastewater, Environmental science & technology, 50 (2016) 5077-5085.

[96] Q. Wu, W.-T. Liu, Determination of virus abundance, diversity and distribution in a municipal wastewater treatment plant, Water research, 43 (2009) 1101-1109.

[97] K. Farkas, D.M. Cooper, J.E. McDonald, S.K. Malham, A. de Rougemont, D.L. Jones, Seasonal and spatial dynamics of enteric viruses in wastewater and in riverine and estuarine receiving waters, Science of The Total Environment, 634 (2018) 1174-1183.

[98] M. Abe, Y. Ueki, T. Miura, S. Kimura, Y. Suzuki, N. Sugawara, Y. Masago, T. Omura, S. Watanabe, Detection of Human Parechoviruses in Clinical and Municipal Wastewater Samples in Miyagi, Japan, in 2012–2014, Japanese Journal of Infectious Diseases, 69 (2016) 414-417.

[99] M. Kitajima, A.T. Rachmadi, B.C. Iker, E. Haramoto, C.P. Gerba, Temporal variations in genotype distribution of human sapoviruses and Aichi virus 1 in wastewater in Southern Arizona, United States, Journal of Applied Microbiology, 124 (2018) 1324-1332.

[100] K. Farkas, M. Marshall, D. Cooper, J.E. McDonald, S.K. Malham, D.E. Peters, J.D. Maloney, D.L. Jones, Seasonal and diurnal surveillance of treated and untreated wastewater for human enteric viruses, Environmental Science and Pollution Research, 25 (2018) 33391-33401.

[101] L. Burutarán, A. Lizasoain, M. García, L.F.L. Tort, R. Colina, M. Victoria, Detection and Molecular Characterization of Aichivirus 1 in Wastewater Samples from Uruguay, Food and Environmental Virology, 8 (2016) 13-17.

[102] G.T. Nguyen, J. Pu, T. Miura, H. Ito, S. Kazama, Y. Konta, A. Van Le, T. Watanabe, Oyster Contamination with Human Noroviruses Impacted by Urban Drainage and Seasonal Flooding in Vietnam, Food and Environmental Virology,

10 (2018) 61-71.

[103] J.A. Vallejo, S. Rumbo-Feal, K. Conde-Perez, A. Lopez-Oriona, J. Tarrio, R. Reif, S. Ladra, B.K. Rodino-Janeiro, M. Nasser, A. Cid, Highly predictive regression model of active cases of COVID-19 in a population by screening wastewater viral load, medRxiv, (2020).

[104] N. Zhang, L. Wang, X. Deng, R. Liang, M. Su, C. He, L. Hu, Y. Su, J. Ren, F. Yu, Recent advances in the detection of respiratory virus infection in humans, Journal of medical virology, 92 (2020) 408-417.

[105] A.H. Bruning, M.M. Leeflang, J.M. Vos, R. Spijker, M.D. de Jong, K.C. Wolthers, D. Pajkrt, Rapid tests for influenza, respiratory syncytial virus, and other respiratory viruses: a systematic review and meta-analysis, Clinical Infectious Diseases, 65 (2017) 1026-1032.

[106] T.-T. Fong, M.S. Phanikumar, I. Xagoraraki, J.B. Rose, Quantitative detection of human adenoviruses in wastewater and combined sewer overflows influencing a Michigan river, Applied and environmental microbiology, 76 (2010) 715-723.

[107] T.J. Wade, R.L. Calderon, E. Sams, M. Beach, K.P. Brenner, A.H. Williams, A.P. Dufour, Rapidly measured indicators of recreational water quality are predictive of swimming-associated gastrointestinal illness, Environmental health perspectives, 114 (2006) 24-28.

[108] X. Wang, J. Li, T. Guo, B. Zhen, Q. Kong, B. Yi, Z. Li, N. Song, M. Jin, W. Xiao, Concentration and detection of SARS coronavirus in sewage from Xiao Tang Shan Hospital and the 309th Hospital of the Chinese People's Liberation

Army, Water science and technology, 52 (2005) 213-221.

[109] F. Carraturo, C. Del Giudice, M. Morelli, V. Cerullo, G. Libralato, E. Galdiero, M. Guida, Persistence of SARS-CoV-2 in the environment and COVID-19 transmission risk from environmental matrices and surfaces, Environmental pollution, (2020) 115010.

[110] R. Wölfel, V.M. Corman, W. Guggemos, M. Seilmaier, S. Zange, M.A. Müller, D. Niemeyer, T.C. Jones, P. Vollmar, C. Rothe, Virological assessment of hospitalized patients with COVID-2019, Nature, 581 (2020) 465-469.

[111] W. Lodder, A.M. de Roda Husman, SARS-CoV-2 in wastewater: potential health risk, but also data source, The Lancet Gastroenterology & Hepatology, 5 (2020) 533-534.

[112] S.H. Wong, R.N. Lui, J.J. Sung, Covid-19 and the digestive system, Journal of gastroenterology and hepatology, 35 (2020) 744-748.

[113] W. Ali, K. Mao, H. Zhang, M. Junaid, N. Xu, A. Rasool, X. Feng, Z. Yang, Comprehensive review of the basic chemical behaviours, sources, processes, and endpoints of trace element contamination in paddy soil-rice systems in rice-growing countries, Journal of Hazardous Materials, (2020) 122720.

[114] C. Yeo, S. Kaushal, D. Yeo, Enteric involvement of coronaviruses: is faecal–oral transmission of SARS-CoV-2 possible?, The lancet Gastroenterology & hepatology, 5 (2020) 335-337.

[115] M.M. Lamers, J. Beumer, J. van der Vaart, K. Knoops, J. Puschhof, T.I. Breugem, R.B. Ravelli, J.P. van Schayck, A.Z. Mykytyn, H.Q. Duimel, SARS-CoV-2

productively infects human gut enterocytes, Science, (2020).

[116] J. Zhou, C. Li, X. Liu, M.C. Chiu, X. Zhao, D. Wang, Y. Wei, A. Lee, A.J. Zhang, H. Chu, Infection of bat and human intestinal organoids by SARS-CoV-2, Nature medicine, (2020) 1-7.

[117] S. Balboa, M. Mauricio-Iglesias, S. Rodriguez, L. Martínez-Lamas, F. Vasallo, B. Regueiro, J. Lema, The fate of SARS-COV-2 in WWTPs points out the sludge line as a suitable spot for monitoring. MedRxiv, in, 2020.

[118] A. Nemudryi, A. Nemudraia, K. Surya, T. Wiegand, M. Buyukyoruk, R. Wilkinson, B. Wiedenheft, Temporal detection and phylogenetic assessment of SARS-CoV-2 in municipal wastewater, MedRxiv, (2020).

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

[120] R. Gonzalez, K. Curtis, A. Bivins, K. Bibby, M. Weir, K. Yetka, H. Thompson, D. Keeling, J. Mitchell, D. Gonzalez, COVID-19 surveillance in Southeastern Virginia using wastewater-based epidemiology, Water research, (2020) 116296.

[121] S. Ding, T.J. Liang, Is SARS-CoV-2 also an enteric pathogen with potential Fecal-Oral transmission: a COVID-19 virological and clinical review, Gastroenterology, (2020).

[122] A. Ronchi, F. Pagliuca, F. Zito Marino, M. Montella, R. Franco, I. Cozzolino, Interventional cytopathology in COVID-19 ERA, Cytopathology.

[123] M.H. Cha, M. Regueiro, D.S. Sandhu, Gastrointestinal and hepatic manifestations of COVID-19: A comprehensive review, World Journal of Gastroenterology, 26 (2020) 2323.

[124] A. Chin, J. Chu, M. Perera, K. Hui, H.-L. Yen, M. Chan, M. Peiris, L. Poon, Stability of SARS-CoV-2 in different environmental conditions, medRxiv, (2020).

[125] P.M. Choi, B.J. Tscharke, E. Donner, J.W. O'Brien, S.C. Grant, S.L. Kaserzon, R. Mackie, E. O'Malley, N.D. Crosbie, K.V. Thomas, Wastewater-based epidemiology biomarkers: past, present and future, TrAC Trends in Analytical Chemistry, 105 (2018) 453-469.

[126] O.E. Hart, R.U. Halden, Computational analysis of SARS-CoV-2/COVID-19 surveillance by wastewater-based epidemiology locally and globally: Feasibility, economy, opportunities and challenges, Science of the Total Environment, (2020) 138875.

[127] A. Bivins, D. North, A. Ahmad, W. Ahmed, E. Alm, F. Been, P. Bhattacharya, L. Bijlsma, A.B. Boehm, J. Brown, Wastewater-Based Epidemiology: Global Collaborative to Maximize Contributions in the Fight Against COVID-19, in, ACS Publications, 2020.

[128] K. Mao, K. Zhang, W. Du, W. Ali, X. Feng, H. Zhang, The potential of wastewater-based epidemiology as surveillance and early warning of infectious disease outbreaks, Current Opinion in Environmental Science & Health, (2020).

[129] K. Mao, H. Zhang, Z. Yang, Can a Paper-Based Device Trace COVID-19 Sources with Wastewater-Based Epidemiology?, Environmental Science &

Technology, 54 (2020) 3733-3735.

[130] C. Prezioso, M.E. Marcocci, A.T. Palamara, G. De Chiara, V. Pietropaolo, The "Three Italy" of the COVID-19 epidemic and the possible involvement of SARS-CoV-2 in triggering complications other than pneumonia, Journal of Neurovirology, (2020) 1-13.

[131] C.G. Daughton, Wastewater surveillance for population-wide Covid-19: The present and future, Science of the Total Environment, (2020) 139631.

[132] T. Hirano, M. Murakami, COVID-19: A new virus, but a familiar receptor and cytokine release syndrome, Immunity, (2020).

[133] O. Onosi, N.S. Upfold, M.D. Jukes, G.A. Luke, C. Knox, The First Molecular Detection of Aichi Virus 1 in Raw Sewage and Mussels Collected in South Africa, Food and Environmental Virology, 11 (2019) 96-100.

[134] V.V. Mabasa, K.D. Meno, M.B. Taylor, J. Mans, Environmental Surveillance for Noroviruses in Selected South African Wastewaters 2015–2016: Emergence of the Novel GII.17, Food and Environmental Virology, 10 (2018) 16-28.

[135] M.N.F. Shaheen, S.E. Abd El-Daim, N.I. Ahmed, E.M. Elmahdy, Environmental monitoring of Aichi virus and human bocavirus in samples from wastewater treatment plant, drain, and River Nile in Egypt, Journal of Water and Health, 18 (2019) 30-37.

[136] H. Hamza, N.M. Rizk, M.A. Gad, I.A. Hamza, Pepper mild mottle virus in wastewater in Egypt: a potential indicator of wastewater pollution and the efficiency of the treatment process, Archives of Virology, 164 (2019) 2707-2713.

[137] E.M. Elmahdy, N.I. Ahmed, M.N.F. Shaheen, E.-C.B. Mohamed, S.A. Loutfy, Molecular detection of human adenovirus in urban wastewater in Egypt and among children suffering from acute gastroenteritis, Journal of Water and Health, 17 (2019) 287-294.

[138] C. Ibrahim, S. Hammami, N. Chérif, S. Mejri, P. Pothier, A. Hassen, Detection of Sapoviruses in two biological lines of Tunisian hospital wastewater treatment, International Journal of Environmental Health Research, 29 (2019) 400-413.

[139] M.F. Varela, I. Ouardani, T. Kato, S. Kadoya, M. Aouni, D. Sano, J.L. Romalde, Sapovirus in Wastewater Treatment Plants in Tunisia: Prevalence, Removal, and Genetic Characterization, Applied and Environmental Microbiology, 84 (2018) e02093-02017.

[140] M. Kitajima, A.T. Rachmadi, B.C. Iker, E. Haramoto, C.P. Gerba, Genetically distinct genogroup IV norovirus strains identified in wastewater, Archives of Virology, 161 (2016) 3521-3525.

[141] W.Q. Betancourt, C.P. Gerba, Rethinking the Significance of Reovirus in Water and Wastewater, Food and Environmental Virology, 8 (2016) 161-173.

[142] J.M. Fioretti, T.M. Fumian, M.S. Rocha, I.d.A.L. dos Santos, F.A. Carvalho-Costa, M.R. de Assis, J.d.S. Rodrigues, J.P.G. Leite, M.P. Miagostovich, Surveillance of Noroviruses in Rio De Janeiro, Brazil: Occurrence of New GIV Genotype in Clinical and Wastewater Samples, Food and Environmental Virology, 10 (2018) 1-6.

[143] T.M. Fumian, J.M. Fioretti, J.H. Lun, I.A.L. dos Santos, P.A. White, M.P.

Miagostovich, Detection of norovirus epidemic genotypes in raw sewage using next generation sequencing, Environment International, 123 (2019) 282-291.

[144] J.M. Fioretti, M.S. Rocha, T.M. Fumian, A. Ginuino, T.P. da Silva, M.R. de Assis, J.d.S. Rodrigues, F.A. Carvalho-Costa, M.P. Miagostovich, Occurrence of human sapoviruses in wastewater and stool samples in Rio De Janeiro, Brazil, Journal of Applied Microbiology, 121 (2016) 855-862.

[145] J. Levican, A. Levican, M. Ampuero, A. Gaggero, JC polyomavirus circulation in one-year surveillance in wastewater in Santiago, Chile, Infection, Genetics and Evolution, 71 (2019) 151-158.

[146] C.F. Estívariz, E.E. Pérez-Sánchez, A. Bahena, C.C. Burns, H.E. Gary, H. García-Lozano, G. Rey-Benito, S. Peñaranda, K.V. Castillo-Montufar, R.S. Nava-Acosta, J.S. Meschke, M.S. Oberste, I. Lopez-Martínez, J.A. Díaz-Quiñonez, Field Performance of Two Methods for Detection of Poliovirus in Wastewater Samples, Mexico 2016–2017, Food and Environmental Virology, 11 (2019) 364-373.

[147] M. Majumdar, S. Sharif, D. Klapsa, T. Wilton, M.M. Alam, M.D. Fernandez-Garcia, L. Rehman, G. Mujtaba, G. McAllister, H. Harvala, Environmental surveillance reveals complex enterovirus circulation patterns in human populations, in: Open forum infectious diseases, Oxford University Press US, 2018, pp. ofy250.

[148] T. Ahmad, Y. Waheed, S. Tahir, S.Z. Safi, K. Fatima, M.S. Afzal, I. Qadri, Frequency of HEV contamination in sewerage waters in Pakistan, The Journal of

Infection in Developing Countries, 4 (2010) 842-845.

[149] Y. Masago, Y. Konta, S. Kazama, M. Inaba, T. Imagawa, K. Tohma, M. Saito, A. Suzuki, H. Oshitani, T. Omura, Comparative Evaluation of Real-Time PCR Methods for Human Noroviruses in Wastewater and Human Stool, PLoS One, 11 (2016) e0160825.

[150] S. Kazama, Y. Masago, K. Tohma, N. Souma, T. Imagawa, A. Suzuki, X. Liu, M. Saito, H. Oshitani, T. Omura, Temporal dynamics of norovirus determined through monitoring of municipal wastewater by pyrosequencing and virological surveillance of gastroenteritis cases, Water Research, 92 (2016) 244-253.

[151] T. Ito, M. Kitajima, T. Kato, S. Ishii, T. Segawa, S. Okabe, D. Sano, Target virus log10 reduction values determined for two reclaimed wastewater irrigation scenarios in Japan based on tolerable annual disease burden, Water Research, 125 (2017) 438-448.

[152] M. Aminipour, M. Ghaderi, N. Harzandi, First Occurrence of Saffold Virus in Sewage and River Water Samples in Karaj, Iran, Food and Environmental Virology, 12 (2020) 75-80.

[153] S. Tavakoli Nick, S.R. Mohebbi, S.M. Hosseini, H. Mirjalali, M. Alebouyeh, Occurrence and molecular characterization of Torque teno virus (TTV) in a wastewater treatment plant in Tehran, Journal of Water and Health, 17 (2019) 971-977.

[154] P. Mancini, G. Bonanno Ferraro, M. Iaconelli, E. Suffredini, B. Valdazo-González, S. Della Libera, M. Divizia, G. La Rosa, Molecular characterization of

human Sapovirus in untreated sewage in Italy by amplicon-based Sanger and next-generation sequencing, Journal of Applied Microbiology, 126 (2019) 324-331.

[155] O. Dienus, E. Sokolova, F. Nyström, A. Matussek, S. Löfgren, L. Blom, T.J.R. Pettersson, P.-E. Lindgren, Norovirus Dynamics in Wastewater Discharges and in the Recipient Drinking Water Source: Long-Term Monitoring and Hydrodynamic Modeling, Environmental Science & Technology, 50 (2016) 10851-10858.

[156] M. Iaconelli, M. Divizia, S. Della Libera, P. Di Bonito, G. La Rosa, Frequent Detection and Genetic Diversity of Human Bocavirus in Urban Sewage Samples, Food and Environmental Virology, 8 (2016) 289-295.

[157] E. Gonzales-Gustavson, M. Rusiñol, G. Medema, M. Calvo, R. Girones, Quantitative risk assessment of norovirus and adenovirus for the use of reclaimed water to irrigate lettuce in Catalonia, Water Research, 153 (2019) 91-99.

[158] J.H. Lun, N.D. Crosbie, P.A. White, Genetic diversity and quantification of human mastadenoviruses in wastewater from Sydney and Melbourne, Australia, Science of the Total Environment, 675 (2019) 305-312.

[159] B. Hughes, D.J. Beale, P.G. Dennis, S. Cook, W. Ahmed, Cross-Comparison of Human Wastewater-Associated Molecular Markers in Relation to Fecal Indicator Bacteria and Enteric Viruses in Recreational Beach Waters, Applied and Environmental Microbiology, 83 (2017) e00028-00017.

List of Figures

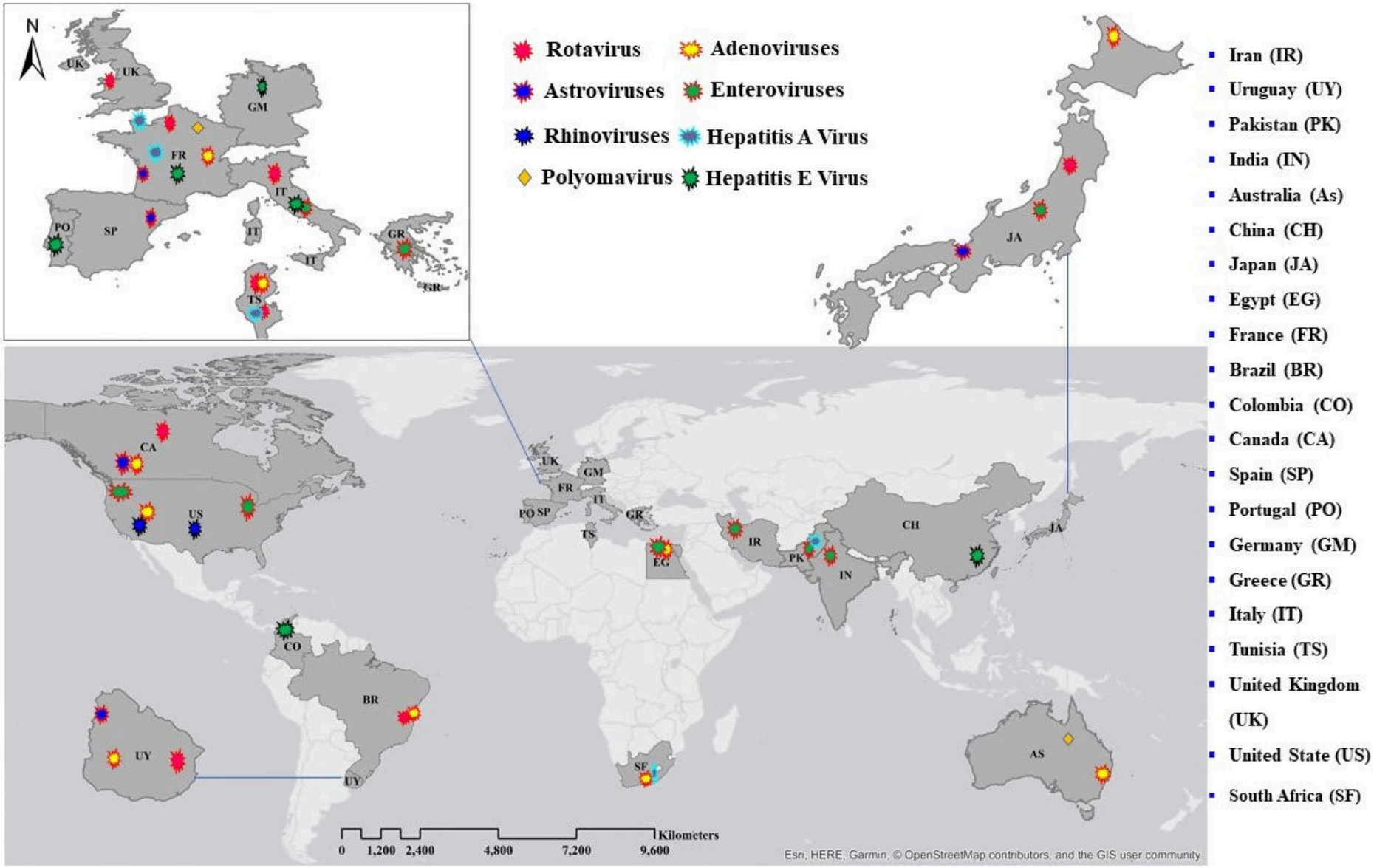

N
Rotavirus
Adenoviruses
Astroviruses
Enteroviruses
Rhinoviruses
Hepatitis A Virus
Polyomavirus
Hepatitis E Virus
Iran (IR)
Uruguay (UY)
Pakistan (PK)
India (IN)
Australia (As)
China (CH)
Japan (JA)
Egypt (EG)
France (FR)
Brazil (BR)
Colombia (CO)
Canada (CA)
Spain (SP)
Portugal (PO)
Germany (GM)
Greece (GR)
Italy (IT)
Tunisia (TS)
United Kingdom (UK)
United State (US)
South Africa (SF)
Kilometers
0 1,200 2,400 4,800 7,200 9,600
Esri, HERE, Garmin, © OpenStreetMap contributors, and the GIS user community

Fig. 1.

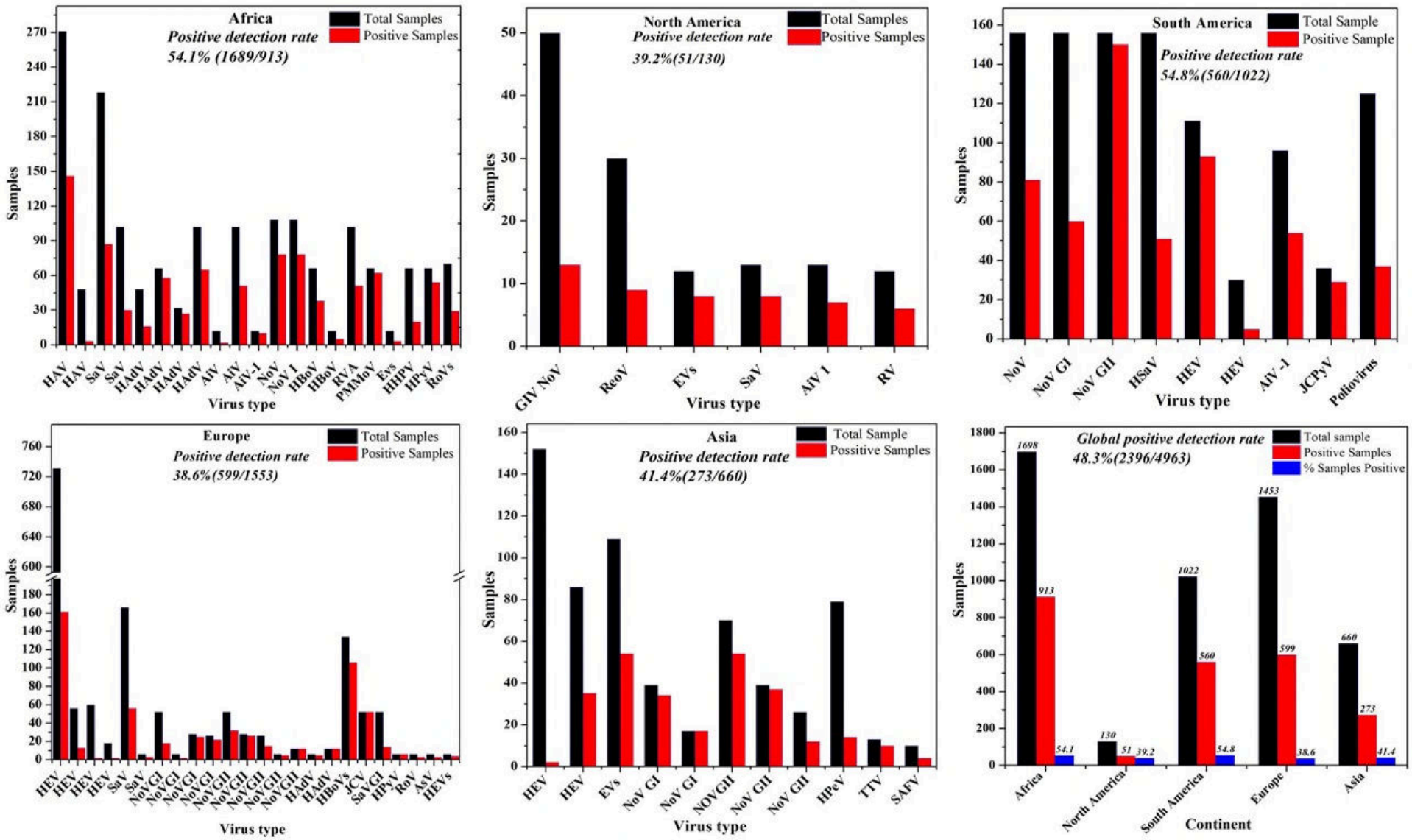

Africa
Positive detection rate
54.1% (1689/913)
Total Samples
Positive Samples
Samples
Virus type
HAV
HAV
SaV
SaV
HAdV
HAdV
HAdV
HAdV
AiV
AiV
AiV-1
NoV
NoV 1
HBoV
HBoV
RVA
PMMoV
Evs
HHPV
HPeV
RoVs
North America
Positive detection rate
39.2%(51/130)
Total Samples
Positive Samples
Samples
Virus type
GIV NoV
ReoV
EVs
SaV
AiV 1
RV
South America
Positive detection rate
54.8%(560/1022)
Total Sample
Positive Sample
Samples
Virus type
NoV
NoV GI
NoV GII
HSaV
HEV
HEV
AiV -1
JCPyV
Poliovirus
Europe
Positive detection rate
38.6%(599/1553)
Total Samples
Positive Samples
Samples
Virus type
HEV
HEV
HEV
HEV
SaV
SaV
NoVGI
NoVGI
NoVGI
NoVGI
NoVGII
NoVGII
NoVGII
NoVGII
NoVGII
HAdV
HAdV
HBoVs
JCV
SaVGI
HPyV
RoV
AstV
HEVs
Asia
Positive detection rate
41.4%(273/660)
Total Sample
Possitive Samples
Samples
Virus type
HEV
HEV
EVs
NoV GI
NoV GI
NOVGII
NoV GII
NoV GII
HPeV
TTV
SAFV
Global positive detection rate
48.3%(2396/4963)
Total sample
Positive Samples
% Samples Positive
Samples
Continent
1698
913
54.1
130
51
39.2
1022
560
54.8
1453
599
38.6
660
273
41.4
Africa
North America
South America
Europe
Asia

Fig. 2.

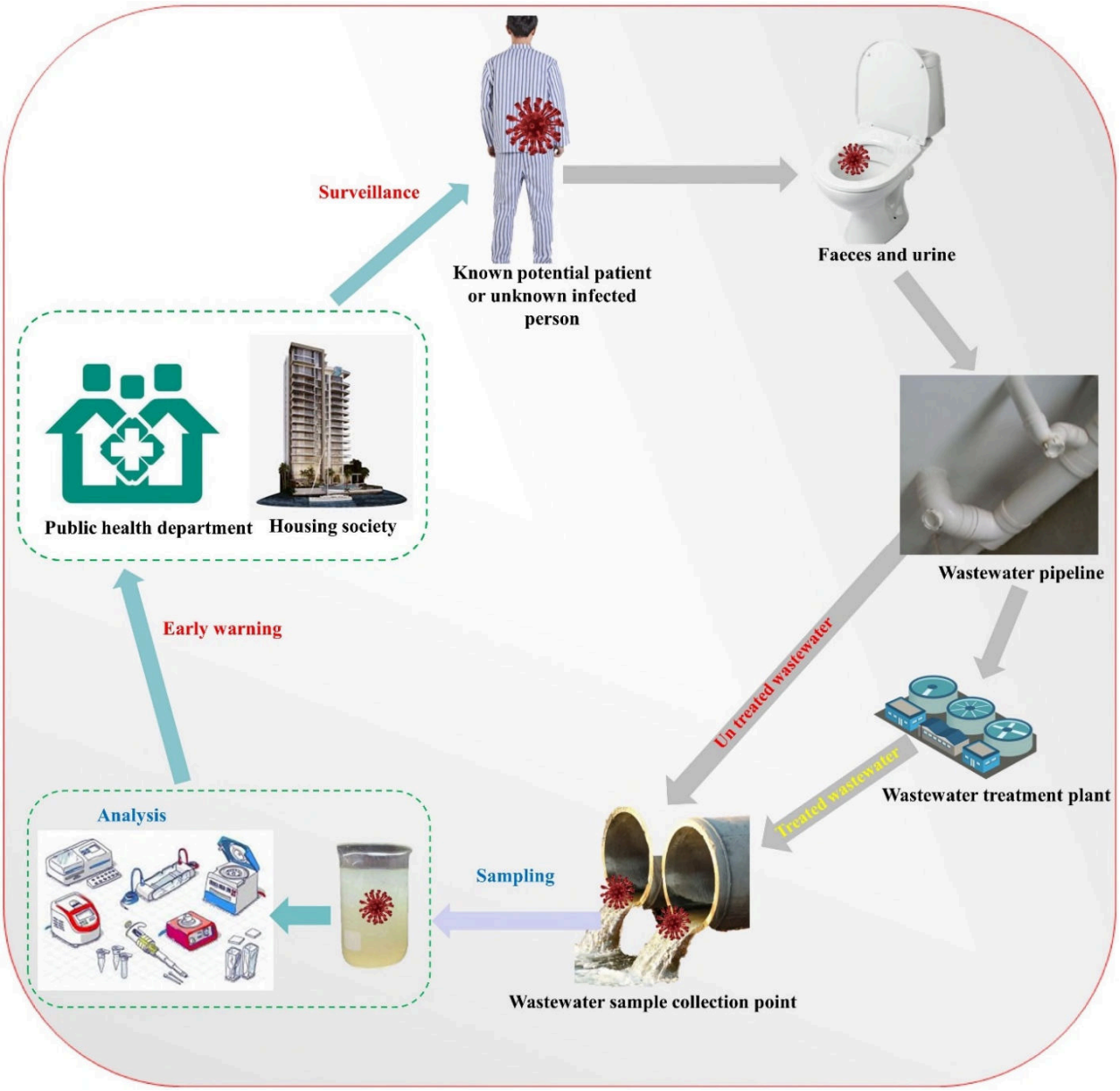
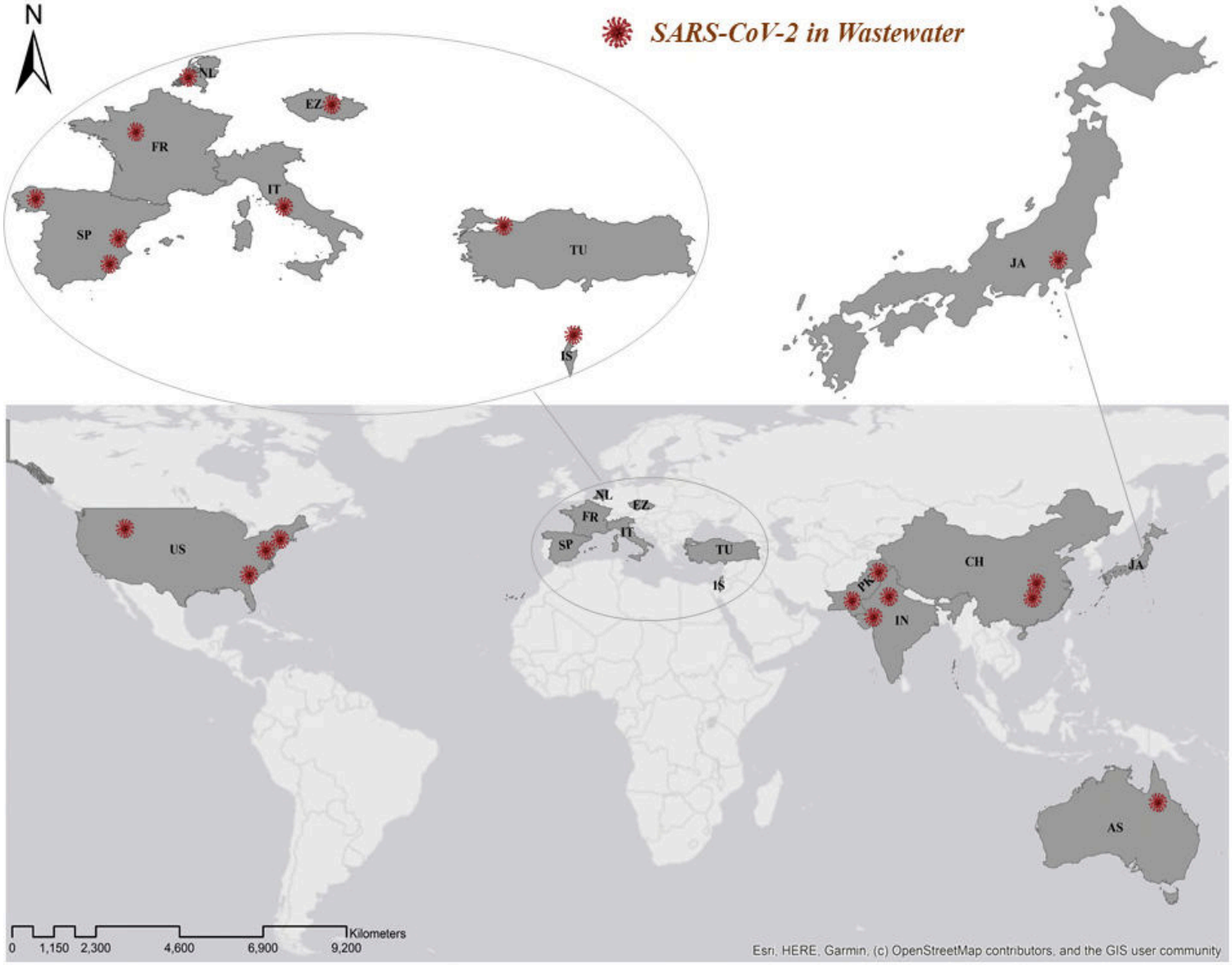

Surveillance
Known potential patient or unknown infected person
Faeces and urine
Public health department
Housing society
Wastewater pipeline
Early warning
Un treated wastewater
Wastewater treatment plant
Treated wastewater
Analysis
Sampling
Wastewater sample collection point

Fig. 3.



SARS-CoV-2 sample % detected +

- **India (IN)** 2/6 (33.3%) (Arora et al., 2020)
- **Australia (AS)** 2/9 (22%) (Ahmed et al., 2020)
- **China (CH)** 28/42 (66.67%) (Chen et al., 2020)
- **Pakistan (PK)** 21/78 (26.9%) (Sharif)
- **Japan (JA)** 1/5 (20%) (Haramoto et al., 2020)
- **France (FR)** 23/23 (100%) (Wurtzer et al., 2020)
- **Spain (SP)** 35/42 (83%) (Randazzo et al., 2020)
- **Italy (IT)** 6/12 (50%) (La Rosa et al., 2020)
- **Netherlands (NL)** 14/24 (85%) (Medema et al., 2020)
- **Turkey (TU)** 5/7 (71.4%) (Kocamemi et al., 2020)
- **United States (US)** 7/7 (100%) (Nemudryi et al., 2020), **&** 10/14 (71%) (Wu et al., 2020)

Fig. 4.

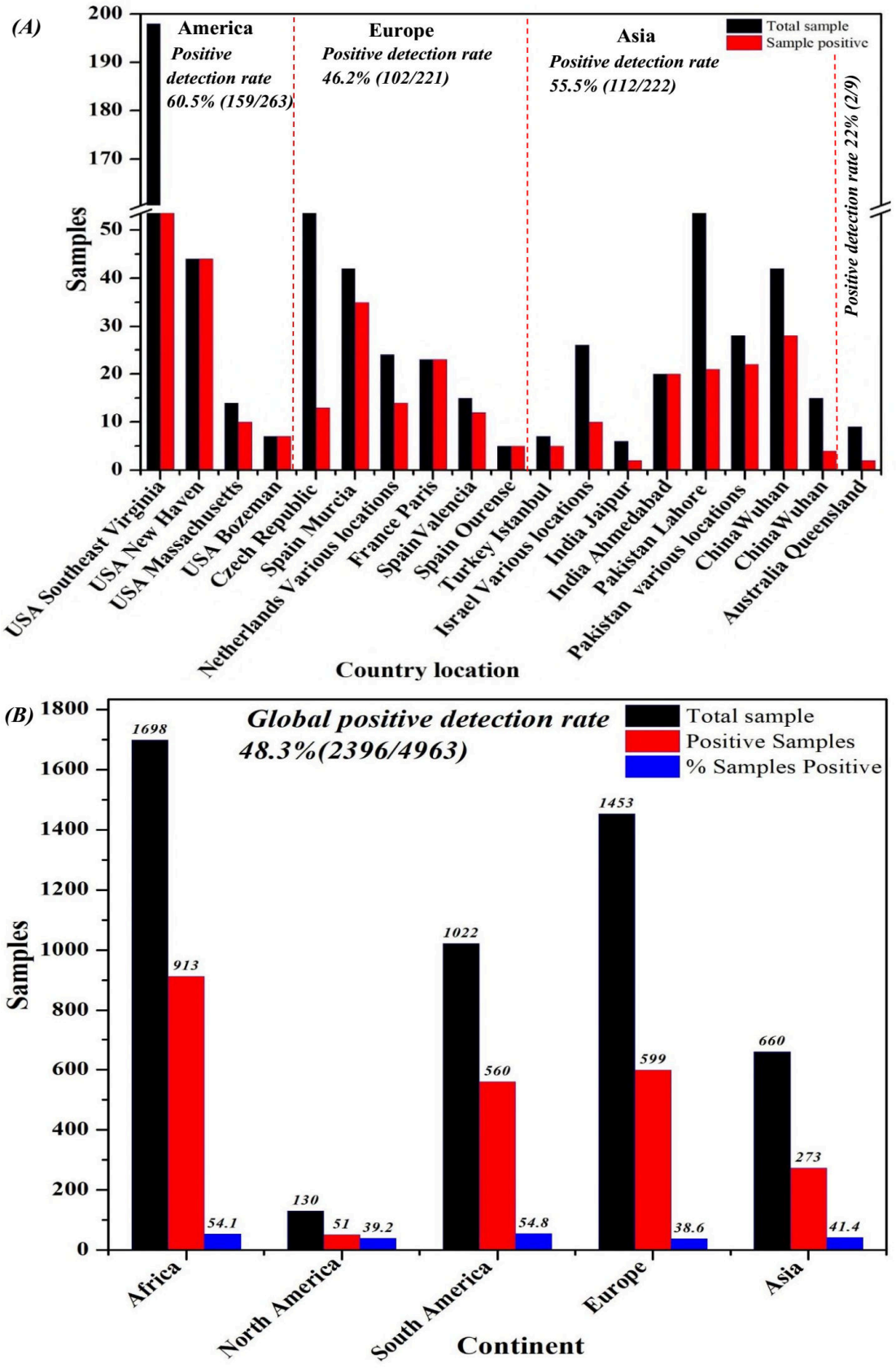

(A)
America
Positive detection rate 60.5% (159/263)
Europe
Positive detection rate 46.2% (102/221)
Asia
Positive detection rate 55.5% (112/222)
Total sample
Sample positive
Positive detection rate 22% (2/9)
Samples
Country location
USA Southeast Virginia
USA New Haven
USA Massachusetts
USA Bozeman
Czech Republic
Spain Murcia
Netherlands Various locations
France Paris
Spain Valencia
Spain Ourense
Turkey Istanbul
Israel Various locations
India Jaipur
India Ahmedabad
Pakistan Lahore
Pakistan various locations
China Wuhan
China Wuhan
Australia Queensland
(B)
Global positive detection rate 48.3%(2396/4963)
Total sample
Positive Samples
% Samples Positive
1698
913
54.1
130
51
39.2
1022
560
54.8
1453
599
38.6
660
273
41.4
Samples
Continent
Africa
North America
South America
Europe
Asia

Fig. 5.

List of Tables

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	[64]
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	[133]
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)	[75]
Tunis, Tunisia	AiV	RNA extracted from (800 µL) wastewater samples using an automatic extractor NucliSENS® Easy Mag™ platform		Total of 102 samples analysed.	NA	[87]

		(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)	51/102 (50%).		
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%).	$(6.7 \times 10^1$ to $5.6 \times 10^{7)}$	[59]
Tunis, Tunisia	RVA	RNA extracted from 800 µL of wastewater samples using an automatic extractor NucliSENS^R Easy Mag^{TM} 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%).	$(3.9 \times 10^1$ to $2.8 \times 10^3)$	[78]
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 qPCR assay conducted; real-time qPCR performed.	Beef extract & $AlCl_3$ method followed by PEG	Total of 66 treated wastewater samples processed, 38/66 (57.5%).	$(6.0 \times 10^3$ to $4.9 \times 10^4)$	[91]
Egypt	HPV, HPyV	Viral nucleic acids extracted from 200 µL of wastewater suspensions using a QIAamp			HPV median (3.9×10^5)	[92]

		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%).	HPyV (5.1×10^{02} to 4.72×10^{03})	
Tunis, Tunisia	HAdV	DNA extracted from 800 µL 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	[42]
South Africa	NoV	Viral nucleic acid recovered from 100 µL 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 JV13I).	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})	[134]

Egypt	AiV and HBoV	Nucleic acids recovered from (240 µL) 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	[135]
Egypt	Pepper mild mottle virus (PMMoV) and HAdV	Nucleic acids recovered from (60 µL) 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 cloning through real-time qRT-PCR.	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 3.3×10^8) influent and (3.9×10^4 to 1.2×10^7) effluent HAdV (1.5× 104 to 1.5×10^7) influent and (2.6×10^4 to 4.4×10^6) effluent	[136]
Egypt	HAdV	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.	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	(2.02 × 106 to7.23×10^6) stool, (8.7×10^5 to 4.3×10^6) raw sewage, (1.22×10^4 to 3.7×10^6) treated sewage, and (1.48×10^6 to 1.77×10^7) sludge	[137]

			Sludge samples ultracentrifugation followed beef extract elution	25/32 (28.3%, 84%, 50%, and 78%) of the raw sewage, treated sewage, and sewage sludge samples, respectively.		
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 1st- and 2nd-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	[94]
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	[138]
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	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)	[139]

		SaV1245R primers and the SaV124TP probe, which mainly target the polymerase-capsid junction in ORF-1.				
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	[140]
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 detection of ReoV RNA in water. These assays were performed using RT-PCR.	Adsorption-elution	Total of 30 wastewater samples analysed, 9/30 (30%).	NA	[141]
Calgary, Canada	RV, AdV	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)	[35]
Canada	NoV	Viral nucleic acid primarily recovered from concentrated (327µL) wastewater samples by cell culture. Virus mixture containing NoV	VIRADEL	Total of 12 wastewater samples analysed.	NoV most numerous (6.6 $\log_{10}$)	[35]

		from clinical stool samples using real-time quantitative PCR (RT-qPCR) and confirmed by in-house RT-qPCR.				
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)	[56]
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	[99]
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 $10^{6)}$	[142]
Rio de Janeiro, Brazil	NoV	Viral RNA recovered from concentrated (140 µL) wastewater samples using the QIAamp	Adsorption of viruses to pre-flocculated skimmed-milk	Total of 156 wastewater samples analysed,	NoV GI and GII (4 to 6.2 $\log_{10}$) and (4.4 to 7.3 $\log_{10}$), respectively	[143]

		Viral RNA Mini Kit (QIAGEN, CA, USA) qPCR) method.	proteins followed centrifugation	NoV GI and GII 38.5% and 96.1%, respectively.		
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 localized 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)	[144]
Antioquia, Colombia	HEV	Viral RNA extracted from concentrated wastewater samples using a commercial kit (QIAamp Viral RNA Mini, QIAgen, Netherlands). RT-nested PCR method used to target the ORF2/3 region (nt 5258–5394) to detect the HEV genome.	Filtration & tangential ultrafiltration	Total of 30 wastewater and drinking water samples analysed, Wastewater 5/30 (16.7%). Drinking water 7/30 (23.3%).	NA	[71]
Uruguay	AiV-1	Nested PCR method used for amplification of the 3CD junction section by Taq DNA polymerase (5 U/ll) with primers 6261/6779, which amplifies a 519-bp region, in the 1st-round PCR and primers C94b/246 k to amplify a 266-bp region in the 2nd-round PCR.	Ultracentrifugation	Total of 96 wastewater samples analysed, 54/96 (56%).	NA	[101]
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	Ultracentrifugation	Total of 72 (36 influents and 36 effluents) wastewater samples analysed,	NA	[145]

		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.		JCPyV 29/36 (80.56%) influent and 18/36 (50%) effluent.		
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)	[74]
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	[146]
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 neutralization tests.	Centrifugation followed PEG	Total of 109 wastewater samples analysed, (50.0%)	NA	[49]
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	Separation-inoculated on rhabdomyosarcoma cell culture flasks.	NA	(7.0 to 7.5 $\log_{10}$)	[147]

		coding region of EV strains analysed using the Sanger method.				
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	[148]
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	[149]
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) synthesized 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	[98]
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	Centrifugation followed PEG	Total of 17 wastewater samples analysed,	Up to (8.7×10^4)	[150]

		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.		NoV GI 17/17(100%).		
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	[151]
Shen Zhen, China	HEV	RNA recovered from concentrated wastewater samples using the UltraPure^{TM} RNA Kit (CWBIO, Beijing, China). Recovered RNA further used for matching DNA (cDNA) synthesis using the HiFiScript 1st strand cDNA Synthesis Kit (CWBIO, 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	[70]
Karaj, Iran	SAFV	RNA extracted from concentrated (100 µL) river and wastewater samples using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA). RT-PCR and RT-qPCR method used a gold standard to quantify SAFV	Centrifugation followed Dextran-PEG	Total of 50 (28 river, 12 treated, and 10 untreated) wastewater samples analysed, 10/28 (35.7%) river,	(2×10^6 to 6.4×10^6) river (1.2×10^6 to 5.2×10^6) treated 2.4× 106 to 6.8× 106) untreated	[152]

		through targeting the 5′ UTR region of the genome.		4/12 (33.3%) treated, 4/10 (40%) untreated.		
Tehran, Iran	TTV	Viral nucleic acids recovered from concentrated (140 µL) final wastewater elute samples using the QIAamp RNA Mini Kit (Qiagen, Germany). Nested PCR used to detect the presence of TTV using 1st-round primers (NG054 and NG147) and 2nd- round amplification using (NG132 and NG133).	Elution with beef extract-glycine	Total of 13 wastewater samples analysed, (76.9%).	NA	[153]
Vietnam	NoV	Viral RNA recovered from concentrate (300 µL) 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)	[102]
Europe Continent						

Italy	HSaV	Nucleic acid recovered from concentrated (100 µL) 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 1st 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	[154]
Portugal	HEV	Nucleic acid recovered from concentrated (140 µL) 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	[72]
French Polynesia	HEVs, NOV, SaV, RoV, HAdV, HPyV	Viral nucleic acids recovered from concentrated (200 µL) 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) detected using RT-qPCR or qPCR assays.	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%). HAV (ND). HPyV 6/6 (100%)	HEVs average (2.3×10^5) and SaV (8.3×10^6). average (7.2×10^2) and GII (1.7×107) HAdV average (1.7×10^5). HEV (LOD) RoV and AsV average (3.3×10^4 2.6×10^3)	[32]

					HPyV average (3.4×10^7).	
Gothenburg, Sweden	NoV	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	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.	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	[155]
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 3f 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	NA	[68]

				and D positive, respectively.		
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	[73]
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	[57]
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 & 6×10^5) NoVGI, GII and SaVGI (NA)	[97]
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)	[156]

		primers targeting the VP1/VP2 region of HBoV.				
Catalonia, Spain	HAdV, NoV	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	[157]
Australia Continent						
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 1st-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)	[158]
Queensland, Australia	HPyV	qPCR amplification method used for EC H8 and HF183 and qPCR amplification in a (20 µL) reaction mixture using (10 µL) of SsoFast EvaGreen supermix (Bio-Rad Laboratories, CA, USA), 400 nM each primer (EC H8 assay), 300 nM each primer (HF183 assay), and 3 µL of template DNA.	Filtration by using electronegative filter followed Tenfold serial dilutions	NA	HPyV average (2.56×10^5) GC/mL	[159]

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

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 gens	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 optimized protocol (Power Fecal 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: ~10^5 treated	[9]
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	[11]
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 µm pore size)	Nucleocapsid gene (N)	Total of 9 untreated wastewater samples analysed.	2/9 (22%) samples detected positive	Max: 1.2 × 10^2	[8]
Italy Rome	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	[10]
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,	Pasteurization (60 °C, 90 min) & Filtration (0.2µm pore size)	Spike protein gene (S)	Total of 14 untreated wastewater samples analysed.	10/14 (71%) samples detected positive	Max: > 2× 10^4	[22]

	Thermo Fisher). SARS-CoV2 quantitative analysis done by RT-qPCR.						
USA Bozeman	Viral RNA genome recovered from concentrated wastewater samples. SARS-CoV2 quantitative analysis done by RT-qPCR.	Filtration (5 μm & 0.4 μm pore size)	Nucleocapsid gene (N)	Total of 7 untreated wastewater samples analysed.	7/7 (100%) samples detected positive	Max: > 3× 10^5	[118]
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	[119]
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	[120]
Spain Murcia	Viral RNA genome recovered from concentrated (150 μL) 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	[89]
Spain Valencia	Viral RNA genome recovered from concentrates (150 μL) 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 to10^6 untreated	[12]
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	< 2.5×10^5 secondary & untreated NA	[117]

Czech Republic	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	NA	Total 112 untreated wastewater samples analysed	13/112 (11.6%) samples detected positive	NA	[13]
Israel Various locations	Viral RNA recovered from concentrated wastewater samples using the RNeasy mini kit (QIAGEN) & Easy MAG (bioMerieux, 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	[16]
Turkey Istanbul	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 $\times 10^4$	[15]
Japan Yamanashi	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	[14]
India Jaipur	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	[86]
India Ahmedabad	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	[17]

Pakistan Various locations	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	[18]
Pakistan Lahore	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	[19]
China Wuhan	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	[20]
China Wuhan	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	[21]

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

Supplementary information

Part 1: Spatial distribution of human viruses in wastewater systems

Part 2: List of Tables

Table S1. Abbreviations used in the current review.

Table S2. Human viruses, genomes, classification, and health symptoms.

Table S3. The primary methods used for virus detection in wastewater systems.

Part 1: Spatial distribution of human viruses in wastewater systems

1.1 Africa

In South Africa, various viruses, including HAdV, RoV, HAV, NoV, and Aichi virus 1 (AiV-1), were detected in wastewater in various areas between 2015 and 2019 [1-4]. For example, NoV is associated with gastroenteritis pandemics and was detected in wastewater-polluted rivers from 2008 to 2011. To prevent both environmental contamination and infectious disease transmission, monitoring of the effluents of WWTPs is critical. Mabasa et al. assessed whether wastewater samples could be used for routine surveillance of NoVs. A total of 108 raw sewage and effluent samples collected monthly from five WWTPs were screened for NoV GI and GII using real-time RT-qPCR. They successfully identified 16 NoV genotypes in raw wastewater in South Africa between 2015 and 2016 [3]. The majority of G1.4 strains detected in all WWTPs share the highest identity (99 to 100% resemblance over 285 nt) with the strains that previously circulated in Italy (JX142184) and South Africa (JN191356). Osuolale et al. evaluated the presence of RoV and EVs in the final effluents of five WWTPs in the Eastern Cape of South Africa using the adsorption–elution method with singleplex RT–PCR assays. The results demonstrated that RoV (up to 10^5GC/L) was present in several effluent samples [2]. Adefisoye et al. (2016) assessed the HAdV genome in wastewater effluents and identified it in 33.3% of positive samples. In this study, HAdV DNA was extracted using DNA extraction kits (Quick gDNATM Mini-Prep; Zymo Research, USA), HAdV RNA was extracted using RNA purification kits (QuickRNATM Mini-Prep; Zymo Research, Irvine, USA), and quantitative detection was performed with TaqMan Probe-Based qPCR assays [1]. HAdV that is increasingly present in treated effluents is discharged into the aqueous environment, increasing the public health risk from water sources for domestic water use. These results highlight the importance of assessing HAdV contamination in the aqueous environment and conducting further screening to evaluate the prevalence and epidemiology of clinically significant HAdV in Eastern Cape, South Africa.

In Tunisia, AiV, Sapovirus (SaV), RoV A, HAV, and enteric AdVs have been detected in wastewater [5-8]. In 2016, Ibrahim et al. detected and quantified genotyped

RAV via RT-PCR, and virus recovery was achieved through the beef extract and $AlCl_3$ method followed by PEG. A total of 102 samples were collected from two biological treatment processes in a semi-industrial pilot plant that receives effluents from the sewage system of Tunis City, Tunisia [5]. The main RVA genotypes G8, G9, G1, G10, G3: G3/G9, G8/G10, and G9/G8 were detected and quantified in 50% of wastewater samples. This study identified the emergence of new and rare G genotypes in Tunisia, i.e., G8 and G10. Earlier, low levels of these genotypes were also detected in wastewater systems in Argentina and Kenya. In the same year, Ouardani et al. analysed HAV in 271 wastewater samples collected from different WWTPs over a period of 13 months. Using the same detection and quantification method as Ibrahim et al., Ouardani et al. found that HAV was endemic in Tunisia and highly prevalent, with 53.9.7% of wastewater testing positive. Further molecular characterization demonstrated that the vast majority of HAV strains belong to subgenotype IA, with cocirculation of subgenotype IB in WWTPs [6]. The following year, they also detected an increased rate of AiV in wastewater via RT-PCR (beef extract and $AlCl_3$ method followed by PEG). Moreover, they detected AiV genotype B, which has been detected in patients from Asia, in Tunisia's wastewater for the first time [7]. Using the same detection and quantification method, SaV was evaluated in four WWTPs for 13 months (December 2009 to December 2010). SaV was positively detected in 26 samples and 61 samples of treated and raw sewage, respectively [9].

Hospital wastewater represents a significant source of dispersal of various toxic pathogenic microorganisms that enter water systems. In 2018, Ibrahim et al. evaluated HAdV exposure rates via nested-PCR to identify the genotypes of these viruses and measure HAdV removal effectiveness in a hospital WWTP in Tunis, Tunisia.. The concentration of viruses was determined using the beef extract and $AlCl_3$ method followed by PEG. HAdVs were detected at the highest frequency, with 64% of the 102 wastewater samples testing positive. In this study, the HAdV detection rate was comparatively higher than that previously reported in Italy and Eastern Cape, South Africa (62.5 and 64%, respectively). [10]. In this study, the HAdV detection rate was higher. It is possible that the nested-PCR-based detection assays are not specific to a

single strain. These researchers also detected SaV in the Tunisian hospital wastewater treatment samples using RT-PCR (beef extract and $AlCl_3$ method followed by PEG) [8]. The results showed that approximately 29.4% of 102 wastewater samples were positive for virus, demonstrating the presence of a wide range of human viruses in wastewater in Tunisia.

In Egypt, human bocaviruses (HBoVs), AiV, pepper mild mottle virus, HAdV, oncogenic papillomavirus, and polyomavirus have been detected. HBoVs are predominantly found in infected respiratory tracts and faecal samples from patients with gastroenteritis. Hamza et al. (2017) identified HBoVs, including HBoV-1, HBoV-2, and HBoV-3, using RT-PCR. The concentration of viruses was determined using the beef extract and $AlCl_3$ method followed by PEG, and 57.5% of the 66 analysed influent wastewater samples were positive. The high prevalence of HBoV in influent wastewater indicates its circulation in the regional population in Greater Cairo, Egypt [11].

In addition to its presence in wastewater samples, Shaeen et al. found that HBoV was widely distributed 41.6% of 12 untreated raw sewage samples. HBoV detection and quantification were achieved using nested-PCR. The concentration of viruses was determined through adsorption-elution using an electronegative membrane, followed by PEG [12]. AiV and HAdV were detected in the River Nile in the North of Giza, Egypt, using qPCR; virus concentrations were determined using beef extract, followed by PEG [12, 13]. Hamza et al. detected other viruses, such as oncogenic papillomavirus and polyomavirus, in wastewater in Egypt [14]. These authors report the first environmental surveillance of both human polyomaviruses (HPyVs) and HPVs in three WWTPs in urban Egypt. The HPyVs and HPVs were detected via nested and semi-nested PCR, and virus concentrations were determined by elution with beef extract-glycine. A high dissemination level was found for both viruses; HPyVs were found in approximately 82.4% of samples, and HPVs were detected in 30.5% of the 66 collected samples. In most studies, enteric viruses and proposed indicators are detected and quantified through RT-PCR and real-time qPCR-based approaches, which are rapid and easy methods enabling strain level detection [15]. However, PCR-based approaches have some drawbacks. qPCR and particularly RT-PCR are frequently hindered by the

organic substances (i.e., polyphenolic compounds) found in wastewater samples. Hence, the use of DNA viruses as an indicator (i.e., HPyV and HAdVs) for wastewater-derived contamination may be more viable than the use of RNA viruses (i.e., AiV and AsVs) because of the more robust molecular detection of target DNA [16]. Based on different published study results, in this review, our findings showed that the total positive detection rate for human viruses in effluent wastewater systems in Africa is approximately 54.1% (913/1689) (Fig. 2). These results suggest that a high incidence of various viruses in the wastewater system indicates their circulation in the population and may cause adverse human health effects.

1.2. North America

In the USA, NoV G IV, ReoV, EV, AiV, and NoV have been detected in wastewater systems [17-22]. In Arizona, Kitajima et al. explored the occurrence and genomic diversity of NoV G IV strains in sewage using RT-PCR, and virus concentration was determined using an electronegative filter. The results demonstrated that NoV G IV was present in 26% of 50 samples; 47 different NoV G IV strains were found, and these were classified as members of the GIV.1 human group and of a unique genomic cluster closely associated with strains previously reported in sewage in Japan [17]. While investigating the genotype distribution and temporal variations through nested-RT-PCR followed by cloning and sequencing process analysis in AiV-1 and SaVs in wastewater in Arizona, Kitajima et al. identified multiple AiV-1 genotypes and SaV strains in sewage collected over a one-year period; the results demonstrated a seasonal change in the prevalent genotypes in communities [22]. Brinkman et al. collected municipal wastewater samples monthly for 1 year to quantify EVs through RT-PCR and identified them via next-generation, high-throughput sequencing. Simultaneously, the virus concentration was determined by filtration and elution with beef extract-Celite, and concentrations ranging from 7.05×10^3 to 8.3×10^5 GC/L were found. The results suggested seasonal patterns of EVs circulating in communities and causing EV disease burden [19]. In Canada, NoVs, RoV, ReoV, SaV, AsV, EVs, AdVs, and JC viruses have also been detected in wastewater [23, 24]. Based on the published study results included in this review, in North America, our results showed that the total positive detection rate

for human viruses in the effluent wastewater system is approximately 39.2% (51/130 samples) (Fig. 2).

1.3. South America

In Brazil, Fumian et al. (2019) investigated NoV GI and GII in 156 wastewater samples cross-collected at three stages (52 samples) from a WWTP in Rio de Janeiro, Brazil. NoV GI and GII was detected through RT-qPCR, and the virus concentration was determined by adsorption to pre-flocculated skimmed milk proteins followed centrifugation [25]. They discovered NoV GII variability in raw sewage samples and examined the NoV occurrence and molecular epidemiology of acute gastroenteritis cases. NoV GI and NoV GII were also measured in final sewage samples. The NoV concentration ranged from 4 to 6.2 $\log_{10}$ GC/L for GI and 4.4 to 7.3 $\log_{10}$ GC/L for GII. The researchers identified 13 NoV genotypes with six leading capsid genotypes during the one-year period. NoVs were the most prevalent type of virus found in wastewater samples (68.5%). Emergent GII.17 was the second most predominant genotype (14.3%) identified in untreated wastewater. Due to the high number of NoV epidemics and the lack of NoV vaccines and antiviral medications, understanding the genotypic variability of NoV at the population level is important. Collecting complementary data from both clinical and ecological (sewage) samples has been shown to be an effective approach to monitoring the movement and appearance of the genotypes associated with NoV epidemics.

To assess the occurrence and molecular epidemiology of HSaV in wastewater and stool samples, a surveillance study was performed in Rio de Janeiro state, Brazil in 2012-2014. HSaV was detected using qPCR, and the virus concentration was determined using the same method used by Fumian et al. (2019). HSaV was detected in 3.5% of 341 stool samples and 33.0% of 156 wastewater samples [26]. Furthermore, for the first time, partial genome sequencing of stool and wastewater samples revealed the high incidence of GI.1, GI.2, GI.6, GII.1, and GV.1 circulation, which causes the majority of human SaV-induced acute gastroenteritis cases, and the ecological distribution of those viruses in Rio de Janeiro, Brazil. Fioretti et al. (2018) successfully identified another human virus, G IV, in 52% of 156 raw sewage samples using real-

time PCR. This first description of GIV norovirus phylogenetic assessment showed the circulation of a new GIV genotype present equally in clinical and ecological samples in Rio de Janeiro, Brazil [27]. In north-western Uruguay, Burutarán et al. found a high prevalence of AiV-1 in the wastewater of 56% of 96 positive samples using nested-PCR [28].

In Colombia, HEV was detected, mainly in samples collected from patients and swine; however, relevant environmental research studies have not been conducted. To determine whether HEV is present in the water supply, samples collected from the main drinking water plants and from the wastewater systems of eight metropolises and two townships in Antioquia state (Columbia) were collected from 2012-2014 [29]. HEV genetic material was detected in 7/30 (23.3%) of the samples from drinking water plants and in 5/30 (16.7%) of the wastewater samples. Viral concentrates were also found in three positive wastewater samples. This study demonstrated the presence of HEV in both drinking water and wastewater in Antioquia state, Colombia. The presence of HEV in ecological waters might pose a severe risk of water-borne transmission to the population in this study area. Furthermore, the results of the study and the potential movement of HEV via humans and swine in Colombia must be considered by the national public health authorities when developing advanced pilot monitoring programmes and establishing HEV contagion diagnoses as part of the country's strategy for combating viral hepatitis. This study was the first report on the occurrence of HEV in ecological samples in Colombia and the second such report in South America.

Although many reports have described genotype distributions worldwide, data on the genetic constitution of JVC in the southernmost parts of South America are very scarce. Levican et al. (2019) detected JCV in wastewater from Santiago, Chile by using qPCR [30]. Sewage samples were collected monthly for one year from three WWTPs that treat approximately 80% of the wastewater produced in Santiago, Chile. The results showed that JCV was abundantly distributed in Santiago, Chile; JCV was detected in 80.56% of the 36 wastewater samples, supporting the use of JCV as a practical indicator for assessment of human and ecological pollution. JCV was detected at high frequencies in influent and effluent samples, with the main WWTPs showing the maximum

detection and viral genome copy loads.

In a phylogenetic examination, the Chilean sequences clustered primarily with genotype 2A. This result is similar to that previously reported for Buenos Aires, Argentina and dissimilar to that indicated by data collected from Brazil. However, the circulation/spread of European subtypes 1 and 4 and African subtypes 3 and 6 has been determined [30]. In Mexico, Estívariz et al. found Sabin polioviruses and non-poliovirus enteroviruses (NPEVs) in multiple wastewater samples collected during 2016 and 2017 using analytical methods they had developed [31]. Based on the individual published study results included in this review, our findings show that overall, the total positive detection rate for various human viruses in effluent wastewater systems in South America is approximately 54.8% (560/1022) (Fig. 2).

1.4 Europe

In Italy, HEV, HBoVs, EVs, and human sapoviruses (HSaVs) were detected in wastewater [32-35]. HEV infection is a severe health issue worldwide. The burden of hepatitis E in Italy appears to be low compared with that in other European countries. Recently, improved surveillance has revealed noticeable geographical differences in HEV prevalence in Italy. For example, the Abruzzo region of Italy is thought to be a high-risk area for HEV transmission and infection. Di Profio et al. further explored the prevalence of HEV in Teramo Province using wastewater analysis in 2016 and 2017 and found HEV in 23.2% of the 56 wastewater samples from four WWTPs by using RT-qPCR [34].

Pennino et al. (2018) investigated the relative abundance and occurrence of various human viruses at different WWTPs in Naples, Italy over a period of five years and found that 22.0% of the 161 influent and effluent samples were virus positive. Among the 140 non-polio EVs detected in wastewater, 30.7% were echoviruses, and 69.3% were coxsackievirus type B (CVB); CVB5 and CVB3 were the main viral types, followed by echovirus 6 and CVB4 [33]. HSaVs are pathogenic factors found in sporadic cases and during acute gastroenteritis outbreaks. There is evidence that HSaV is distributed worldwide, although the epidemiology of HSaV in Italy is unknown. A total of 166 raw sewage samples from 16 WWTPs in Italy were analysed, and 33.7%

of the samples tested positive [35].

Iaconelli et al. (2016) evaluated the epidemiology of HBoVs in sewage samples and reported that approximately 79.1% of the 25 wastewater samples collected from WWTPs in Italy were positive [32]. Six wastewater samples from Tahiti, France, located at the central part of the Pacific Ocean, were investigated for the presence of enteric viruses; if viruses were present, the diversity, infectivity, and integrity of the HAdVs were studied. EVs, SaVs, and HPyV were detected in all samples. NoV GI, NoV GII, HAdVs, RoVs, AsVs, and HEVs (Table 1) were also observed on occasion [36].

A hepatitis E outbreak on a small, isolated island provided an opportunity to explore the relationship between hepatitis E cases and the concentration of the virus in wastewater. Miura et al. demonstrated that if 1 to 4% of inhabitants connected to a WWTP were infected with HEV, HEV could be detected in untreated wastewater. Such small-scale infections can contaminate wastewater, increasing the risk of further virus transmission [37].

In the UK, Farkas et al. monitored various enteric viruses, including AdV, JCV, NoVs, SaVs, HAV and HEV, in wastewater in North Wales. Significantly elevated concentrations of JCV and AdV were detected in most samples. NoVs and SaV were also detected at high concentrations in the wastewater, and their occurrence was significantly correlated with common or local gastroenteritis outbreaks [38]. In another study, the results confirmed the presence of HAdV, NoV GI, and NoV GII in raw and treated wastewater, while SaV GI was detected only in raw wastewater [39].

In Sweden, Dienus et al. (2016) evaluated the impact of WWTPs on the Göta älv River [40]. High concentrations of NoV GI and NoV GII were also detected in samples collected primarily at WWTPs and from drinking water, and the intake over a period of one year was measured. The average NoV GI and GII genome levels were between 6.2 and 6.8 $\log_{10}$ genome equivalents (g.e.)/L in received wastewater and between 5.3 and 5.9 $\log_{10}$ g.e./L in treated wastewater. In the samples from WWTPs, diversity decreased to between 0.4 and 1.1 $\log_{10}$ units. However, the concentrations of NoV in source water ranged from below the detection limit (bdl) to 3.8 $\log_{10}$ g.e./L. The spread of NoV in

the river was predicted using a three-dimensional hydrodynamic model, and the results suggested that the NoV GI and GII genome levels in drinking water may infrequently be up to 2.8 and 1.9 $\log_{10}$ units higher, respectively, than the concentrations that were measured during the monitoring project [40].

In Catalonia, Spain, Gonzales-Gustavson et al. (2019) examined the concentrations and elimination of NoV GII and HAdV as significantly abundant viral pathogens detected in wastewater subjected to a variety of tertiary treatments at two WWTPs for one year [41]. The results showed that the quantitative risk of HAdV and NoV GII to lettuce irrigated with tertiary waste from WWTPs was higher than the World Health Organization (WHO) recommendation of 10^{-6} disability-adjusted life years for similar viruses.

A WWTP with constructed wetlands exhibited a greater average viral decrease than that observed at WWTPs where conventional treatment was employed; nonetheless, the WWTP with constructed wetlands showed more significant variability than conventional WWTPs. Studies in other areas, including Portugal, have reported that HEV circulates at significant levels in both humans and swine; nonetheless, research has not yet focused on the occurrence of HEV in wastewater. Matos et al. conducted the first measurements of HEV in wastewater from northern and central Portugal [42]. This report was the first study of the occurrence of HEV in sewage in Portugal. In Germany, Beyer et al. found that 84 to 100% of collected influent wastewater was positive for HEV and that HEV G-3c was the most prevalent genotype [43]. Based on the different published study results included in this review, in Europe, the positive detection rate for various human viruses in effluent wastewater systems is approximately 38.6% (599/1553). The results show that the high incidence of various viruses in the wastewater system may have adverse human health effects.

1.5. Asia

In Japan, NoVs and human parechoviruses (HPeVs) have been detected in wastewater [44-47]. For example, Kazama et al. investigated NoV GI, and NoV GII in urban wastewater in the town of Matsushima in north-eastern Japan's Miyagi Prefecture from November 2012 to March 2013. A virologic observation of gastroenteritis cases

was simultaneously performed in the same area. A total of fourteen diverse genotypes were detected, and up to eight genotypes per wastewater sample were detected in significant amounts. The NoV GII.4 Sydney 2012 variant, which appeared to dominate during the period of our study, was also measured in wastewater [46]. In Miyagi Prefecture, Japan from April 2012 and March 2014., Abe et al. demonstrated that 18% of the 79 wastewater samples were positive for HPeV. The results of the current study indicate clear seasonality was observed: all positive samples were collected between July and December during the study period [45]. Ito et al. further calculated the viral target values for recycled wastewater irrigation in Japan under two exposure conditions using different levels of indigenous viruses in unprocessed wastewater and a distinct acceptable yearly disease burden [47].

In Iran**,** TTV, a ssDNA virus that is mainly transmitted through the faecal-oral route and may be excreted in the absence of clinical symptoms, was detected in wastewater. An assessment of the prevalence and molecular characteristics of TTV in effluent wastewater in Iran from 2017-2018 found TTV in 76.9% of the 13 wastewater samples, and TTV GI and TTV GIII were identified by phylogenetic analysis [48]. Saffold virus is an emerging virus that can lead to acute gastroenteritis; it was initially found in infant stool samples from America. For detection of Saffold virus in wastewater in Karaj, Iran, 50 treated and untreated wastewater samples were collected; the results indicated that 33.3% of influent and 40% of effluent wastewater samples contained the virus, suggesting that Saffold virus infection poses a health risk for the Iranian people [49].

In Shenzhen, China, Li et al. investigated the occurrence of HEV in wastewater samples and showed that 1.32% of 152 samples obtained from livestock sewage plants were positive [50]. The results of the current study indicate that the possibility of sporadic HEV infections should be emphasized because viruses can still circulate in sewage in China. This report is the first study to include a molecular characterization of HEV in wastewater in China.

In Vietnam, Nguyen et al. (2018) evaluated NoV contamination of oysters polluted by discharged sewage for 17 months [51]; they demonstrated co-contamination with NoV GI and NoV GII and detected NoV GI more frequently than NoV GII. They also

explored the genetic diversity of NoV GI and NoV GII and reported the presence of six genotypes in the collected samples. These results showed that wastewater is an important contributor to NoV contamination of oysters in this area. Based on the different published study results included in this review, in Asia, the total positive detection rate for various human viruses in effluent wastewater systems is approximately 41.4% (373/660) (Fig. 2). The high incidence of various viruses in wastewater systems in Asia is likely to result in human health effects that are comparatively higher than the effects that occur in North America and Europe and lower than those that occur in South America and Africa.

1.6. Australia

Lun et al. identified the genetic variability of HAdV at the local population level using wastewater samples collected from Sydney and Melbourne from 2016 to 2017 [52]. HAdV was detected in wastewater collected over the two-year study period at an average level of 1.8×10^7 GC/L. A total of six main groups of HAdV were detected in the wastewater samples, representing nineteen different serotypes. This study broadened insights into the epidemiology of HAdV in Australia. Based on the studies included in this review, the positive detection rate for various human viruses in effluent wastewater systems in Australia is very low compared with the rates on other continents. Due to the limited number of studies (Table 1), further testing is needed to assess the prevalence and distribution of human viruses in wastewater systems. In this review, the current data suggest that all viral indicators are present in the raw wastewater system at high concentrations. Therefore, they are potentially good indicators of wastewater contamination.

Table S*1*. Abbreviations used in the current review.

Name	Abbreviation	Name	Abbreviation
Aichi virus	AiV	Norovirus	NoV
Astrovirus	AsV	Norovirus genotype I	NoV GI
Adenovirus	AdV	Norovirus genotype II	NoV GII
BK polyomavirus	BKPyV	Novel coronavirus pneumonia	COVID-19
Coxsackievirus type B	CVB	Reovirus	ReoV
Double-stranded DNA	dsDNA	Rotavirus	RoV
Enterovirus	EV	Rotavirus A	RoV A
Genogroups I, II…	GI, GII…	Sapovirus	SaV
Hepatitis A virus	HAV	Sapovirus GI	SaV GI
Hepatitis E virus	HEV	Severe acute respiratory syndrome	SARS
Human adenovirus	HAdV	Severe acute respiratory syndrome coronavirus 2	SARS-CoV-2
Human parechovirus	HPeV	Single-stranded DNA	ssDNA
Human bocavirus	HBoVs	Single-stranded RNA	ssRNA
Human polyomavirus	HPyV	Torque teno virus	TTV
Human sapovirus	HSaV	United States of America	USA
Human papillomavirus	HPV	United Kingdom	UK
JC polyomavirus	JCV	Wastewater treatment plant	WWTP
Non-poliovirus enterovirus	NPEV	Polymerase chain reaction	PCR
Genogroup IV norovirus	GIV NoV	Human enteroviruses	HEVs
Enteric viruses	EVs	Saffold virus	SAFV
Angiotensin-converting enzyme 2	ACE2	Wastewater-based epidemiology	WBE
Polyethylene glycol	PEG	Virus adsorption and elution	VIRADEL

Table S2. Human viruses: genomes, classification, and health symptoms.

Virus type	Genome	General classification	Human health symptoms	References
Human adenoviruses (HAdVs)	HAdVs are non-enveloped positive-sense double-stranded DNA (dsDNA) icosahedral viruses 70 to 90 nm in diameter made up of 252 capsomeres.	HAdVs are classified as genus *Mastadenovirus* and include seven diverse known HAdV types: HAdV-A to HAdV-G.	Symptoms of HAdV infection include respiratory and conjunctival infections ranging from the common cold to severe pneumonia, croup, and bronchitis. Some types of HAdVs cause gastroenteritis, conjunctivitis, cystitis, and neurological illness.	[16, 53, 54]
Aichi virus (AiV)	AiV is a non-enveloped positive-sense single-stranded RNA (ssRNA) icosahedral virus ≈30 nm in diameter and approximately 8.2 kb in length.	AiV is a species in the genus *Kobuvirus* and a member of the Picornaviridae family; it includes the diverse genotypes A–C. AiV-.	Symptoms of AiV infection include fever, nausea, diarrhoea, vomiting, and abdominal pain.	[55, 56]
Astroviruses (AsVs)	AsVs are non-enveloped ssRNA icosahedral viruses with a mean diameter of 28 nm.	AsVs are classified within the family *Astroviridae* and comprise two genera: *Mamastrovirus* species infect both humans and animals, and *Avastrovirus* species infect poultry and other birds.	AsVs cause mild gastroenteritis after a 3-4-day incubation period and a great variety of other symptoms, such as nausea dehydration, vomiting, and diarrhoea.	[57, 58]
BK polyomavirus (BKPyV)	BKPyV is a non-enveloped dsDNA icosahedral capsid virus with a diameter of 40 to 44 nm.	BKPyV is classified within the family Polyomaviridae and the genus *Betapolyomavirus.*	BKPyV, an emerging pathogen in kidney and bone marrow transplant recipients, causing related nephropathy and haemorrhagic cystitis.	[59, 60]
Human bocaviruses (HBoVs)	HBoVs are non-enveloped ssDNA viruses 18-26 nm in diameter with a 5300-nucleotide genome.	HBoVs are classified within the Parvoviridae family and the genus *Bocavirus.*	HBoVs cause colds, upper respiratory infections, and gastroenteritis. HBoV infection symptoms frequently appear as a cold with fever, runny nose, and cough.	[61, 62]

Coxsackievirus type B (CVB)	CVB is a ssRNA virus comprising approximately 7400 nucleotides encoding a 250-kDa polyprotein.	B (CVB) non-polio enterovirus belongs to the EV family and the genus *Picornaviridae* (EVs B (1) subtype) and includes six diverse serotypes of B (CVB): 1-6 (2).	B (CVB) infection symptoms include severe diseases: myocarditis, chronic dilated cardiomyopathy, diabetes, heart failure, encephalitis, aseptic meningitis, and diabetes.	[63, 64]
Hepatitis A virus (HAV)	HAV is a non-enveloped positive-stranded RNA virus with a 27 to 30 nm diameter icosahedral capsid and a 7.48-kb genome.	HAV is classified within the Picornavirus family.	HAV symptoms include fever, diarrhoea, abdominal discomfort, malaise, nausea, jaundice, loss of appetite, and dark-coloured urine.	[6]
Hepatitis E virus (HEV)	HEV is non-enveloped, has a ssRNA genome, and exists as a virion approximately 32 to 34 nm in diameter.	HEV is classified within the Hepeviridae family, in the genus *Orthohepevirus*. Five members of Orthohepevirus A can infect humans. The genotypes HEV-1 and HEV-2 are known human viruses.	HEV infection symptoms include mild fever, anorexia, nausea, vomiting, abdominal pain, itching, skin rash, joint pain, jaundice, and hepatomegaly.	[65, 66]
JC polyomavirus (JCV)	JCV is a non-enveloped icosahedral capsid with a dsDNA genome and consists of 5,130 nucleotide pairs.	Human JCV is classified within the Polyomaviridae family, which also includes simian virus 40 (SV40), murine polyomavirus, and human BKV.	JCV is well known and causes progressive multifocal leukoencephalopathy (PML), an often-lethal illness of the human brain.	[67, 68]
Non-poliovirus enterovirus (NPEV)	NPEV is a non-enveloped virus with a positive-sense ssRNA genome of approximately 7.5 kb.	NPEV is classified within the Picornaviridae family and the genus *Enterovirus* (consisting of 15 species).	NPEV infection symptoms include common cold symptoms, febrile illness, aseptic meningitis, myocarditis, encephalitis, and poliomyelitis-like acute flaccid paralysis.	[69, 70]
Norovirus (NoV)	NoV is a non-enveloped, positive-sense ssRNA virus approximately 27-38 nm in diameter with arch-like capsomeres.	NoV is classified within the Caliciviridae family. NoV is genetically classified into five genogroups; NoV GII and NoV GI are associated with human diseases, although	NoV infection symptoms include headaches, fever, vomiting, abdominal cramps, diarrhoea, body aches, and stomach pain.	[71]

		NoV GIV has rarely been implicated in outbreaks.		
Human papillomavirus (HPV)	HPV is a non-enveloped, dsDNA virus approximately 8 kb in size.	HPV is classified within the Papillomaviridae family and contains approximately 29 genera comprising 189 PV types, mainly isolated from humans.	HPV infection symptoms include warts, particularly genital warts (small bumps, clusters of bumps, or stem-like protrusions); they also affect the vulva or cervix in women and the penis or scrotum in men and increase the risk of developing cancer.	[72, 73].
Human parechovirus (HPeV)	HPeV has a non-enveloped positive-sense ssRNA genome enclosed in an icosahedral capsid. The HPeV virion is 28 nm in diameter.	HPeV is classified within the Picornaviridae family in the genus *Parechovirus* and further divided into two types: HPeV A and Parechovirus B. HPeV A is subdivided into 19 genotypes (HPeV-1 to -19), and Parechovirus B comprises Ljungan viruses (1 to 4).	HPeV symptoms include fever, diarrhoea, cold, sepsis-like syndrome, meningitis, flaccid paralysis, encephalitis, seizures, and hepatitis.	[74]
Poliovirus	Polioviruses are non-enveloped positive-sense ssRNA viruses approximately 34 to 39 nm in diameter with icosahedral symmetry.	Poliovirus, also known as poliomyelitis, is a serotype of the species EV C in the family Picornaviridae.	Poliovirus infection symptoms include sore throat, fever, tiredness, nausea, headache, stomach pain, and poliomyelitis (polio).	[75]
Reovirus (ReoV)	ReoV is a non-enveloped dsRNA virus.	ReoV is classified within the Reoviridae family.	ReoV infection symptoms include pneumonia, meningitis, myocarditis, encephalitis, choledochal cysts, and biliary atresia.	[76, 77]
Rotaviruses (RoVs)	RoVs have an icosahedral non-enveloped segmented dsRNA genome and are approximately 100 nm in diameter.	RoVs are classified in the Reoviridae family of the genus *Rotavirus.* RoVs are serologically classified into seven groups	RoV infection symptoms include abdominal pain, watery diarrhoea, vomiting, and fever.	[65, 78]

		(A–G). Human RoV pathogens fall into groups A, B, and C.		
Human Saffold virus (SAFV)	Human SAFV is a non-enveloped, icosahedral ssRNA virus with a genome approximately of 7.8 kb in length.	Human SAFV viruses are classified within the Picornavirus family in the genus *Cardiovirus.*	Human SAFV affects the endocrine and cardiovascular systems; infection symptoms include high fever, loss of appetite, and neurological symptoms, including headache.	[79, 80].
Sapovirus (SaV)	SaV is a non-enveloped positive sense ssRNA virus with an icosahedral structure and is approximately 30 to 38 nm in diameter.	SaV belongs to the genus Sapovirus within the family Caliciviridae.	Common SaV infection symptoms include vomiting, cramps, diarrhoea, myalgia, headache, chills, nausea, and abdominal pain.	[81]
Torque teno virus (TTV)	TTV is a non-enveloped negative-sense ssDNA virus approximately 30 to 50 nm in diameter.	TTV is classified within the Circoviridae family in the genus *Anellovirus*.	TTV symptoms include hepatitis-associated aplastic anaemia, liver failure, and cryptogenic cirrhosis.	[56, 82]
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)	SARS-CoV-2 is a positive-sense ssRNA virus with a single linear RNA genome and is approximately 50 to 200 nm in size.	SARS-CoV-2 is classified within the broad family of viruses known as coronaviruses.	Common symptoms of SARS-CoV-2 infection include headache, loss of taste, sore throat, congestion, runny nose, nausea, vomiting, diarrhoea, fever, chills, cough, shortness of breath, fatigue, and body aches. It is the strain of coronavirus that causes COVID-19.	[83, 84].[85, 86]

Table S3. The primary methods used for virus detection in wastewater systems.

Method type	Method description	Advantages	Disadvantages
Transmission electron microscopy (TEM)	TEM is the earliest imaging technique with nanometre-scale resolution and is mainly applied to quantify, identify, and classify viruses based on morphology. TEM involves negative staining—the virus particles are primarily adsorbed on a pre-treated specimen support. After the staining and drying steps, the samples are analysed under an electron microscope. Information from negative staining comprises virus count, size, and structure [87].	TEM can be used to study the morphology of viruses present in activated sludge and anaerobically digested sludge samples [88]. TEM results can show the various morphologic types of different viral communities in sludge samples. TEM can identify viruses in emerging infectious diseases when the virus morphologies are well known [89].	TEM is highly selective for host-specific infectious virus and fails to enable an actual viral count [90]. TEM cannot be used to evaluate a large number of samples. TEM requires high level of expertise, well-trained personnel and advanced equipment. TEM analyses are not yet automated [91].
Nucleic acid staining with fluorescent dyes	In this viral quantification method, wastewater samples are passed through filters (commonly 0.22 μm), and then, the nucleic acids in the virus particles are stained with a highly fluorescent dye. Staining with this type of dye allows the formation of fluorescent dots with dimensions larger than actual virus particles [15].	Through this method, stained viral particles can easily be counted even at lower magnifications, thus obviating the requirement to use TEM [15]. Epifluorescence microscopy (EFM) can count viruses that are not arable in laboratories. Through DNase treatment, EFM can distinguish virus particles with nucleic acids from virus-like particles without nucleic acids [92].	This efficiency of this method is very low, mainly detecting RNA viruses and single-stranded DNA viruses [93].

Flow cytometry (FCM)	FCM involves two processing steps. Initially, the sample is diluted with buffer solutions and stained with fluorescent dyes, which bind selectively to DNA or RNA. Therefore, the fluorescence intensity of the DNA/dye and RNA/dye complexes is correlated with the DNA/RNA content in the sample, which is injected into the flow cytometer [94]. The adjacent sheath fluid hydrodynamics effect allows the virus particles to enter a stream in single file. Each particle intersects with a beam of monochromatic light, normally from an argon-ion laser. The scattering and fluorescence produced by interactions of each particle with the incident laser beam is collected by detectors and examined as the scatter and fluorescence intensity, correspondingly [95].	FCM has a particularly high quantification speed and higher sensitivity and accuracy than TEM [90, 96].	FCM requires single-particle viral nucleic acids for analysis, and the strength of flow cytometry is also its main disadvantage. FCM fails to provide information about viral inter-cellular distribution, such as protein distribution [97].
Immunofluorescence assay (IFA)	In IFA, the viral sample is taken from the infected cell culture and adsorbed onto a microscope slide. Viral protein antigen can be detected by sequentially incubating the fixed sample with a particular antibody and a fluorescent chemical-conjugated secondary antibody that identifies the protein of interest. Below visual innervation, the fluorophore-conjugated antibody fluoresces. When viewed under a fluorescence microscope, the antigen-antibody complex appears to be a fluorescent particle [15]	IFA can be used to quantify the infectivity of viruses [98]. IFA has comparatively higher sensitive than other cell culture-based methods and rapid testing [99].	IFA requires adequate equipment and a highly trained technicians to obtain precise results [99].
Enzyme-linked immunosorbent assay (ELISA)	ELISA is a method that can detect the occurrence of microbial antigens in different matrices. It depends on the antigen-binding principle and elicits a change in colour or fluorescence due to the resulting enzyme activity. The	ELISA is a comparatively simple method with a high specificity and sensitivity due to the antigen-antibody reaction and high efficiency because it does not require complicated sample pre-	ELISA is a labour-intensive and expensive method because it is very sophisticated and requires expensive cell culture media to obtain a specific

	difference in colour or fluorescence is correlated with the concentration of the probed antigen in the sample [15, 100].	treatment. ELISAs are safe and eco-friendly because radioactive substances and large amounts of organic solvents are not needed [101].	antibody. ELISAs are associated with a high chance of false positive or negative outcomes [101].
Pulsed-field gel electrophoresis (PFGE)	In the PFGE method, a pulsating electric field is used to separate high molecular weight DNA fragments according to their molecular size. The alternating electric fields produced among two individual electrodes trigger the molecules to periodically reorient parallel to the applied electric field. The ability of DNA molecules to reorient themselves and respond to the applied modulated electric field depends on their molecular size and charge [15].	PFGE is highly effective in assessing epidemiologic similarity and can be used as a universal basic subtyping process for different microbes, with only the choice of the restriction enzyme and electrophoresis conditions optimized for every genus. PFGE produces very stable and reproducible DNA restriction patterns [102].	PFGE is time-consuming, cannot distinguish among unrelated isolates, and cannot optimize separation in each part of a gel simultaneously [103]. Bands of the same size may not originate from the same part of the chromosome. Thus, differences in the restriction site can shift the results [104]. PFGE cannot distinguish isolates to the same degree achieved through whole-genome sequencing [105].
Molecular methods			
Conventional polymerase chain reaction (cPCR)	Generally, PCR is an in vitro amplification process in which a DNA fragment is copied from a DNA extract. In virus detection, this method amplifies or clones a piece of the viral genome. A pair of oligonucleotides (primers), which describe the beginning site for DNA polymerase, is applied to flank the DNA fragment that is to be replicated. Each oligonucleotide is intended to become affixed to a specific target DNA edge, dependent on the provided sequence. Amplification through PCR requires a thermostable enzyme;	PCR has comparatively high sensitivity, can be performed within 4-8 hours, is more cost-effective with selective use than culture and staining, and can be used to test for antimicrobial resistance. PCR is also capable of identifying a small amount of virus [106].	Compared with culture and staining, the possibly lower specificity of cPCR and the necessity for a narrow list of causative agents to create specific primers lead cPCR to become less cost-effective once performed for multiple organisms due to supply costs, equipment fees, and training costs [106].

	usually Taq Polymerase, to make new DNA strands from existing DNA extract strands. PCR proceeds through three steps: 1st denaturation/separation of dsDNA at temperature > 90 °C, 2nd annealing of primers at a temperature close to the melting temperature, and 3rd elongation at ⩾72 ° C. Repetition of these steps generates multiple copies of DNA sequences [15].		
Reverse transcription-polymerase chain reaction (RT-PCR)	For virus RNA detection, the amplification of nucleic acids begins with DNA production from RNA using a reverse transcriptase enzyme [107]. In RT-PCR, the produced DNA is cDNA, which acts as a new template that is then amplified via PCR; the technique is described in cPCR [15, 108]. Reverse transcription allows cDNAs to be created from RNA viruses, such as NoV, AiV, HAV, HEV, astrovirus, RoV and CoVs, which can occur in wastewater systems. RT-PCR can also amplify the nucleic acids of HEVs, which are positive-strand RNA viruses [15].	Compared with PCR-based quantification approaches, RT-PCR eliminates post-amplification handling, has simpler automation, and can process a large number of samples. RT-PCR has a dynamic range of template determination (about six orders of magnitude). Thus, it offers vast potential for quantifying a range of viruses in wastewater systems [109].	Disadvantages of RT-PCR include its complexity and problems related to its specificity, sensitivity, and reproducibility. Furthermore, it suffers from the troubles inherent in cPCR when used as a quantitative method [109].

Real-time polymerase chain reaction (qPCR)	qPCR is a technique that allows simultaneous PCR amplification of nucleic acids and detection of the products. qPCR is categorized as a quantitative approach because it allows quantification of the target sequences compared to the cPCR technique, which offers qualitative data via gel electrophoresis. In qPCR, the PCR products are marked by binding with fluorogenic probes or fluorescent dyes. A real-time thermocycler is applied to examine the fluorescence emission through PCR amplification. Quantification is based on the correlation between the fluorescence emission intensity and the quantity of the PCR product amplification after each cycle. qPCR has been used to detect and quantify HAdV, JCPyV, NoV GII, s, EV, RoV, reovirus, Sapovirus, and SARS-CoV-2 in wastewater systems [15].	qPCR is more time efficient, sensitive, and specific than cPCR [110]. cPCR is more costly than qPCR because of the many chemicals and agarose gel electrophoresis needed [111]. qPCR requires a smaller amount of template material. Furthermore, with qPCR, the analytes can be confirmed through a melting curve analysis [112].	qPCR instrumentation is costly compared with cPCR; multiplexing is still limited in RT-PCR. Kits are not obtainable for all types of genetic material and disorders. The technical and standardized protocols are limited. Increased expertise and technical skills are required for developing a novel qPCR assay [113].

References

[1] M.A. Adefisoye, U.U. Nwodo, E. Green, A.I. Okoh, Quantitative PCR Detection and Characterisation of Human Adenovirus, Rotavirus and Hepatitis A Virus in Discharged Effluents of Two Wastewater Treatment Facilities in the Eastern Cape, South Africa, Food and Environmental Virology, 8 (2016) 262-274.

[2] O. Osuolale, A. Okoh, Human enteric bacteria and viruses in five wastewater treatment plants in the Eastern Cape, South Africa, Journal of Infection and Public Health, 10 (2017) 541-547.

[3] V.V. Mabasa, K.D. Meno, M.B. Taylor, J. Mans, Environmental Surveillance for Noroviruses in Selected South African Wastewaters 2015–2016: Emergence of the Novel GII.17, Food and Environmental Virology, 10 (2018) 16-28.

[4] O. Onosi, N.S. Upfold, M.D. Jukes, G.A. Luke, C. Knox, The First Molecular Detection of Aichi Virus 1 in Raw Sewage and Mussels Collected in South Africa, Food and Environmental Virology, 11 (2019) 96-100.

[5] C. Ibrahim, N. Cherif, S. Hammami, P. Pothier, A. Hassen, Quantification and Genotyping of Rotavirus A within Two Wastewater Treatment Processes, CLEAN – Soil, Air, Water, 44 (2016) 393-401.

[6] I. Ouardani, S. Turki, M. Aouni, J.L. Romalde, Detection and Molecular Characterization of Hepatitis A Virus from Tunisian Wastewater Treatment Plants with Different Secondary Treatments, Applied and Environmental Microbiology, 82 (2016) 3834-3845.

[7] C. Ibrahim, S. Hammami, S. Mejri, I. Mehri, P. Pothier, A. Hassen, Detection of Aichi virus genotype B in two lines of wastewater treatment processes, Microbial Pathogenesis, 109 (2017) 305-312.

[8] C. Ibrahim, S. Hammami, N. Chérif, S. Mejri, P. Pothier, A. Hassen, Detection of Sapoviruses in two biological lines of Tunisian hospital wastewater treatment, International Journal of Environmental Health Research, 29 (2019) 400-413.

[9] M.F. Varela, I. Ouardani, T. Kato, S. Kadoya, M. Aouni, D. Sano, J.L. Romalde, Sapovirus in Wastewater Treatment Plants in Tunisia: Prevalence, Removal, and Genetic Characterization, Applied and Environmental Microbiology, 84 (2018)

e02093-02017.

[10] C. Ibrahim, A. Hassen, P. Pothier, S. Mejri, S. Hammami, Molecular detection and genotypic characterization of enteric adenoviruses in a hospital wastewater, Environmental Science and Pollution Research, 25 (2018) 10977-10987.

[11] H. Hamza, M. Leifels, M. Wilhelm, I.A. Hamza, Relative abundance of human bocaviruses in urban sewage in Greater Cairo, Egypt, Food and environmental virology, 9 (2017) 304-313.

[12] M.N.F. Shaheen, S.E. Abd El-Daim, N.I. Ahmed, E.M. Elmahdy, Environmental monitoring of Aichi virus and human bocavirus in samples from wastewater treatment plant, drain, and River Nile in Egypt, Journal of Water and Health, 18 (2019) 30-37.

[13] E.M. Elmahdy, N.I. Ahmed, M.N.F. Shaheen, E.-C.B. Mohamed, S.A. Loutfy, Molecular detection of human adenovirus in urban wastewater in Egypt and among children suffering from acute gastroenteritis, Journal of Water and Health, 17 (2019) 287-294.

[14] H. Hamza, I.A. Hamza, Oncogenic papillomavirus and polyomavirus in urban sewage in Egypt, Science of The Total Environment, 610 (2018) 1413-1420.

[15] M.V.A. Corpuz, A. Buonerba, G. Vigliotta, T. Zarra, F. Ballesteros Jr, P. Campiglia, V. Belgiorno, G. Korshin, V. Naddeo, Viruses in wastewater: occurrence, abundance and detection methods, Science of the Total Environment, 745 (2020) 140910.

[16] K. Farkas, D.I. Walker, E.M. Adriaenssens, J.E. McDonald, L.S. Hillary, S.K. Malham, D.L. Jones, Viral indicators for tracking domestic wastewater contamination in the aquatic environment, Water research, (2020) 115926.

[17] M. Kitajima, A.T. Rachmadi, B.C. Iker, E. Haramoto, C.P. Gerba, Genetically distinct genogroup IV norovirus strains identified in wastewater, Archives of Virology, 161 (2016) 3521-3525.

[18] W.Q. Betancourt, C.P. Gerba, Rethinking the Significance of Reovirus in Water and Wastewater, Food and Environmental Virology, 8 (2016) 161-173.

[19] N.E. Brinkman, G.S. Fout, S.P. Keely, Retrospective Surveillance of Wastewater

To Examine Seasonal Dynamics of Enterovirus Infections, mSphere, 2 (2017) e00099-00017.

[20] K.-Y. Lim, Y. Wu, S.C. Jiang, Assessment of Cryptosporidium and norovirus risk associated with de facto wastewater reuse in Trinity River, Texas, Microbial Risk Analysis, 5 (2017) 15-24.

[21] J.W. Zabinski, K.J. Pieper, J.M. Gibson, A Bayesian Belief Network Model Assessing the Risk to Wastewater Workers of Contracting Ebola Virus Disease During an Outbreak, Risk Analysis, 38 (2018) 376-391.

[22] M. Kitajima, A.T. Rachmadi, B.C. Iker, E. Haramoto, C.P. Gerba, Temporal variations in genotype distribution of human sapoviruses and Aichi virus 1 in wastewater in Southern Arizona, United States, Journal of Applied Microbiology, 124 (2018) 1324-1332.

[23] Y. Qiu, B.E. Lee, N.J. Ruecker, N. Neumann, N. Ashbolt, X. Pang, A one-step centrifugal ultrafiltration method to concentrate enteric viruses from wastewater, Journal of Virological Methods, 237 (2016) 150-153.

[24] Y. Qiu, Q. Li, B.E. Lee, N.J. Ruecker, N.F. Neumann, N.J. Ashbolt, X. Pang, UV inactivation of human infectious viruses at two full-scale wastewater treatment plants in Canada, Water Research, 147 (2018) 73-81.

[25] T.M. Fumian, J.M. Fioretti, J.H. Lun, I.A.L. dos Santos, P.A. White, M.P. Miagostovich, Detection of norovirus epidemic genotypes in raw sewage using next generation sequencing, Environment International, 123 (2019) 282-291.

[26] J.M. Fioretti, M.S. Rocha, T.M. Fumian, A. Ginuino, T.P. da Silva, M.R. de Assis, J.d.S. Rodrigues, F.A. Carvalho-Costa, M.P. Miagostovich, Occurrence of human sapoviruses in wastewater and stool samples in Rio De Janeiro, Brazil, Journal of Applied Microbiology, 121 (2016) 855-862.

[27] J.M. Fioretti, T.M. Fumian, M.S. Rocha, I.d.A.L. dos Santos, F.A. Carvalho-Costa, M.R. de Assis, J.d.S. Rodrigues, J.P.G. Leite, M.P. Miagostovich, Surveillance of Noroviruses in Rio De Janeiro, Brazil: Occurrence of New GIV Genotype in Clinical and Wastewater Samples, Food and Environmental Virology, 10 (2018) 1-6.

[28] L. Burutarán, A. Lizasoain, M. García, L.F.L. Tort, R. Colina, M. Victoria, Detection and Molecular Characterization of Aichivirus 1 in Wastewater Samples from Uruguay, Food and Environmental Virology, 8 (2016) 13-17.

[29] P.A. Baez, M.C. Lopez, A. Duque-Jaramillo, D. Pelaez, F. Molina, M.-C. Navas, First evidence of the Hepatitis E virus in environmental waters in Colombia, PLoS One, 12 (2017) e0177525.

[30] J. Levican, A. Levican, M. Ampuero, A. Gaggero, JC polyomavirus circulation in one-year surveillance in wastewater in Santiago, Chile, Infection, Genetics and Evolution, 71 (2019) 151-158.

[31] C.F. Estívariz, E.E. Pérez-Sánchez, A. Bahena, C.C. Burns, H.E. Gary, H. García-Lozano, G. Rey-Benito, S. Peñaranda, K.V. Castillo-Montufar, R.S. Nava-Acosta, J.S. Meschke, M.S. Oberste, I. Lopez-Martínez, J.A. Díaz-Quiñonez, Field Performance of Two Methods for Detection of Poliovirus in Wastewater Samples, Mexico 2016–2017, Food and Environmental Virology, 11 (2019) 364-373.

[32] M. Iaconelli, M. Divizia, S. Della Libera, P. Di Bonito, G. La Rosa, Frequent Detection and Genetic Diversity of Human Bocavirus in Urban Sewage Samples, Food and Environmental Virology, 8 (2016) 289-295.

[33] F. Pennino, A. Nardone, P. Montuori, S. Aurino, I. Torre, A. Battistone, R. Delogu, G. Buttinelli, S. Fiore, C. Amato, M. Triassi, Large-Scale Survey of Human Enteroviruses in Wastewater Treatment Plants of a Metropolitan Area of Southern Italy, Food and Environmental Virology, 10 (2018) 187-192.

[34] F. Di Profio, I. Melegari, A. Palombieri, V. Sarchese, A. Arbuatti, P. Fruci, F. Marsilio, V. Martella, B. Di Martino, High prevalence of hepatitis E virus in raw sewage in Southern Italy, Virus Research, 272 (2019) 197710.

[35] P. Mancini, G. Bonanno Ferraro, M. Iaconelli, E. Suffredini, B. Valdazo-González, S. Della Libera, M. Divizia, G. La Rosa, Molecular characterization of human Sapovirus in untreated sewage in Italy by amplicon-based Sanger and next-generation sequencing, Journal of Applied Microbiology, 126 (2019) 324-331.

[36] L. Kaas, L. Ogorzaly, G. Lecellier, V. Berteaux-Lecellier, H.-M. Cauchie, J.

Langlet, Detection of Human Enteric Viruses in French Polynesian Wastewaters, Environmental Waters and Giant Clams, Food and Environmental Virology, 11 (2019) 52-64.

[37] T. Miura, S. Lhomme, J.-C. Le Saux, P. Le Mehaute, Y. Guillois, E. Couturier, J. Izopet, F. Abranavel, F.S. Le Guyader, Detection of Hepatitis E Virus in Sewage After an Outbreak on a French Island, Food and Environmental Virology, 8 (2016) 194-199.

[38] K. Farkas, D.M. Cooper, J.E. McDonald, S.K. Malham, A. de Rougemont, D.L. Jones, Seasonal and spatial dynamics of enteric viruses in wastewater and in riverine and estuarine receiving waters, Science of The Total Environment, 634 (2018) 1174-1183.

[39] K. Farkas, M. Marshall, D. Cooper, J.E. McDonald, S.K. Malham, D.E. Peters, J.D. Maloney, D.L. Jones, Seasonal and diurnal surveillance of treated and untreated wastewater for human enteric viruses, Environmental Science and Pollution Research, 25 (2018) 33391-33401.

[40] O. Dienus, E. Sokolova, F. Nyström, A. Matussek, S. Löfgren, L. Blom, T.J.R. Pettersson, P.-E. Lindgren, Norovirus Dynamics in Wastewater Discharges and in the Recipient Drinking Water Source: Long-Term Monitoring and Hydrodynamic Modeling, Environmental Science & Technology, 50 (2016) 10851-10858.

[41] E. Gonzales-Gustavson, M. Rusiñol, G. Medema, M. Calvo, R. Girones, Quantitative risk assessment of norovirus and adenovirus for the use of reclaimed water to irrigate lettuce in Catalonia, Water Research, 153 (2019) 91-99.

[42] A. Matos, J.R. Mesquita, D. Gonçalves, J. Abreu-Silva, C. Luxo, M.S. Nascimento, First detection and molecular characterization of hepatitis E virus in water from wastewater treatment plants in Portugal, Annals of Agricultural and Environmental Medicine, 25 (2018) 364-367.

[43] S. Beyer, R. Szewzyk, R. Gnirss, R. Johne, H.-C. Selinka, Detection and Characterization of Hepatitis E Virus Genotype 3 in Wastewater and Urban Surface Waters in Germany, Food and Environmental Virology, (2020).

[44] Y. Masago, Y. Konta, S. Kazama, M. Inaba, T. Imagawa, K. Tohma, M. Saito, A.

Suzuki, H. Oshitani, T. Omura, Comparative Evaluation of Real-Time PCR Methods for Human Noroviruses in Wastewater and Human Stool, PLoS One, 11 (2016) e0160825.

[45] M. Abe, Y. Ueki, T. Miura, S. Kimura, Y. Suzuki, N. Sugawara, Y. Masago, T. Omura, S. Watanabe, Detection of Human Parechoviruses in Clinical and Municipal Wastewater Samples in Miyagi, Japan, in 2012–2014, Japanese Journal of Infectious Diseases, 69 (2016) 414-417.

[46] S. Kazama, Y. Masago, K. Tohma, N. Souma, T. Imagawa, A. Suzuki, X. Liu, M. Saito, H. Oshitani, T. Omura, Temporal dynamics of norovirus determined through monitoring of municipal wastewater by pyrosequencing and virological surveillance of gastroenteritis cases, Water Research, 92 (2016) 244-253.

[47] T. Ito, M. Kitajima, T. Kato, S. Ishii, T. Segawa, S. Okabe, D. Sano, Target virus log10 reduction values determined for two reclaimed wastewater irrigation scenarios in Japan based on tolerable annual disease burden, Water Research, 125 (2017) 438-448.

[48] S. Tavakoli Nick, S.R. Mohebbi, S.M. Hosseini, H. Mirjalali, M. Alebouyeh, Occurrence and molecular characterization of Torque teno virus (TTV) in a wastewater treatment plant in Tehran, Journal of Water and Health, 17 (2019) 971-977.

[49] M. Aminipour, M. Ghaderi, N. Harzandi, First Occurrence of Saffold Virus in Sewage and River Water Samples in Karaj, Iran, Food and Environmental Virology, 12 (2020) 75-80.

[50] H. Li, W. Li, R. She, L. Yu, Q. Wu, J. Yang, F. Hu, M.H. Soomro, R. Shi, W. Hao, Y. Zhao, J. Mao, Hepatitis E Virus Genotype 4 Sequences Detected in Sewage from Treatment Plants of China, Food and Environmental Virology, 9 (2017) 230-233.

[51] G.T. Nguyen, J. Pu, T. Miura, H. Ito, S. Kazama, Y. Konta, A. Van Le, T. Watanabe, Oyster Contamination with Human Noroviruses Impacted by Urban Drainage and Seasonal Flooding in Vietnam, Food and Environmental Virology, 10 (2018) 61-71.

[52] J.H. Lun, N.D. Crosbie, P.A. White, Genetic diversity and quantification of human mastadenoviruses in wastewater from Sydney and Melbourne, Australia, Science of the Total Environment, 675 (2019) 305-312.

[53] Y.S. Ahi, S.K. Mittal, Components of adenovirus genome packaging, Frontiers in microbiology, 7 (2016) 1503.

[54] B. Ghebremedhin, Human adenovirus: Viral pathogen with increasing importance, European Journal of Microbiology and Immunology, 4 (2014) 26-33.

[55] D. D'Souza, S. Joshi, Foodborne viruses of human health concern, (2016).

[56] M.V. Yates, Emerging Viruses, in: Microbiology of Waterborne Diseases, Elsevier, 2014, pp. 529-533.

[57] B. Prevost, F.S. Lucas, K. Ambert-Balay, P. Pothier, L. Moulin, S. Wurtzer, Deciphering the diversities of astroviruses and noroviruses in wastewater treatment plant effluents by a high-throughput sequencing method, Appl. Environ. Microbiol., 81 (2015) 7215-7222.

[58] S. Cordey, D.-L. Vu, M. Schibler, A.G. L'Huillier, F. Brito, M. Docquier, K.M. Posfay-Barbe, T.J. Petty, L. Turin, E.M. Zdobnov, Astrovirus MLB2, a new gastroenteric virus associated with meningitis and disseminated infection, Emerging infectious diseases, 22 (2016) 846.

[59] F. Helle, E. Brochot, L. Handala, E. Martin, S. Castelain, C. Francois, G. Duverlie, Biology of the BKPyV: an update, Viruses, 9 (2017) 327.

[60] G.R. Ambalathingal, R.S. Francis, M.J. Smyth, C. Smith, R. Khanna, BK polyomavirus: clinical aspects, immune regulation, and emerging therapies, Clinical microbiology reviews, 30 (2017) 503-528.

[61] R.W. Körner, M. Söderlund-Venermo, S. van Koningsbruggen-Rietschel, R. Kaiser, M. Malecki, O. Schildgen, Severe human bocavirus infection, Germany, Emerging infectious diseases, 17 (2011) 2303.

[62] M. Guido, M.R. Tumolo, T. Verri, A. Romano, F. Serio, M. De Giorgi, A. De Donno, F. Bagordo, A. Zizza, Human bocavirus: Current knowledge and future challenges, World journal of gastroenterology, 22 (2016) 8684.

[63] V. Stone, M. Hankaniemi, O. Laitinen, A. Sioofy-Khojine, A. Lin, I.D. Lozano,

M. Mazur, V. Marjomäki, K. Loré, H. Hyöty, A hexavalent Coxsackievirus B vaccine is highly immunogenic and has a strong protective capacity in mice and nonhuman primates, Science Advances, 6 (2020) eaaz2433.

[64] K.S. Kim, G. Hufnagel, N.M. Chapman, S. Tracy, The group B coxsackieviruses and myocarditis, Reviews in medical virology, 11 (2001) 355-368.

[65] S.S. Long, C.G. Prober, M. Fischer, Principles and practice of pediatric infectious diseases E-Book, Elsevier Health Sciences, 2017.

[66] J. Denner, Hepatitis E virus (HEV)—The Future, Viruses, 11 (2019) 251.

[67] U. Neu, M.S. Maginnis, A.S. Palma, L.J. Ströh, C.D. Nelson, T. Feizi, W.J. Atwood, T. Stehle, Structure-function analysis of the human JC polyomavirus establishes the LSTc pentasaccharide as a functional receptor motif, Cell host & microbe, 8 (2010) 309-319.

[68] C.S. Tan, I.J. Koralnik, Beyond progressive multifocal leukoencephalopathy: expanded pathogenesis of JC virus infection in the central nervous system, Lancet neurology, 9 (2010) 425.

[69] C.O. Owino, J.J.H. Chu, Recent advances on the role of host factors during non-poliovirus enteroviral infections, Journal of biomedical science, 26 (2019) 47.

[70] M.D. Fernandez-Garcia, O. Kebe, A.D. Fall, K. Ndiaye, Identification and molecular characterization of non-polio enteroviruses from children with acute flaccid paralysis in West Africa, 2013–2014, Scientific reports, 7 (2017) 1-11.

[71] M.V. Yates, Norovirus, in: Microbiology of Waterborne Diseases, Elsevier, 2014, pp. 515-522.

[72] H.-U. Bernard, R.D. Burk, Z. Chen, K. Van Doorslaer, H. Zur Hausen, E.-M. de Villiers, Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments, Virology, 401 (2010) 70-79.

[73] A. Harari, Z. Chen, R.D. Burk, Human papillomavirus genomics: past, present and future, in: Human Papillomavirus, Karger Publishers, 2014, pp. 1-18.

[74] L. Olijve, L. Jennings, T. Walls, Human parechovirus: an increasingly recognized cause of sepsis-like illness in young infants, Clinical microbiology reviews, 31 (2018).

[75] S. Modrow, D. Falke, U. Truyen, H. Schätzl, Viruses with single-stranded, positive-sense RNA genomes, Molecular Virology, (2013) 185.

[76] R.L. DeBiasi, K.L. Tyler, Orthoreoviruses and Orbiviruses, Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, (2015) 1848.

[77] J.W. Kim, K.C. Pituch, A. Xiao, J.S. Young, W.K. Panek, M.E. Muroski, A. Rashidi, J.R. Kane, D. Kanojia, M.S. Lesniak, Gene/Viral Treatment Approaches for Malignant Brain Cancer, in: Handbook of Brain Tumor Chemotherapy, Molecular Therapeutics, and Immunotherapy, Elsevier, 2018, pp. 211-227.

[78] B.W. Mahy, M.H. Van Regenmortel, Desk encyclopedia of human and medical virology, Academic Press, 2010.

[79] T.S. Nielsen, A.Y. Nielsen, J. Banner, J. Hansen, U. Baandrup, L.P. Nielsen, Saffold virus infection associated with human myocarditis, Journal of Clinical Virology, 74 (2016) 78-81.

[80] S.Z.K. Tan, M.Z.Y. Tan, M. Prabakaran, Saffold virus, an emerging human cardiovirus, Reviews in medical virology, 27 (2017) e1908.

[81] T. Oka, Q. Wang, K. Katayama, L.J. Saif, Comprehensive review of human sapoviruses, Clinical microbiology reviews, 28 (2015) 32-53.

[82] M. Schaechter, Encyclopedia of microbiology, Academic Press, 2009.

[83] D. Fox, What you need to know about the Wuhan coronavirus, Nature, (2020).

[84] N. Chen, M. Zhou, X. Dong, J. Qu, F. Gong, Y. Han, Y. Qiu, J. Wang, Y. Liu, Y. Wei, Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study, The lancet, 395 (2020) 507-513.

[85] C. Wu, Y. Liu, Y. Yang, P. Zhang, W. Zhong, Y. Wang, Q. Wang, Y. Xu, M. Li, X. Li, Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods, Acta Pharmaceutica Sinica B, (2020).

[86] R. Castagnoli, M. Votto, A. Licari, I. Brambilla, R. Bruno, S. Perlini, F. Rovida, F. Baldanti, G.L. Marseglia, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents: a systematic review, JAMA pediatrics, (2020).

[87] P. Roingeard, P.I. Raynal, S. Eymieux, E. Blanchard, Virus detection by transmission electron microscopy: Still useful for diagnosis and a plus for biosafety, Reviews in medical virology, 29 (2019) e2019.

[88] Q. Wu, W.-T. Liu, Determination of virus abundance, diversity and distribution in a municipal wastewater treatment plant, Water research, 43 (2009) 1101-1109.

[89] M. Laue, Electron microscopy of viruses, in: Methods in cell biology, Elsevier, 2010, pp. 1-20.

[90] M. Brown, S. Camézuli, R.J. Davenport, E. Petelenz-Kurdziel, L. Øvreås, T. Curtis, Flow cytometric quantification of viruses in activated sludge, water research, 68 (2015) 414-422.

[91] D.F. Barreto-Vieira, O.M. Barth, Negative and positive staining in transmission electron microscopy for virus diagnosis, InTech, 2015.

[92] A.C. Ortmann, C.A. Suttle, Determination of virus abundance by epifluorescence microscopy, in: Bacteriophages, Springer, 2009, pp. 87-95.

[93] F. Ferreira, F. Guimaraes, T. Fumian, M. Victoria, C. Vieira, S. Luz, T. Shubo, J.P.G. Leite, M.P. Miagostovich, Environmental dissemination of group A rotavirus: P-type, G-type and subgroup characterization, Water Science and Technology, 60 (2009) 633-642.

[94] A. Adan, G. Alizada, Y. Kiraz, Y. Baran, A. Nalbant, Flow cytometry: basic principles and applications, Critical reviews in biotechnology, 37 (2017) 163-176.

[95] M. Brown, C. Wittwer, Flow cytometry: principles and clinical applications in hematology, in, Oxford University Press, 2000.

[96] L. Ma, G. Mao, J. Liu, H. Yu, G. Gao, Y. Wang, Rapid quantification of bacteria and viruses in influent, settled water, activated sludge and effluent from a wastewater treatment plant using flow cytometry, Water Science and Technology, 68 (2013) 1763-1769.

[97] F. Porichis, M.G. Hart, M. Griesbeck, H.L. Everett, M. Hassan, A.E. Baxter, M. Lindqvist, S.M. Miller, D.Z. Soghoian, D.G. Kavanagh, High-throughput detection of miRNAs and gene-specific mRNA at the single-cell level by flow cytometry, Nature communications, 5 (2014) 1-12.

[98] B. Calgua, C.R.M. Barardi, S. Bofill-Mas, J. Rodriguez-Manzano, R. Girones, Detection and quantitation of infectious human adenoviruses and JC polyomaviruses in water by immunofluorescence assay, Journal of virological methods, 171 (2011) 1-7.

[99] L. Vaz-de-Lima, M. Souza, T. Matsumoto, M. Hong, M. Salgado, M. Barbosa, N. Sato, H. Requejo, C. Oliveira, R. Pecchini, Performance of indirect immunofluorescence assay, immunochromatography assay and reverse transcription-polymerase chain reaction for detecting human respiratory syncytial virus in nasopharyngeal aspirate samples, Memórias do Instituto Oswaldo Cruz, 103 (2008) 463-467.

[100] N. Boonham, J. Kreuze, S. Winter, R. van der Vlugt, J. Bergervoet, J. Tomlinson, R. Mumford, Methods in virus diagnostics: from ELISA to next generation sequencing, Virus research, 186 (2014) 20-31.

[101] S. Sakamoto, W. Putalun, S. Vimolmangkang, W. Phoolcharoen, Y. Shoyama, H. Tanaka, S. Morimoto, Enzyme-linked immunosorbent assay for the quantitative/qualitative analysis of plant secondary metabolites, Journal of natural medicines, 72 (2018) 32-42.

[102] M. Tamburro, M.L. Sammarco, I. Fanelli, G. Ripabelli, Characterization of Listeria monocytogenes serovar 1/2a, 1/2b, 1/2c and 4b by high resolution melting analysis for epidemiological investigations, International Journal of Food Microbiology, 310 (2019) 108289.

[103] R.V. Goering, Pulsed field gel electrophoresis: a review of application and interpretation in the molecular epidemiology of infectious disease, Infection, Genetics and Evolution, 10 (2010) 866-875.

[104] R. Hagman, I. Kühn, Escherichia coli strains isolated from the uterus and urinary bladder of bitches suffering from pyometra: comparison by restriction enzyme digestion and pulsed-field gel electrophoresis, Veterinary microbiology, 84 (2002) 143-153.

[105] S.J. Salipante, D.J. SenGupta, L.A. Cummings, T.A. Land, D.R. Hoogestraat, B.T. Cookson, Application of whole-genome sequencing for bacterial strain

typing in molecular epidemiology, Journal of clinical microbiology, 53 (2015) 1072-1079.

[106] H.Y. Liu, G.C. Hopping, U. Vaidyanathan, Y.C. Ronquillo, P.C. Hoopes, M. Moshirfar, Polymerase Chain Reaction and Its Application in the Diagnosis of Infectious Keratitis, Medical Hypothesis, Discovery and Innovation in Ophthalmology, 8 (2019) 152.

[107] F. Cobo, Suppl 1: application of molecular diagnostic techniques for viral testing, The open virology journal, 6 (2012) 104.

[108] R. Schinazi, J. Kohler, B. Kim, Reverse Transcription, (2013).

[109] G. Bleve, L. Rizzotti, F. Dellaglio, S. Torriani, Development of reverse transcription (RT)-PCR and real-time RT-PCR assays for rapid detection and quantification of viable yeasts and molds contaminating yogurts and pasteurized food products, Applied and Environmental Microbiology, 69 (2003) 4116-4122.

[110] T.b. Yang, J. Liu, J. Chen, Compared with conventional PCR assay, qPCR assay greatly improves the detection efficiency of predation, Ecology and Evolution, 10 (2020) 7713-7722.

[111] O.C. Moreira, J.D. Ramírez, E. Velázquez, M.F.D. Melo, C. Lima-Ferreira, F. Guhl, S. Sosa-Estani, J.A. Marin-Neto, C.A. Morillo, C. Britto, Towards the establishment of a consensus real-time qPCR to monitor Trypanosoma cruzi parasitemia in patients with chronic Chagas disease cardiomyopathy: a substudy from the BENEFIT trial, Acta tropica, 125 (2013) 23-31.

[112] S. Nutz, K. Döll, P. Karlovsky, Determination of the LOQ in real-time PCR by receiver operating characteristic curve analysis: application to qPCR assays for Fusarium verticillioides and F. proliferatum, Analytical and bioanalytical chemistry, 401 (2011) 717-726.

[113] J.W.-F. Law, N.-S. Ab Mutalib, K.-G. Chan, L.-H. Lee, Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations, Frontiers in microbiology, 5 (2015) 770.

Highlights

- Occurrence of viruses in wastewater around the world.
- Temporal patterns of viruses in wastewater systems are described.
- Recent evidence of SARS-CoV-2 in the wastewater system.
- Qualitative and quantitative analysis of viruses in wastewater.
- Wastewater-based epidemiology as a new surveillance tool for infectious disease.

Graphical Abstract