Occurrence of human pathogenic viruses in drinking water and in its sources: A review

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Virus occurrence in sources for drinking water production and in drinking water: a review

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Corresponding Author:	Marta Gea Università degli Studi di Torino: Universita degli Studi di Torino Torino, (TO) Italy ITALY
First Author:	Marco Panizzolo
Order of Authors:	Marco Panizzolo
	Marta Gea
	Elisabetta Carraro
	Giorgio Gilli
	Silvia Bonetta
	Cristina Pignata
Abstract:	Microbiological quality of drinking water (DW) is crucial for Public Health. Many diseases linked to DW consumption are due to viruses. The aim of this review was to describe virus presence detected using molecular methods in sources for DW production and in DW. Four water types were considered: surface water used for DW production (SW-D), groundwater used for DW production (GW-D), water used for human consumption (DW) and bottled water (BW). The considered viruses were human pathogens; moreover plant pathogens proposed as novel viral indicators were presented. Studies published in the last 10 years were analysed and 79 articles were included in the review. Regarding virus occurrence in SW-D, GW-D, DW, high percentages of positive samples were reported for adenovirus, polyomavirus and pepper mild mottle virus. The most searched viruses were adenovirus, enterovirus, norovirus GI/GII and rotavirus. These viruses were frequently detected in SW-D, while they were rarely found in GW-D, suggesting that GW may be safer as a DW source. These viruses were detected also in DW, posing a possible threat for human health. Considering global occurrence, the lowest percentages of positive samples were found in Europe, while the highest percentages in Asia and South America. Only three articles assessed viruses in BW. Considering detection methods, filtration was the most applied concentration method, while nucleic acid extraction and molecular detection were generally performed using spin columns with silica membrane and quantitative PCR respectively. This review highlighted some critical issues such as method standardization lack and need for legislation updates.
Suggested Reviewers:	Marcello Iaconelli ISS: Istituto Superiore Di Sanita marcello.iaconelli@iss.it Research activity focused on environmental monitoring and human pathogenic viruses.
	Mark A. Borchardt Marshfield Clinic Research Institute mark.borchardt@ars.usda.gov Expert in viruses in drinking water
	Fernando R. Spilki FEEVALE University: Universidade FEEVALE fernandors@feevale.br

	expert in removal of viral agents in water and sewage and contamination of water, soil and foods by enteric viruses.
	Tahir Ahmad COMSATS University Islamabad Baig42@gmail.com Expert in environmental virology
	Walter Randazzo University of Valencia: Universitat de Valencia walter.randazzo@uv.es Expert in analytical and detection methods for virology, environmental contamination and public health
	Annalaura Carducci University of Pisa: Universita degli Studi di Pisa annalaura.carducci@unipi.it Expert in environmental virology, water quality and molecular biology.

UNIVERSITÁ DEGLI STUDI DI TORINO
DIPARTIMENTO DI SCIENZE DELLA SANITA' PUBBLICA E PEDIATRICHE
DEPARTMENT OF PUBLIC HEALTH AND PEDIATRICS
DIRETTORE: PROF. SSA CARLA MARIA ZOTTI
Piazza Polonia, 94 – 10126 Torino (Italia)
Codice Fiscale 80088230018 – P.IVA IT02099550010

February 22nd, 2022

Dear Editor,

We send the review *"Virus occurrence in sources for drinking water production and in drinking water: a review"* by Marco Panizzolo, Marta Gea, Elisabetta Carraro, Giorgio Gilli, Silvia Bonetta, Cristina Pignata on JOURNAL OF ENVIRONMENTAL SCIENCES.

Many studies showed that microbial water quality used for human consumption is critical for Public Health. Outbreaks linked to the consumption of contaminated or improperly treated water have been reported all over the world. Among all the waterborne pathogens, viruses are of major concern. Indeed, they can induce gastroenteritis through the faecal-oral route and, due to their peculiar characteristics compared to other pathogens, they are not efficiently removed by drinking water treatments. Virus presence in drinking water is among the main causes of death in developing countries and it induces a consistent percentage of drinking water outbreaks in high-income countries. Therefore, virus detection at all phases of the integrated water cycle, from wastewater to drinking water, has a key role for human health. However, a complete overview of viral occurrence in sources for drinking water production and in drinking water from all over the world is still lacking.
The aim of the submitted review was to describe available data about virus occurrence in sources for drinking water production and in drinking water using molecular methods. Water types considered were: surface water used for drinking production, groundwater used for drinking water production, water used for human consumption (drinking water) and bottled water. Two virus types were considered: human pathogens and plant pathogens proposed as novel viral indicators. Scientific studies published in the last 10 years from all over the world were analyzed and 79 articles were finally included in the review.

We believe that the paper fits the aims and scope of the Journal, specifically, fits the following subjects:

- Aquatic environments
- Environmental microbiology

The study does not involve human subjects. All of the authors have read and approved the paper and it has not been published previously nor is it being considered by any other peer-reviewed journal. All authors are aware of and accept responsibility for the manuscript. All figures and tables were produced by the authors. Lastly, all authors declare no conflicting interests.

Hoping that the manuscript may fulfil the scientific standards of JOURNAL OF ENVIRONMENTAL SCIENCES, our best regards.

Marta Gea and Co-authors

UNIVERSITÁ DEGLI STUDI DI TORINO
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Piazza Polonia, 94 – 10126 Torino (Italia)
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Virus occurrence in sources for drinking water production and in drinking water: a review

Marco Panizzolo[1#], Marta Gea[1#*], Elisabetta Carraro[1], Giorgio Gilli[1], Silvia Bonetta[2], Cristina Pignata[1]

[1]Department of Public Health and Pediatrics, University of Torino, Piazza Polonia 94, 10126 Torino, Italy
[2]Department of Life Sciences and Systems Biology, University of Torino, via Accademia Albertina 13, 10123 Torino, Italy

[#]Marco Panizzolo and Marta Gea contributed equally to this work.

*__Corresponding author__:

Marta Gea

marta.gea@unito.it

Department of Public Health and Pediatrics,

University of Torino,

Piazza Polonia 94, 10126 Torino, Italy.

Phone: +39 011 6703190

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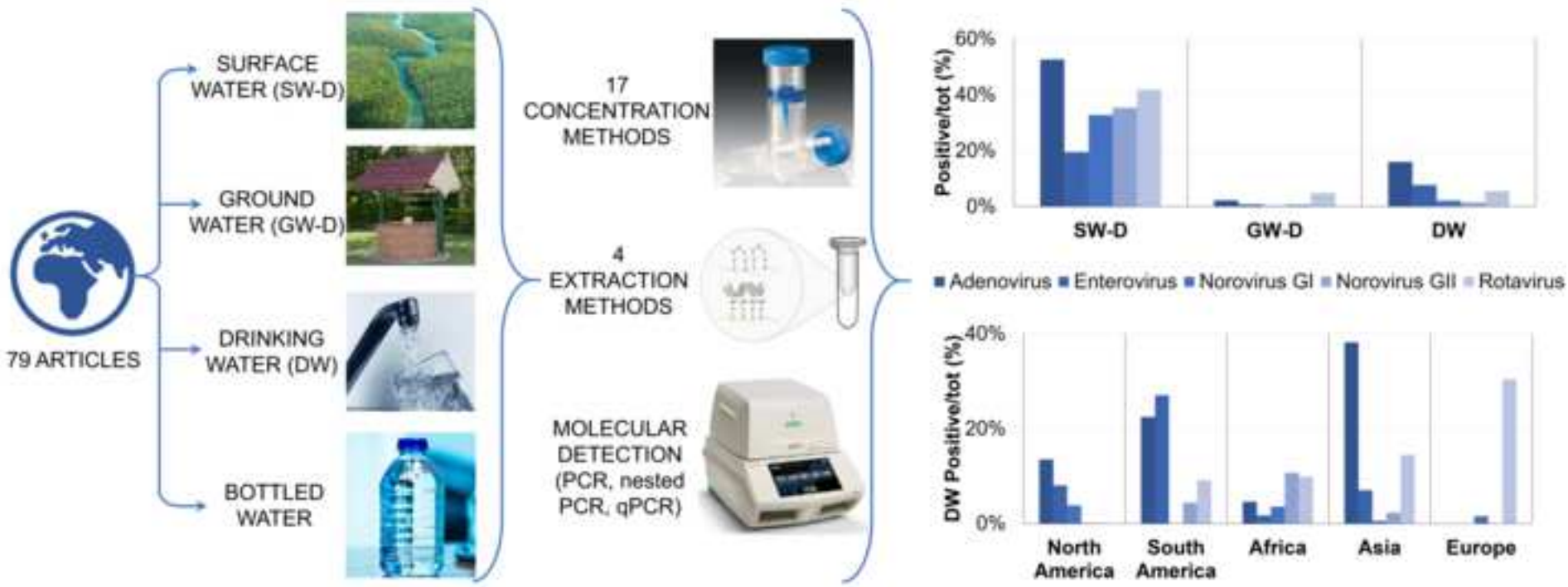
Highlights (max 5, max 85 characters, including spaces, per bullet point)

1. Adenovirus, enterovirus, norovirus, rotavirus were the most searched viruses
2. High % of positive samples for adenovirus, polyomavirus, pepper mild mottle virus
3. Viruses frequently detected in surface water, rarely in ground and drinking water
4. Viruses more detected in drinking water from Asia/South America than from Europe
5. Detection usually performed with filtration (negative filter) and quantitative PCR

Graphical Abstract



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Marco Panizzolo[1#], Marta Gea[1#*], Elisabetta Carraro[1], Giorgio Gilli[1], Silvia Bonetta[2], Cristina Pignata[1]

[1]Department of Public Health and Pediatrics, University of Torino, Piazza Polonia 94, 10126 Torino, Italy
[2]Department of Life Sciences and Systems Biology, University of Torino, via Accademia Albertina 13, 10123 Torino, Italy

[#]Marco Panizzolo and Marta Gea contributed equally to this work.

*__Corresponding author__:
Marta Gea
marta.gea@unito.it
Department of Public Health and Pediatrics,
University of Torino,
Piazza Polonia 94, 10126 Torino, Italy.
Phone: +39 011 6703190

Other e-mail addresses:

marco.panizzolo@unito.it

elisabetta.carraro@unito.it

giorgio.gilli@unito.it

silvia.bonetta@unito.it

cristina.pignata@unito.it

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Marco Panizzolo[1#], Marta Gea[1#*], Elisabetta Carraro[1], Giorgio Gilli[1], Silvia Bonetta[2], Cristina Pignata[1]

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*Corresponding author:

Marta Gea

marta.gea@unito.it

Department of Public Health and Pediatrics,

University of Torino,

Piazza Polonia 94, 10126 Torino, Italy.

Phone: +39 011 6703190

ABSTRACT (max 250 words)

Microbiological quality of drinking water (DW) is crucial for Public Health. Many diseases linked to DW consumption are due to viruses. The aim of this review was to describe virus presence detected using molecular methods in sources for DW production and in DW. Four water types were considered: surface water used for DW production (SW-D), groundwater used for DW production (GW-D), water used for human consumption (DW) and bottled water (BW). The considered viruses were human pathogens; moreover plant pathogens proposed as novel viral indicators were presented. Studies published in the last 10 years were analysed and 79 articles were included in the review.

Regarding virus occurrence in SW-D, GW-D, DW, high percentages of positive samples were reported for adenovirus, polyomavirus and pepper mild mottle virus. The most searched viruses were adenovirus, enterovirus, norovirus GI/GII and rotavirus. These viruses were frequently detected in SW-D, while they were rarely found in GW-D, suggesting that GW may be safer as a DW source. These viruses were detected also in DW, posing a possible threat for human health. Considering global occurrence, the lowest percentages of positive samples were found in Europe, while the highest percentages in Asia and South America. Only three articles assessed viruses in BW.

Considering detection methods, filtration was the most applied concentration method, while nucleic acid extraction and molecular detection were generally performed using spin columns with silica membrane and quantitative PCR respectively.

This review highlighted some critical issues such as method standardization lack and need for legislation updates.

Keywords (max 6): drinking water, enteric virus, human health, microbial water quality, molecular methods, surface water.

Abbreviations:

AdV = adenovirus

AiV = aichivirus

AstV = astrovirus

BW = bottled water (water used for human consumption)

DW = drinking water (water used for human consumption, not bottled)

EPA = Environmental Protection Agency

EV = enterovirus

GW = groundwater

GW-D = groundwater used as a source for DW production

HAV = hepatitis A virus

HEV = hepatitis E virus

NoV = norovirus

PMMoV = pepper mild mottle virus

PyV = polyomavirus

RoV = rotavirus

SW = surface water

SW-D = surface water used as a source for DW production

SaV = sapovirus

TMV = tobacco mosaic virus

TTV = torque teno virus

PCR = polymerase chain reaction

qPCR = quantitative PCR

QMRA = Quantitative Microbial Risk Assessment

WSP = Water Safety Plan

Contents:

1. INTRODUCTION

Fresh water is an essential resource for life on our planet and just a small part is accessible because the most one is present in aquifers or in form of ice. Water scarcity increases and so its reuse is essential (Cocoran et al., 2010). Moreover, water quality as well as water quantity is important. Nowadays, 20% of world's population have no access to drinking water (DW) and 40% suffer the consequences of improperly treated water (Cocoran et al., 2010; Seelen et al., 2019).

Microbiological water quality used for human consumption is considered by many studies to be critical for Public Health. Outbreaks linked to the DW consumption have been reported worldwide and the main causes for contamination were identified as intrusion of animal faeces or wastewaters due to heavy rain in groundwaters (GWs), wastewaters discharge into the DW source, malfunctioning of the disinfection equipment at DW treatment plants and cross-connections, pipe breaks and wastewater intrusion into the distribution system (Ligon and Bartram, 2016; Moreira and Bondelind, 2017). Waterborne outbreaks were reported, for examples, in China (Shang et al., 2017; Xue et al., 2014), Denmark (Van Alphen et al., 2014), Albania (Donia et al., 2011), Spain (Blanco et al., 2017), Switzerland (Breitenmoser et al., 2011), Italy (Giammanco et al., 2014), Philippines (Rebato et al., 2019), India (Tripathy et al., 2019) and United States (Beer et al., 2015). In these studies, through retrospective

111 investigations and environmental analyses, pathogenic viruses were hypothesised as
112 possible causative agents.
113 Viruses can induce viral gastroenteritis through the faecal-oral route. In developing countries
114 diarrhoeal diseases due to virus presence in DW are one of the main causes of death
115 (Fayomi et al., 2019; WWAP, 2017). Similarly, in high income countries data concerning DW
116 outbreaks show that in 2013-2014 7% of outbreaks in the United States were caused by
117 viruses (CDC, 2021), while in the European Union Member States in 2019 most of DW
118 outbreaks with strong-evidence were related to norovirus (NoV) and other calicivirus (ECDC,
119 2021).
120 Viruses are naturally present in environmental matrices such as in water where their
121 presence can be promoted by the discharge of not properly treated wastewater (Gibson et
122 al., 2011; Masciopinto et al., 2019; Okoh et al., 2010; Upfold et al., 2021). Moreover, several
123 studies have shown that DW treatments do not always succeed in removing viruses (Kato et
124 al., 2018; Salvador et al., 2020; Ye et al., 2012); therefore, detection of viruses at all phases
125 of the integrated water cycle (from wastewater to DW) has a key role for human health.
126 In literature, virus presence within wastewaters have been investigated by many reviews
127 (Bhatt et al., 2020; Corpuz et al., 2020; Foladori et al., 2020; Sano et al., 2016), whereas
128 virus occurrence in DW has been considered by just few reviews which were focused on DW
129 treatment systems and DW related outbreaks (Chen et al., 2021; Moreira and Bondelind,
130 2017). An overview of viral presence in water used as a source for DW production and in DW
131 is still lacking. Consequently, the aim of this review is to report the recent available
132 knowledge about virus occurrence in sources for DW production and in DW. Water types
133 considered were surface water used for DW production (SW-D), GW used for DW production
134 (GW-D), DW and bottled water (BW). Moreover, two virus types were considered: human
135 pathogens and plant pathogens proposed as novel viral indicators. Scientific studies
136 published in the last 10 years from all over the world were analysed and data of virus
137 presence assessed using molecular methods were summarized and discussed. In addition,
138 virus characteristics, concentration methods, nucleic acid extraction and molecular detection

techniques reported in these studies were detailed. To the best of our knowledge, this is the first review that describes the most recent data on the worldwide virus occurrence in water used as sources for DW production and in water used for human consumption (DW).

2. SEARCH CRITERIA

In order to find information about the virus presence in sources for DW production and in DW, a literature search was performed in PubMed, Scopus and Web of Science. These databases were selected to be the most relevant and used for research on environmental topics. The search terms “virus” and “presence” or “detection” were combined with “drinking water” or “bottled water” or “mineral water”. Article search was set in the last 10 years and were chosen only articles published between 2011 and 2021. The search gave 798 results in PubMed, 379 results in Scopus and 367 results in Web of Science (total = 1,544 results). Two authors of the review independently screened the 1,544 publications. Using PRISMA approach, 79 articles were finally included in this review (Fig. 1).

The search was limited to environmental monitoring articles that analysed human pathogens or plant pathogens proposed as novel viral indicators. The articles were included when they were written in English and met the following criteria: i) the analysed water type was water used as a source for DW production or water used as DW, ii) origin of sources for DW production was reported (surface water-SW or GW), iii) the detection of viruses was performed using molecular methods, iv) viruses were not spiked intentionally into the samples, v) data could be extrapolated for each viral agent and for each water type.

3. VIRUS TYPES AND CHARACTERISTICS IN SOURCES FOR DW PRODUCTION AND IN DW

Viruses are obligatory intracellular parasites able to spread and be environmentally transmitted through air, inert surfaces or waters. One of the main vehicles of viral transmission is water through faecal-oral route. Inevitably, all water types can be subject to contamination starting with SW (rivers, lakes), GW (wells, springs) and finally the seas and

oceans. Therefore, it is essential to study virus's resistance within these matrices (Pinon and Vialette, 2019; Shoham et al., 2012).

Without host cells, viruses may decrease in number or remain stable. Their reduction can occur depending on the water type (SW, GW, DW) and on various environmental conditions such as temperature, sunlight (UV) and disinfection products (chlorine and derivatives).

Water type influences the persistence of viral agents. In fact, GW, unlike SW, is a more stable environment with few changes in chemical and physical parameters over time, and thus constitutes a favourable matrix (Espinosa et al., 2008). Temperature is one of the most studied environmental condition and has been recognised as the most influential factor that affect viral persistence (Espinosa et al., 2008; Pinon and Vialette, 2019).

In general, it has been shown that most viral agents can survive for years at low temperatures, whereas at higher temperatures viruses are reduced within a few days. In some studies, resistance to different temperature ranges was tested for certain viral agents in different water matrices. A reduction of 5 log units of polioviruses and echoviruses was found after one month in ocean water at around 21-26°C, while the same reduction was seen after over 2 months when ocean water had temperatures between 4 and 16°C. Moreover, in mineral waters 1 log unit decrease of poliovirus and hepatitis A virus (HAV) was demonstrated in about 11 months at 4°C, in contrast, the same reduction at 23°C took about 1 month (Pinon and Vialette, 2019). Finally, Ogorzaly et al. (2010) study, carried out on DW and GW, showed a decrease of 2-1 log units of astrovirus (AstV) and adenovirus (AdV) with increasing water temperature from 4 to 20°C in half to a third of the time, respectively.

Another important factor that significantly influences viral viability is sunlight (UV). It is well known that UV promotes a significant reduction in viral particles, indeed the study by Flannery et al. (2013) showed a reduction of 1 log unit after 4 hours exposure to light simulating winter conditions (10°C), while the same reduction was induced by 15 minutes exposure to light simulating summer conditions (17°C). UV effectiveness is also confirmed by Garver et al. (2013) study that showed a reduction of 2-3 logs in 3 hours in deep water (less UV) compared to 3-4 logs in 1.5 hours in superficial water (more UV).

Other factors responsible for viral reduction may be the presence of disinfectants, pH extremes, or heavy metals (Pinon and Vialette, 2019).

In parallel to the environmental factors, some viral characteristics can affect viral survival such as aggregation tendency, genome type and capsid composition. Actually, under adverse conditions, viruses tend to aggregate with each other and with organic matter to form aggregate suspension material, making their elimination difficult. Indeed, disinfectants are unable to penetrate the viral capsid so merely bind to the biofilm of organic matter in the aggregates (Espinosa et al., 2008). Moreover, viruses are characterized by a different persistence according to their genome type. For example, due to its double-stranded DNA genome, AdV showed greater stability in water than enterovirus (EV) which has RNA genome (Mena and Gerba, 2008). Due to the higher persistence in the environment, AdV has been suggested as a possible indicator of the viral contamination water (Verani et al., 2019). Finally, a comparison of rotavirus (RoV) and AstV, both of which are RNA viruses, showed that RoVs are more persistent in GW, as they have a triple-layer capsid and a double-stranded RNA genome, whereas AstV have a single-layer capsid and a single-stranded RNA genome (Espinosa et al., 2008).

In the 79 included articles, virus analysis in sources for DW production and in DW was mainly focused on the detection of two virus types namely human pathogens and indicators. The first viruses are able to infect hosts causing diseases, while the second ones are used as indicators of faecal contamination. The articles included in this review mainly investigated the following viruses: AdV, HAV, EV, aichivirus (AiV), hepatitis E virus (HEV), sapovirus (SaV), NoV, torque teno virus (TTV), RoV, AstV, polyomavirus (PyV), pepper mild mottle virus (PMMoV) and tobacco mosaic virus (TMV). The characteristics of these viruses are described in Table 1.

3.1 Human pathogens

The integrated water cycle plays a key role in reduction and elimination of pathogenic viruses, decreasing their spread in environment. Wastewater treatments are often unable to

eliminate viruses and fail to prevent their release in the environment; moreover, pathogenic viruses could be also resistant to DW treatments threatening Public Health. Among the human pathogens, enteric viruses are obligate parasites that infect and replicate within the human gastrointestinal tract (Upfold et al., 2021). Depending on the type of viral agent colonising the gastrointestinal tract, different possible diseases may occur. Enteric viruses are one of the main causes of waterborne diseases transmissible via the faecal-oral route so their detection in water used as DW source or in DW is of crucial importance in order to assess the risk for human health.

3.2 Plant pathogens proposed as novel viral indicators

Currently the monitoring of microbial water quality is generally performed quantifying bacterial indicators of faecal contamination. Since they are easy to identify, more present and resistant than pathogens, bacterial indicators are used to assess the faecal contamination and to estimate the presence of microbial pathogens. However, numerous studies showed that the concentration of indicator bacteria is not related to the concentration of pathogenic viruses, suggesting that these indicators are unsuitable to define the presence of human viral pathogens in water (Liang et al., 2015). Indeed, environmental conditions affect differently bacteria and viruses (Kitajima et al., 2018).

Bacteriophages (e.g. coliphages) have been proposed as alternative indicators instead of bacteria. Coliphages are viruses that infect *Escherichia coli* and other coliforms (Leclerc et al., 2000). Environmental transport and survival of coliphages is similar to enteric viruses. However, coliphages show a greater persistence than human enteric viruses in environment since their replication in bacterial hosts can continue after being shed in faeces. In addition, only a small percentage of human or animal faecal samples test positive for coliphages so these viruses may be too sparse to be detected in some environmental waters (Griffin et al., 2008). Therefore, other viruses were suggested by the scientific community as possible viral indicators of faecal contamination. In particular, two plant pathogens were proposed as

alternative viral faecal indicators: PMMoV and TMV (Kitajima et al., 2018; Tandukar et al., 2020a). These two viruses were analysed by some research articles included in this review. PMMoV is a plant pathogen globally distributed that causes significant economic and crop losses worldwide (e.g. in the United States, Japan and China). Its presence may be indicative of faecal contamination because is the most abundant virus type in human faecal samples (Kitajima et al., 2018). However, its application as a viral indicator has limitations, since studies show conflicting results on the correlation between concentrations of this virus and concentrations of human enteric viruses (Kitajima et al., 2018; Tandukar et al., 2020a). As PMMoV, TMV is a plant pathogen. TMV was discovered in the 19th century when a new infection was affecting tobacco plants causing characteristic patterns, such as mosaic-like mottling and discoloration on the leaves (Tandukar et al., 2020a). Similarly to PMMoV, this virus is excreted by a large proportion of healthy people.

PMMoV and TMV are widely distributed in SW, in GW and even in DW. They are used as indicators of faecal contamination in wastewater, SW and also in DW because their presence is high in human faeces and in sewage. In the analysed articles the presence of these viruses was studied in sources for DW production and in DW (Haramoto et al., 2013; Kuroda et al., 2015; Tandukar et al., 2020a, 2018).

4. VIRUS OCCURRENCE IN SOURCES FOR DW PRODUCTION AND IN DW

4.1 Virus occurrence in SW-D, GW-D, DW

In the 79 included articles, water samples coming from all over the world were analysed (Fig. 2). In particular, 24 articles analysed samples from Asia, 17 from South America, 13 from Africa, 13 from Europe, 11 from North America and 1 from Oceania (79 total articles). The articles assessed virus occurrence in three different water types.

- SW-D was analysed by 43 articles, SW-D was collected from rivers, estuarine bays, dams, lagoons, ponds, lakes and other reservoirs.
- GW-D was analysed by 17 articles. GW-D was collected from wells and springs.

- DW was analysed by 45 articles. These articles analysed different water types (e.g. tap water, DW treatment plant effluents, SW used as DW without any treatment).

The mean of sample volume analysed was significantly different according to the water types (Kruskal-Wallis test followed by pairwise comparisons, SW-D *vs* GW-D, SW-D *vs* DW, GW-D *vs* DW, $p<0.05$). Mean values were 43.58 ± 114.83 L ranging from 0.050 L to 2340 L for SW-D, 321.91 ± 407.99 L ranging from 0.250 L to 1783 L for GW-D and 242.05 ± 467.26 L ranging from 0.050 L to 3400 L for DW. In particular, volumes were higher for GW-D/DW samples than SW-D probably because a lower viral presence was expected.

Table 2 presents the cumulative percentages of positive samples for each viral agent (total positive samples/total samples). For NoV the cumulative percentages were calculated dividing data according to the viral subtype (cumulative percentages were calculated independently for NoV GI, NoV GII, NoV GIII, NoV GIV).

The percentages of positive samples were compared among virus types. For some viral types a small number of samples was analysed, therefore the percentages could not reflect the real occurrence of these viruses in sources for DW production and in DW. For this reason, comparison was performed considering viruses that were analysed in at least 100 samples. The highest percentages of positive samples were found for PMMoV (85.31%), AdV (52.61%), PyV (44.25%), AiV (43.86%), RoV (41.81%) in SW-D, for PMMoV (5.94%), RoV (4.88%), AdV (2.21%), PyV (0.97%), EV (0.78%) in GW-D, for PMMoV (28.33%), AdV (15.96%), PyV (12.94%), AiV (11.27%), EV (7.73%) in DW.

AdV and PyV were among the human pathogenic viruses that showed the highest percentages of positive samples. This result can be explained considering that AdV and PyV are characterized by a DNA genome which is generally more stable in the environment and less affected by the physico-chemical treatments applied to obtain DW with respect to RNA genome (Ye et al., 2012).

For the three water types, the percentages of PMMoV positive samples were the highest compared to the percentages of the other viruses. This finding is interesting since PMMoV has been proposed as a possible viral indicator of human faecal contamination in several

studies (Kitajima et al., 2018; Zhang et al., 2006). Indeed, the high percentages of positive samples found in SW-D, GW-D and DW confirm that this virus is more persistent in water than other enteric viruses, including AdV and PyV (Hamza et al., 2011; Haramoto et al., 2013), suggesting that it could be an excellent candidate as an indicator and it could be used as a possible process control to measure the removal of enteric viruses during water treatments (Symonds et al., 2018). The higher presence of this virus with respect to human pathogenic viruses could also be due to the fact that, while other enteric viruses are more abundant in water when there is an increase of infected individuals, PMMoV presence seems not to be characterized by seasonal variations (Haramoto et al., 2013).

Virus detection in SW-D, GW-D, DW is reported divided by reference in Table S.1, S.2, S.3, respectively.

4.2 Comparison of virus occurrence among the water types

The percentages of positive samples were compared among the water types. The comparison was performed considering viruses that were searched in the highest number of samples and that were analysed by most articles (i.e. the most searched viruses for number of total samples and number of total articles). This choice was adopted to compare data that could reflect the real virus occurrence and could be considered representative of the global situation. As can be seen in Table 2, AdV, EV, NoV GI, NoV GII and RoV were most searched viruses in all water types. These viruses were the most searched probably because are important foodborne pathogens (Koopmans and Duizer, 2004).

In Fig. 3 are reported the percentages of positive samples in SW-D, GW-D and DW of these viruses. As can be seen, all the five viruses were frequently detected in SW-D. This result could be explained considering that these enteric viruses are excreted in large quantities in the faeces of infected individuals (symptomatic and asymptomatic), which are conveyed to sewage treatment plants. Since the water treatments of these plants can be not efficient to remove all viruses, they may be released into SW (Bhatt et al., 2020). Moreover, the high presence of enteric viruses in SW is not only due to municipal wastewaters but may also

result from livestock slurry from livestock farms, which are sometimes not conveyed to the wastewater treatment plants but directly discharged in SW (Haramoto et al., 2018). Among the water types, the percentages of positive samples in GW-D samples were the lowest. This result suggests that GWs are more protected from possible sources of contamination, making them safer when they are used to produce DW. Nevertheless, GW, if not properly protected, are susceptible and can easily be polluted from some contamination sources. After a period of heavy rainfall, GW located in proximity to livestock farms can be contaminated by livestock slurry leaching into the ground or due to damage or deficiency of pipes conveying wastewater effluents to the plants (Gibson and Schwab, 2011a; Gotkowitz et al., 2016). Percentages of positive samples in GW-D were also lower than in DW. This result is not surprising considering that the DWs include not only treated GWs but also treated SWs.

The percentages of positive samples were higher in SW-D than in DW. This may be attributable to the fact that DW are generally treated with physico-chemical processes which can reduce viral presence in this water type (Asami et al., 2016; Atabakhsh et al., 2019; Jacob et al., 2015; Kato et al., 2018; Tandukar et al., 2020b; Ye et al., 2012).

Even if at lower percentages compared to SW-D, the five enteric viruses were detected also in DW. Since high percentages of positive samples in water used for human consumption may be a source of risk to the population, the presence of these viruses in DW might pose a possible threat to human health. Indeed, the ingestion of water contaminated by enteric viruses can lead to sporadic episodes of viral gastroenteritis, which, if not treated with appropriate care, could lead to death in children (Wang et al., 2016). It is important to highlight that in this review were presented only data of virus presence analysed using molecular methods; therefore, the percentages of positive samples do not necessarily mean that these samples contain active and pathogenic viruses but only that in these samples the viral genomic material was detected (Rachmadi et al., 2016). Indeed, many studies compared virus infectivity and virus detection using molecular methods in water samples and

found that samples in which viral genomes were detected did not always contain infectious viral particles (Iaconelli et al., 2017; Salvador et al., 2020).

4.3 Comparison of virus occurrence in DW among the continents

Considering virus detection in DW samples, the percentages of positive samples were compared among the continents. As for the comparison among the water types, the comparison was performed considering the most searched viruses for number of total samples and number of total articles (see paragraph 4.2). In Fig. 4 are reported the percentages of positive sample in DW samples divided according to continents. It should be noted that the number of studies is not the same across continents. Indeed, there are fewer studies in Europe than in the other continents. The global distribution of the samples is probably not homogenous because in some continents such as Europe the risk associated with water consumption is not considered a major health concern, so the research articles focused on this topic are limited. On the contrary, in developing countries diseases associated with water consumption are a major issue, thus this research topic is more investigated. Comparing the percentages among the continents, except for RoV, the lowest percentages of positive samples were found in Europe. In contrast, the highest percentages of positive samples were found in Asia and South America.

The different virus occurrence in Europe with respect to Asia and South America could be due to several factors. Indeed, in developing countries the quality of sources for DW production could be lower due to a higher discharge of not properly treated wastewaters; moreover, technologies used for DW treatment could be less efficient in virus removal. Finally, water distribution networks could be less monitored and more prone to breakdowns that may cause the intrusion of contaminated water in DW distribution systems. Regarding RoV, the unexpected percentage of positive samples in Europe could be explained considering that only one study carried out in Slovenia assessed RoV occurrence in European DW (Steyer et al., 2011), so this percentage could be not representative of the whole European occurrence of this virus.

The results obtained analysing African samples are interesting. Indeed, although the number of articles is higher than in Europe and the articles analysed samples coming from different African countries (giving a complete picture of virus occurrence throughout the continent), the percentages of positive samples were less than 11%. These percentages seem to be too low if compared with the incidence of viral gastrointestinal diseases transmitted by water consumption in this continent, so further studies are needed to clarify this discrepancy. Finally, considering the occurrence of viruses in North America, the percentages of positive samples were quite low suggesting that the DW quality is quite good in this continent.

4.4 Virus occurrence in BW

Three articles assessed the virus presence in BW (Da Silva Luz et al., 2020; Dos Santos et al., 2015; Kuroda et al., 2015). The analysed BW was produced using GW as source (water from wells/springs) and samples were collected in Brazil and Vietnam from bottles containing different water volumes (0.5, 1.5, 19, 20 L). The analysed water volume ranged from 0.5 L to 100 L, while the percentages of positive samples ranged from 81.69% (AdV) to 0% (PMMoV, NoV GII, AiV). It's important to highlight that these percentages of positive samples were calculated considering only three articles which analysed few samples collected in Brazil (2 articles) and in Vietnam (1 article). The limited number of articles on virus occurrence in BW is probably due to the low frequency of outbreaks linked to the consumption of BW. However, since also in this water type genomes of some viruses were found (e.g. AdV, EV, NoV, RoV), more studies to assess the real virus occurrence in BW are needed. Virus detection in BW is reported in Table S.4.

5. METHODS FOR VIRUS CONCENTRATION AND DETECTION IN SOURCES FOR DW PRODUCTION AND IN DW

5.1. Virus concentration methods

In the analysed studies, different methods/methodologies of concentration, extraction and identification of viral particles were reported. Many of these concentration methods were

established in the 1980s and have not been changed. They include the use of negatively and positively charged membranes, glass wool filters and ultrafiltration. These methods can be applied alone or can be followed by a secondary concentration that allows for a higher concentration of the treated water sample (Ikner et al., 2012).

Concentration methods used in the analysed articles are described below.

- Adsorption-elution method, also known as filtration method, is based on the absorption of organisms on a solid membrane utilising the ionic properties of the micro-organisms (bacteria/viruses) to be concentrated. The filters mainly used in this technique can be membrane filters (cellulose) or glass filters; moreover, filters could be with neutral charge or could have electropositive/electronegative charge using electrostatic forces to concentrate viruses (Cashdollar and Wymer, 2013; Ikner et al., 2012). The adsorption phase (with filters) is followed by an elution phase using a specific fluid which is variable according to the analysed virus type (Cai et al., 2015; Ruhanya, 2016). For instance, the Environmental Protection Agency (EPA) has proposed a procedure to detect human enteric viruses in water whose first step is based on adsorption-elution method (i.e. filtration through electropositive filters, followed by elution using a solution of glycine and beef extract) (Fout et al., 2015).
- Tangential flow filtration system consists in flowing the liquid parallel to the filtering medium to reduce the probability of clogging of the latter and thus enhance its filtering capacity. This method is still used today to concentrate micro-organisms present in a matrix (e.g. water). It is essential to adopt an appropriate membrane according to the type of microbial agent researched (Cai et al., 2015). In the analysed studies, 30 kDa and 100 kDa filter membranes were used.
- Ultrafiltration is commonly used as water treatment technology for the removal of human pathogens and can be considered as a special form of filtration that uses positive pressure to promote the flow of water through a membrane (Reeve et al., 2016). This method allows to retain not only particles and macromolecules but also

micro-organisms such as viruses and bacteria. The membranes used in ultrafiltration process have pores with diameters ranging from 1 to 10^{-3} µm (Shao et al., 2011).

- Polyethylene glycol is a biocompatible polymer used for protein precipitation. Its properties promote virus precipitation sequestering water molecules from the outer layer of their pericapsids/capsids to promote virus-virus interactions and thus virus concentration (Corpuz et al., 2020).
- Skimmed milk flocculation is based on three physical processes, i.e. adsorption, sedimentation and dissolution. The first two steps consist in the adsorption of viruses on pre-flocculated skimmed milk proteins and precipitation of flakes with adsorbed viruses. After sedimentation, sediment is dissolved using a buffer solution. This methodology does not require the use of special equipment and long processing steps, making its use advantageous (Corpuz et al., 2020).

In the 79 articles analysed, 7 different primary concentration methods were used, which were or were not followed by other 4 types of secondary concentration methods for a total of 17 different combinations of primary-secondary concentration methods (Table 3). The most frequently used combinations of primary-secondary methods were filtration with negatively charged membranes (applied in 17 articles), filtration with negatively charged membranes followed by a secondary concentration using ultrafiltration (applied in 11 articles), filtration with positively charged membranes (applied in 10 articles), filtration with positively charged membranes followed by a secondary concentration with polyethylene glycol (applied in 7 articles). The other methods were reported in less than 6 articles. The filtration with negatively charged membranes was the most applied method probably because it has numerous advantages. Indeed, this method is cheap and it allows high recoveries for viruses. Moreover, since electronegative filters are less influenced by clogging, this method is suitable also for turbid waters (Cashdollar and Wymer, 2013).

5.2 Virus detection methods

Virus detection can be performed using both cell culture and molecular methods. Cell culture methods are based on virus ability to grow in cell cultures causing morphological changes. Viruses are inoculated on cell monolayers and the number of infectious viruses is quantified counting the plaque forming units (cell destruction areas caused by viruses) (Gerba et al., 2017). Cell culture methods are able to quantify viruses that potentially can replicate in humans causing the disease. Before the development of molecular methods, virus detection in environmental matrices was exclusively performed using these methods (Gerba and Betancourt, 2019). However, the main disadvantage of cell culture methods is that they can not be applied to analyse all virus types because some of them, such as NoV, can not replicate in cell culture (Fout et al., 2015); therefore, in order to assess the presence of these viruses in environmental matrices, only molecular methods can be applied. Moreover, these detection methods seem to underestimate virus concentration at least by 2-3 orders of magnitude (Chen et al., 2021). The underestimation could be due to viral aggregation; indeed, many aggregated viruses can form a single plaque forming unit so they can be counted as one infectious viral particle. Moreover, one group of viruses may grow faster than another or interfere with the replication of another group of viruses, causing an underestimation of the viral particle number (Gerba and Betancourt, 2019).

Therefore, nowadays, virus detection in waters is generally performed using molecular methods, which are based on the detection of viral genomes. Due to the low environmental stability of genomes, especially for RNA viruses a positive molecular result indicates that viral particles are intact; however, this result can be obtained also for viruses that have been inactivated by chemical disinfection, heat or proteases (Kopecka et al., 1993). As a consequence, one of the most important disadvantages of molecular methods is that they can not distinguish between infectious and inactivated viruses. On the contrary, these methods have numerous advantages. They are able to detect low virus concentrations so they are more sensitive than cell culture methods. In addition, they are characterized by high specificity and rapidity. Finally, in contrast to cell culture methods, they potentially allow the

detection of all virus types, detecting also viruses that are hardly propagated using cell cultures such as RoV and NoV (Carducci et al., 2003; Corpuz et al., 2020). Detection of viral genomes is performed through extraction of nucleic acids followed by amplification of specific nucleic acid fragments using polymerase chain reaction (PCR).

In the 79 analysed articles, different methods for nucleic acid extraction were reported. In some articles more than one extraction type was used. The different methods are described below.

- Nucleic acids can be purified through the binding with silica membrane. The principle of this method is the following. DNA binds specifically to the silica-gel membrane, while contaminants pass through. Then unwanted materials are generally removed with washing steps and finally the remaining nucleic acids are eluted in either water or a buffer. This extraction type can be performed using both spin columns or vacuum columns. This method was the most applied for nucleic acid extraction. Indeed 71 articles applied it using spin columns, while one using vacuum columns.
- Magnetic beads separation is a method based on specific interaction between nucleic acids and magnetizable particles. Briefly, after a lysis step to release the nucleic acids, viral genomes bind to magnetizable particles in the presence of a binding buffer. The other molecules are washed with a water-based wash buffer and finally the nucleic acids are eluted in an elution buffer (Nargessi and Ou, 2010). This extraction type was applied by 13 articles.
- Nucleic acids can be purified through the binding with glass fibre or glass powder. For example, nucleic acids can be immobilized through the binding to the surface of the glass fibre fleece in the presence of a chaotropic salt. Sample is mixed with a chaotropic salt and applied to the glass fibre fleece. Nucleic acids bind to the glass fleece, while contaminating substances are removed through washing steps. Nucleic acids are finally eluted in a small volume of low-salt buffer or water. Among the analysed articles, 4 applied this extraction method.

- Organic extraction is a method that uses organic solvents. According to this method, samples are mixed with a reagent composed by a monophasic solution of guanidine thiocyanate and phenol. Then chloroform is added and the homogenate is allowed to separate into different phases containing RNA, DNA and proteins. The phases are separated and finally the nucleic acids are isolated through precipitation with organic solvents (e.g. isopropanol, ethanol). 4 included articles reported this extraction method.

After extraction of nucleic acids, the molecular detection of viruses is performed through the amplification of specific nucleic acid fragments using PCR. For RNA viruses, viral genome is reverse transcribed through a reverse transcriptase-PCR before PCR to obtain the cDNA. Molecular detection methods can provide both qualitative and quantitative data depending on the PCR type. Qualitative data can be obtained using conventional PCR or nested/semi-nested PCR (performed by two successive conventional PCR), whose products are subjected to agarose gel electrophoresis (Corpuz et al., 2020). On the contrary, quantitative data can be obtained using quantitative PCR (qPCR). Virus quantification can be affected by some factors that can cause data variability. For example the quantification can be influenced by recovery efficiency of the applied extraction method, by PCR inhibitory substances within the samples or by PCR conditions (number of replicates, primer/probe design, thermal cycling conditions) (Gerba et al., 2018). In addition to providing quantitative data, another qPCR advantage is that it has a high sensitivity, therefore it can detect even small amounts of nucleic acids (Corpuz et al., 2020).

Regarding molecular detection in the 79 analysed articles, conventional PCR, qPCR, nested PCR or semi-nested PCR were applied for the identification of viral particles. Overall, the most applied detection method was qPCR (60/79 articles, 75.9%), followed by nested/semi-nested PCR and conventional PCR (17/79 articles, 21.5% and 10/79 articles, 12.7%, respectively). The use of qPCR was frequent, probably because it has a higher sensitivity than the other molecular methods. The higher sensitivity was confirmed by Assis et al. (2015) and Dos Santos et al. (2015) studies. These studies applied conventional PCR and qPCR to

554 detect the same virus type; the results showed that a higher number of positive samples was
555 found using qPCR than using conventional PCR. The qPCR was frequently applied also
556 because it can provide quantitative data. However, it is important to highlight that even if
557 using qPCR, the number of genomic copies/L can be quantified, many of the included
558 articles did not report virus concentrations. In some articles virus concentrations were not
559 reported because data were under the quantification limit.
560 Methods applied for virus concentration, nucleic acid extraction and molecular analyses are
561 reported in Table S.5 divided by reference, while data on detection limit/quantification limit
562 are shown in Tables S1-S4. One of the main problems related to the monitoring of viruses in
563 water is linked to the fact that different methods can be applied to detect them. Furthermore,
564 even if quality assurance/quality control is important to assure data quality, in environmental
565 monitoring studies, this information is often not reported.
566

567 ## 6. CONCLUSIONS

568 The microbiological water quality for human consumption is crucial for Public Health. As long
569 as viruses are one of the most important causative agents of waterborne diseases, their
570 detection in sources for DW production and in DW has a key role in healthcare. In this
571 review, scientific studies of the last 10 years from all over the world were analysed in order to
572 summarize data of virus presence assessed using molecular methods in sources for DW
573 production and in DW. Water types considered were SW-D, GW-D, DW and BW.
574 In the 79 articles finally included in the review different virus types were searched. However,
575 only some of them are important for Public Health because they may cause waterborne
576 outbreaks. Therefore, in order to collect more data in short times, the authors of this review
577 believe that it could be more appropriate to focus research on these viruses (i.e. AdV, EV,
578 NoV GI, NoV GII, RoV).
579 As highlighted by this review, data on virus presence in water for human consumption are
580 very heterogeneous. This finding could be related to the methods used for virus detection;
581 indeed, different combinations of primary-secondary concentration methods and different

582 nucleic acid extraction methods were carried out. This evidence raises an important question
583 about a lack of standardization of methodologies for virus detection. It is not easy to compare
584 data collected using different methodologies and it would be desirable to standardise
585 methodologies in order to make data more comparable.
586 The comparison of virus detection among the water types showed that in SW-D viruses were
587 frequently detected, while the percentages of positive samples in GW-D were the lowest. It is
588 crucial to investigate viral presence in sources for DW production (SW-D and GW-D),
589 because a higher presence in SW and GW could lead to a higher presence in DW. In
590 particular, the assessment of virus occurrence in SW is important because the use of this
591 water as DW source will probably increase in the next years. Indeed, climate change and
592 global population growth will lead to more DW demand and less water availability.
593 Consequently, to produce DW it will be necessary to increase the use of sources most
594 vulnerable to contamination, such as SW.
595 Even if at lower percentages compared to SW-D, viruses were detected also in DW, where
596 they might pose a possible threat to human health. Although these percentages do not
597 necessarily mean that these samples contain viable pathogenic viruses (because they were
598 found through molecular detection methods), this evidence suggests the need for regulatory
599 updates. Indeed, the monitoring of enteric viruses together with coliphages and phages, is
600 considered important for the assessment of DW treatments effectiveness. However, the only
601 parameter proposed by WHO guidelines for verification of microbial quality of DW is the
602 monitoring of *Escherichia coli* or thermotolerant coliform bacteria, whereas for viruses no
603 guidelines values have been proposed yet (WHO, 2017). Even the new European legislation
604 (The European Parliament and the Council of the European Union, 2020) requires only the
605 search for *E. coli* and fecal enterococci to establish the DW requirement for water intended
606 for human consumption. *Clostridium perfringens* and *Legionella* spp. must only be analysed
607 on the basis of the risk assessment. Finally, the legislation provides for the search of somatic
608 coliphages in untreated waters if specifically indicated in the risk assessment.

As had already been proposed by the WHO (2004), an excellent way to take into account the risk associated with the presence of viruses in DW could be the application of the Water Safety Plans (WSP) which have also been introduced by the new European legislation (The European Parliament and the Council of the European Union, 2020) and will be mandatory for all Member States since 2029. It is an approach based on the risk assessment and management throughout all the water supply chain, from catchment to consumer. The main limitation derives from the fact that very often water companies do not have data on the presence of enteric viruses from source to tap (Masciopinto et al., 2019; van den Berg et al., 2019) and therefore the risk assessment and management are based on the presence of the microbiological indicators required by the regulations, even if the correlation between *E. coli*, faecal enterococci and bacteriophages and the presence of viruses is often absent or very low (Edge et al., 2013; Goh et al., 2019; Lee et al., 2014; Payment and Locas, 2011). This further strengthens the need to include the search for enteric viruses or alternative indicators in the monitoring programs, in order to obtain objective data for the application of WSPs. Considering the comparison of virus detection in DW among the continents, this review showed that the number of studies is not homogeneously distributed across the continents. Indeed, few studies have assessed DW collected in Europe while, to our knowledge, there is no study on Oceanian DW. This finding highlighted the need to analyse additional samples from these geographical regions. Moreover, the results obtained analysing African samples were unexpected, so the authors believe that they are worth of further studies. Finally, the bibliographic research performed in this review demonstrated that only three articles assessed virus presence in BW, underlining another research gap.

In order to estimate the potential human health risk due to virus exposure through DW, reliable data on virus occurrence in this matrix are needed. However, virus concentration data in DW are still limited, so future studies are needed to fulfil this research gap. Besides studies focused on viral occurrence in DW, future research should also investigate virus distribution in other environmental matrices, such as SW and GW. Indeed, these data

636 together with DW data could be used as inputs to perform Quantitative Microbial Risk
637 Assessment (QMRA), allowing a more precise estimate of human health risk.
638
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649
650 ## 10. REFERENCES
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Table captions

Table 1. Virus characteristics. AdV= adenovirus, HAV=hepatitis A virus, EV=enterovirus, AiV=aichivirus, HEV=hepatitis E virus, SaV=sapovirus, NoV=norovirus, TTV=torque teno virus, RoV=rotavirus, AstV=astrovirus, PyV=polyomavirus, PMMoV=pepper mild mottle virus, TMV=tobacco mosaic virus.

Table 2. Total number of articles that investigated each viral agent and cumulative percentages of positive samples divided by type of water and type of virus. - = not assessed; AdV = adenovirus; AiV = aichivirus; ASFLV = asfarvirus-like virus; AstV = astrovirus; CosV = cosavirus; EV = enterovirus; HAV = hepatitis A virus; HCV = hepatitis C virus; HEV = hepatitis E virus; KV = klassevirus; NoV = norovirus; PMMoV = pepper mild mottle virus; PyV = polyomavirus; ReV = reovirus; RoV = rotavirus; SaliV = salivirus; SaV = sapovirus; TMV = tobacco mosaic virus; TTV = torque teno virus.

Table 3. Primary and secondary concentration methods reported in the analysed articles (total articles= 79, two concentration methods were applied by the study of Kuroda et al., 2015). TFF = tangential flow filtration; UF = ultrafiltration; F = filtration; F- = filtration with electronegative charged membrane; F+ = filtration with electropositive charged membrane; PEG = precipitation with polyethylene glycol; SMF = skimmed milk flocculation.

Figure captions

Figure 1. Flow chart of study selection process.

Figure 2. Number of articles that analysed SW-D, GW-D, DW or BW performed in each continent (Africa, Asia, Europe, Oceania, North America, South America). Total articles = 79 (58 analysed one water type, 14 analysed two water types, 6 analysed three water types, 1 analysed four water types). Satellite image from European Space Agency.

Figure 3. Percentages of positive samples in SW-D, GW-D and DW of the most searched viruses (AdV, EV, NoV GI, NoV GII, RoV).

Figure 4. Percentages of positive samples in DW of the most searched viruses (AdV, EV, NoV GI, NoV GII, RoV) divided by continent.

Figure 1

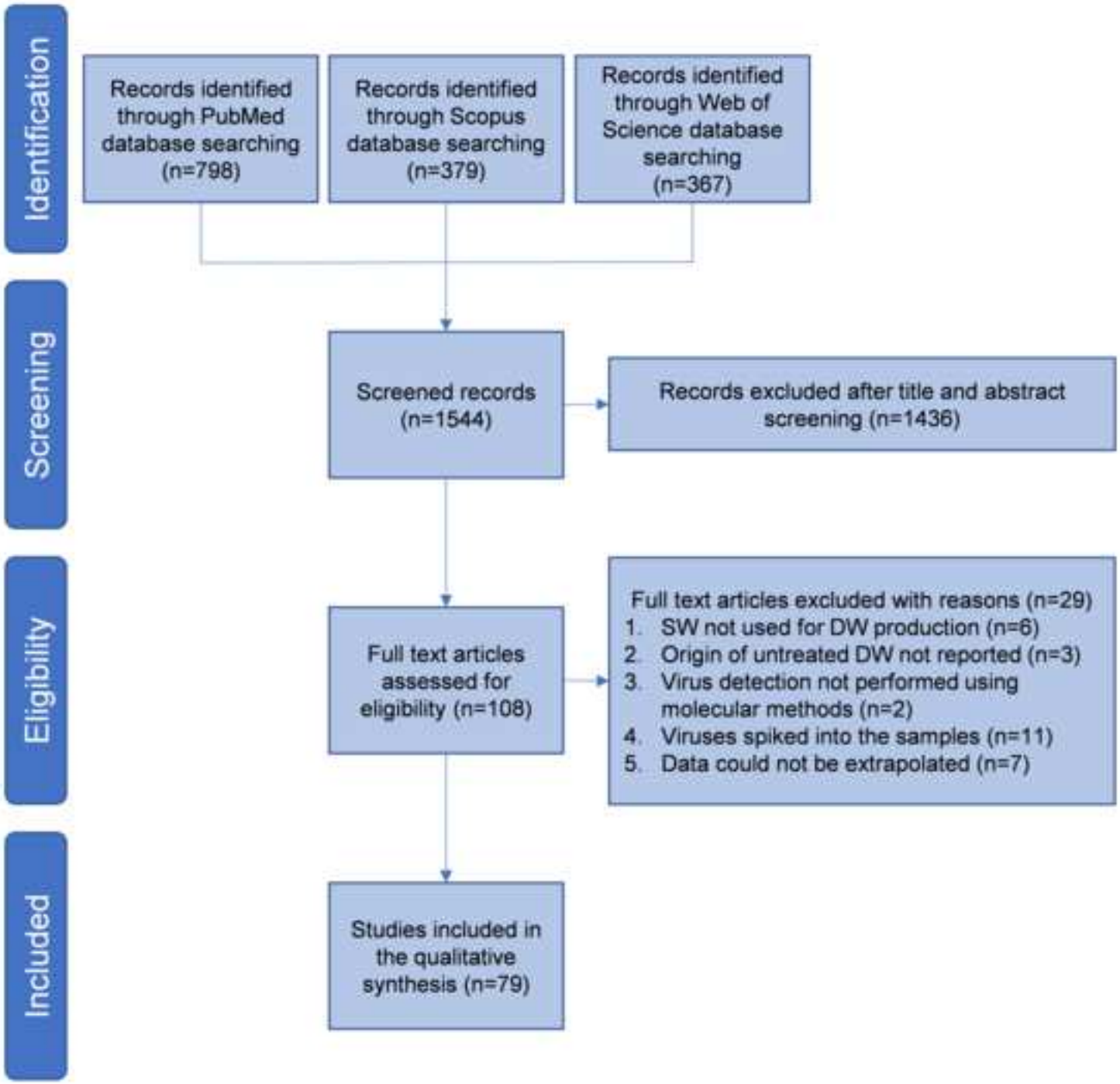

Figure 2

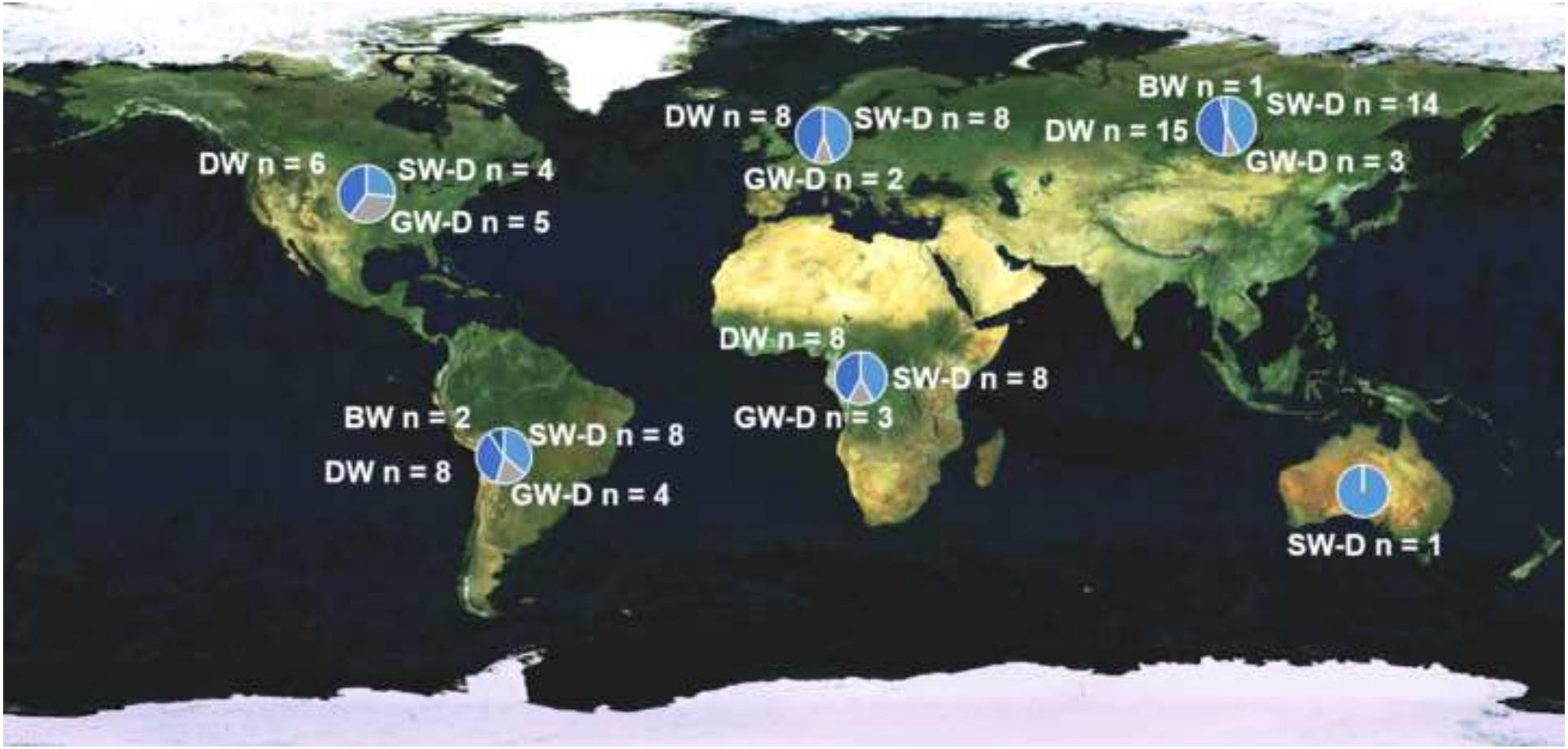

Figure 3

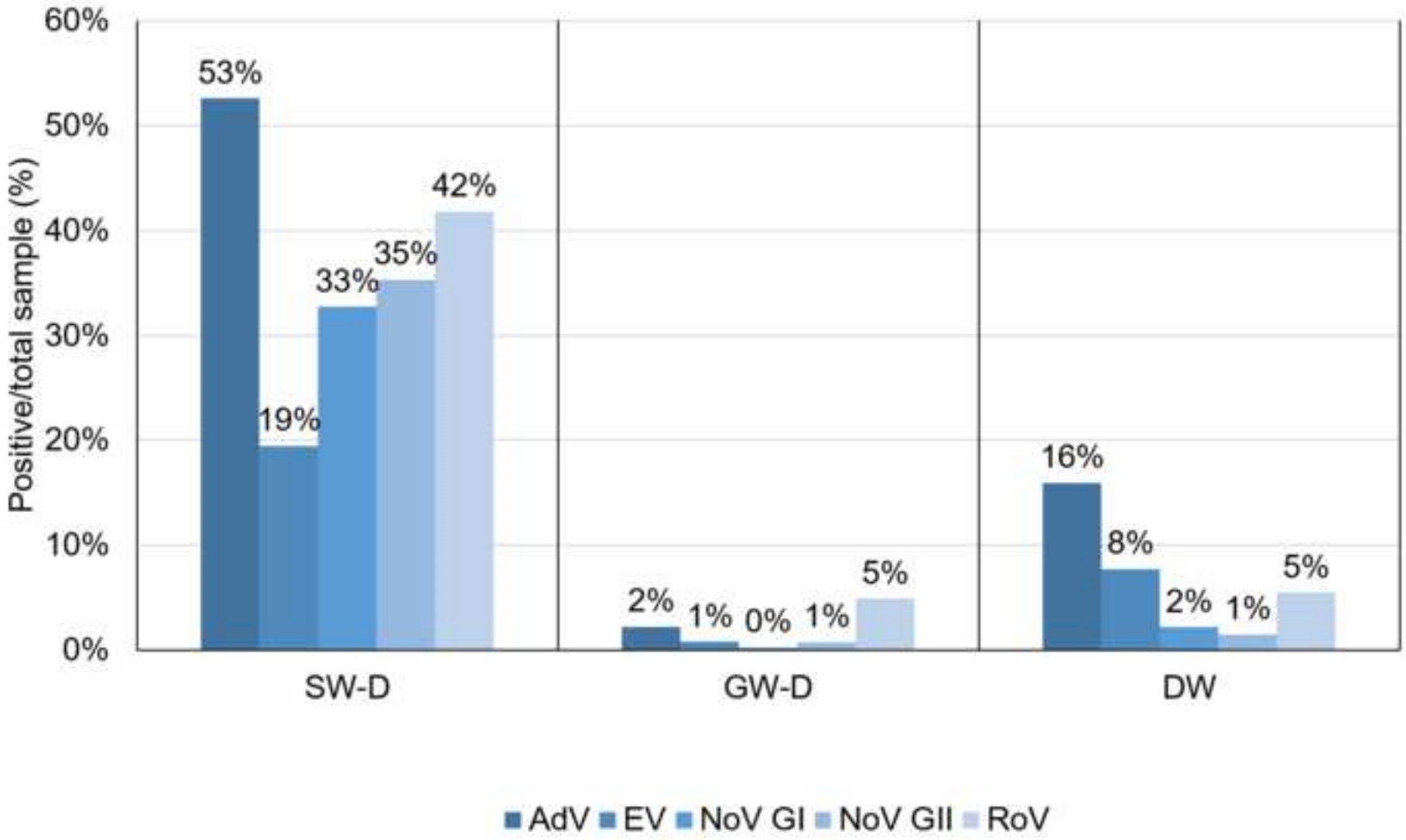

Figure 4

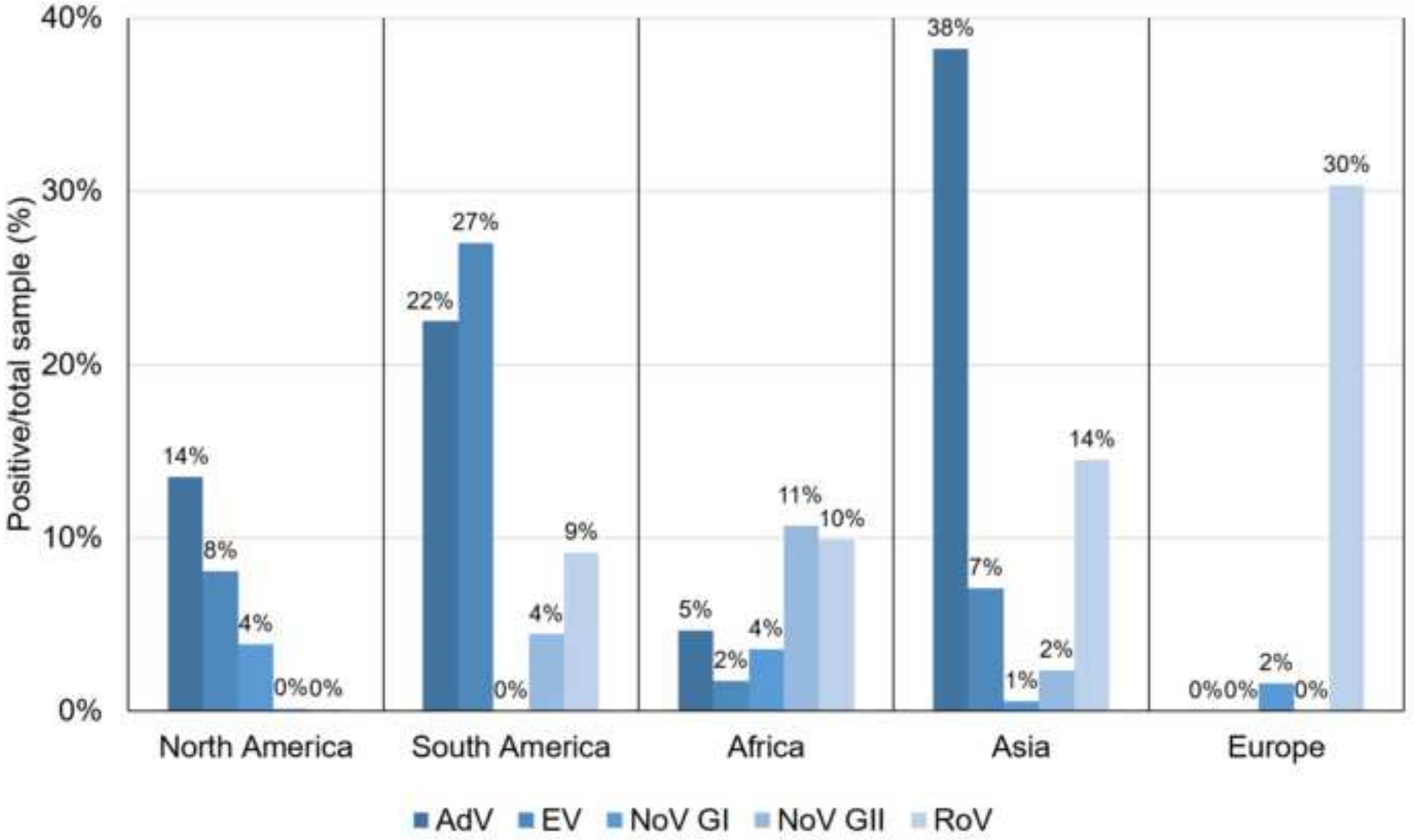

Table 1. Virus chararacteristics.

Human pathogens						
Virus type	**Family**	**Genome**	**Persistence in water supply[a]**	**Disease**	**Vaccine**	**Reference**
AdV	Adenoviridae	double-stranded DNA	Long	pharyngitis, cystitis, gastroenteritis	yes	Broderick et al., 2017; Okoh et al., 2010; Upfold et al., 2021; Vellinga et al., 2005
AiV	Picornaviridae	single-stranded and positive-sense RNA	NA	gastroenteritis	no	Upfold et al., 2021
AstV	Astroviridae	single-stranded and positive-sense RNA	Long	gastroenteritis, respiratory diseases, encephalitis, meningitis, acute flaccid paralysis	no	Bosch et al., 2014; Upfold et al., 2021; Vu et al., 2017
EV	Picornaviridae	single-stranded and positive-sense RNA	Long	gastroenteritis, myocarditis, pericarditis, encephalitis	yes	Baggen et al., 2018; Li et al., 2021; Lugo and Krogstad, 2016
HAV	Picornaviridae	single-stranded and positive-sense RNA	Long	hepatitis	yes	Okoh et al. 2010; Smith and Simmonds, 2018; Upfold et al., 2021
HEV	Hepeviridae	single-stranded and positive-sense RNA	Long	hepatitis	yes	Larrue et al., 2021; Upfold et al., 2021
NoV	Caliciviridae	single-stranded and positive-sense RNA	Long	gastroenteritis	no	Carter et al., 2005; Okoh et al., 2010; Upfold et al., 2021
PyV	Polyomaviridae	double-stranded DNA	NA	progressive multifocal leukoencephalopathy, nephropathy, pulmonary infections, possible oncogenic viruses	no	Calgua et al., 2013
RoV	Reoviridae	double-stranded RNA	Long	gastroenteritis	yes	Crawford et al., 2017; Okoh et al., 2010; Steele et al., 2003; Upfold et al., 2021
SaV	Caliciviridae	single-stranded and positive-sense RNA	Long	diarrhoea, nausea, myalgia	no	Upfold et al., 2021
TTV	Anelloviridae	single-stranded and negative-sense DNA	NA	partially unknown, co-factor in several diseases	no	Charest et al., 2015; Jiménez-Melsiò et al., 2013; Shoeib et al., 2011

Indicators				
Virus type	**Family**	**Genome**	**Host**	**Reference**
PMMoV	Virgoviridae	single-stranded and positive-sense RNA genome	plant	Haramoto et al., 2018; Kitajima et al., 2018
TMV	Virgaviridae	single-stranded and positive-sense RNA	plant	Tandukar et al., 2020a

AdV= adenovirus, HAV=hepatitis A virus, EV=enterovirus, AiV=aichivirus, HEV=hepatitis E virus, SaV=sapovirus, NA = not available, NoV=norovirus, TTV=torque teno virus, RoV=rotavirus, AstV=astrovirus, PyV=polyomavirus, PMMoV=pepper mild mottle virus, TMV=tobacco mosaic virus. [a] = detection period for infective stage in water at 20° C: short, up to 1 week; moderate, 1 week to 1 month,; long, over 1 month (WHO, 2017).

Table 2. Total number of articles that investigated each viral agent and cumulative percentages of positive samples divided by type of water and type of virus.

	SW-D			GW-D			DW		
virus type	**positive/total**	**%**	**n° articles**	**positive/total**	**%**	**n° articles**	**positive/total**	**%**	**n° articles**
AdV	755/1435	52.61%	29	102/4607	2.21%	13	535/3352	15.96%	29
AiV	50/114	43.86%	6	1/9	11.11%	2	16/142	11.27%	6
ASFLV	1/12	8.33%	1	-	-	-	-	-	-
AstV	48/132	36.36%	3	-	-	-	7/145	4.83%	3
CosV	-	-	-	-	-	-	9/18	50.00%	1
EV	106/544	19.49%	18	13/1667	0.78%	11	266/3439	7.73%	24
HAV	63/268	23.51%	7	0/994	0.00%	3	17/2335	0.73%	10
HCV	3/30	10.00%	1	-	-	-	-	-	-
HEV	26/176	14.77%	5	0/15	0.00%	2	24/80	30.00%	5
KV	2/12	16.67%	1	-	-	-	-	-	-
NoV GI	311/950	32.74%	21	4/1634	0.24%	10	97/4454	2.18%	20
NoV GII	463/1310	35.34%	26	13/1768	0.74%	11	71/4794	1.48%	22
NoV GIII	56/173	32.37%	2	-	-	-	-	-	-
NoV GIV	0/64	0.00%	1	-	-	-	-	-	-
PMMoV	273/320	85.31%	8	64/1078	5.94%	4	34/120	28.33%	7
BK/JC/MC/KI/WU PyV	273/617	44.25%	11	15/1541	0.97%	6	22/170	12.94%	6
ReV	0/16	0.00%	1	-	-	-	-	-	-
RoV	347/830	41.81%	14	57/1168	4.88%	4	201/3671	5.48%	18
SaliV	-	-	-	-	-	-	13/18	72.22%	1
SaV	5/144	3.47%	3	-	-	-	1/20	5.00%	1
TMV	8/12	66.67%	1	-	-	-	17/30	56.67%	2
TTV	16/79	20.25%	2	-	-	-	19/69	27.54%	3

- = not assessed; AdV = adenovirus; AiV = aichivirus; ASFLV = asfarvirus-like virus; AstV = astrovirus; CosV = cosavirus; DW = drinking water (water used for human consumption, not bottled); EV = enterovirus; GW-D = groundwater used as a source for DW production; HAV = hepatitis A virus; HCV = hepatitis C virus; HEV = hepatitis E virus; KV = klassevirus; NoV = norovirus; PMMoV = pepper mild mottle virus; PyV = polyomavirus; ReV = reovirus; RoV = rotavirus; SaliV = salivirus; SaV = sapovirus; SW-D = surface water used as a source for DW production; TMV = tobacco mosaic virus; TTV = torque teno virus.

Table 3. Primary and secondary concentration methods reported in the analysed articles (total articles= 79, two concentration methods were applied by the study of Kuroda et al., 2015).

Primary concentration	Secondary concentration	Number of articles	Reference
F-	None	17	Ahmad et al., 2018; Bortagaray et al., 2020; Canh et al., 2021; de Souza et al., 2018; Dos Santos et al., 2015; Gad et al., 2019; Kato et al., 2018; Kishida et al., 2012; Kluge et al., 2014; Miagostovich et al., 2020; Miura et al., 2019; Rashid et al., 2021; Rizk and Allayeh, 2018; Spilki et al., 2013; Staggemeier et al., 2015; Tandukar et al., 2020b; Vecchia et al., 2013
	UF	11	Asami et al., 2016; Assis et al., 2015; Da Silva Luz et al., 2020; Diston et al., 2015; Fongaro et al., 2015, 2013; Garcia et al., 2012; Haramoto et al., 2013, 2012; Kuroda et al., 2015; Tandukar et al., 2018
	PEG	1	Mackowiak et al., 2018
F+	None	10	Bonanno Ferraro et al., 2021; Ferrer et al., 2015; Iaconelli et al., 2017; Joung et al., 2013; Jung et al., 2011; Salvador et al., 2020; Silva et al., 2015; Steyer et al., 2011; Sylvestre et al., 2021; Varughese et al., 2018
	PEG	7	Grøndahl-Rosado et al., 2014; Kiulia et al., 2014; Opere et al., 2021; Pérez-Sautu et al., 2012; Potgieter et al., 2020; Shi et al., 2021; Ye et al., 2012
	F	1	Lee et al., 2018
	UF	1	Teixeira et al., 2020
F	UF	6	Chigor and Okoh, 2012a, 2012b; Dienus et al., 2016; Kuroda et al., 2015; Malla et al., 2019; Sangsanont et al., 2016
	None	5	Ferguson et al., 2012; Guerrero-Latorre et al., 2011; Hssaine et al., 2011; Kittigul and Pombubpa, 2021; Mattioli et al., 2013
	PEG	3	Borchardt et al., 2012; Gotkowitz et al., 2016; Lambertini et al., 2011
TFF	UF	1	Aw and Gin, 2011
	PEG	1	Marie and Lin, 2017
UF	PEG	5	Charest et al., 2015; Cuevas-Ferrando et al., 2020; Murphy et al., 2020; Stokdyk et al., 2020; Williamson et al., 2011
	UF	4	Gibson et al., 2011; Gibson and Schwab, 2011a, 2011b; Hata et al., 2021
	None	3	Jacob et al., 2015; Knappett et al., 2011; Shoeib et al., 2011
PEG	F-	1	Ahmad et al., 2015
SMF	None	3	Calgua et al., 2013; Gamazo et al., 2018; Vieira et al., 2016

TFF = tangential flow filtration; UF = ultrafiltration; F = filtration; F- = filtration with electronegative charged membrane; F+ = filtration with electropositive charged membrane; PEG = precipitation with polyethylene glycol; SMF = skimmed milk flocculation.

Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Editorial Department
P. O. Box 2871
Beijing 100085
China
Tel: 86-10-62920553
E-mail: jesc@rcees.ac.cn

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Author(s): Marco Panizzolo, Marta Gea, Elisabetta Carraro, Giorgio Gilli, Silvia Bonetta, Cristina Pignata

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First Author: Marco Panizzolo

Corresponding author: Marta Gea

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