Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 25(4): 1049 – 1084 (2021)
www.ejabf.journals.ekb.eg

Suitability of some viruses as indices of viral pollution of water

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ARTICLE INFO
Article History:
Received: June 18, 2021
Accepted: July 29, 2021
Online: Aug. 27, 2021

Keywords:
viral index,
viral indicator,
enteric viruses,
drinking water,
irrigation water

ABSTRACT

Water quality is of great importance for public health. Any defect in water treatment may lead to the presence of pathogens such as pathogenic bacteria and viruses in different types of water such as drinking or irrigation water. Traditional regulations (usually used to determine water quality from a microbiological point of view) became non-sufficient because Coliform bacteria, *Escherichia coli (E. coli)*, and Coliphages are not sufficient as indicators of water quality. They do not express accurately the presence/absence of enteric viruses. Any mistake in the estimation of the presence/absence of enteric viruses in water samples may lead to a big threat to human health. However, enteric viruses cause many diseases such as hepatitis, gastroenteritis, conjunctivitis, myocarditis, fever, rash, and may be diabetes. So, it is necessary to find viral indices to express correctly the presence/absence of enteric viruses in drinking water samples. Several scientific types of research were done to investigate viral indices expressing the viral pollution of the water. The objective of this review is to estimate the most suitable viral index/indices to express the viral pollution of water.

INTRODUCTION

Water quality through the presence of pathogenic enteric microorganisms may affect human health. Coliform bacteria, *E. coli*, and coliphages are normally used as indicators of water quality. However, the presence of the above-mentioned indicators does not always suggest the presence of human enteric viruses. It is important to study human enteric viruses in water. Human enteric viruses can tolerate fluctuating environmental conditions and survive in the environment for long periods becoming causal agents of diarrhoeal diseases. Therefore, the potential of human pathogenic viruses as significant indicators of water quality is emerging. Human adenoviruses and other viruses have been proposed as suitable indices for the effective identification of such organisms of human origin contaminating water systems **(Lin and Ganesh, 2013).**

Traditionally, indicator micro-organisms have been used to suggest the presence of pathogens **(Berg, 1978).** To eliminate the ambiguity in the term 'microbial indicator', the following three groups (Table 1) are now recognized. The presence or absence of

indicator organisms is fundamental to most drinking water quality guidelines, water supply operating licenses, and agreements between bulk water suppliers and retail water companies **(Colford *et al.,* 2006).** To be an effective indicator of fecal contamination, an organism must be consistently present in feces and high numbers. Although the bacterial indicators including total coliforms, fecal coliforms, *E. coli*, fecal streptococci, and enterococci have been used to access quality in water quality management and health risk assessments because they are much easier and less costly to detect and enumerate than the pathogens themselves **(Meays *et al.,* 2004),** there is no direct correlation between numbers of any indicator and enteric pathogens **(Grabow, 1996).**

Waterborne pathogens transmit diseases to around 250 million people each year resulting in 10 to 20 million deaths around the globe **(Wilkes et *al.,* 2009).** The assessment of the microbiological quality of drinking water aspires to protect consumers from illnesses due to the consumption of water that may contain pathogens such as bacteria, viruses, and protozoa, thereby thwarting water-related illness outbreaks. An indicator of microbial water quality is generally one specific species or group of microorganisms, which must have entered the water system at the same time as feces, but this indicator is easier to measure than the full range of microorganisms that pose the health risk **(WHO, 1997).** Many studies **(Liang *et al.,* 2006; Hewitt *et al.,* 2007; Maunula *et al.,* 2009)** have associated the outbreaks of waterborne gastroenteritis with a diversity of enteric bacteria and viruses, although recreational exposure to polluted water has often been more linked to viral infections **(Vantarakis and Papapetropoulou, 1999).**

None of the bacterial indicators currently used for monitoring meet all ideal criteria established for water quality **(Ashbolt *et al.,* 2001; Stevens *et al.,* 2001; Bitton, 2005).** The concentration of fecal bacteria can provide some level of indication of enteric viruses when the contamination originates from human sources. This relationship may not exist when the source of pollution is of animal origin **(Payment *et al.,* 2000).**

The survival and incidence of bacterial viruses (phages) in water environments resemble those of human viruses more closely than most other bacterial indicators commonly used. The enumeration of bacteriophages that infect coliform bacteria (coliphages) has been widely accepted as a tool in water quality assessment **(DWAF, 1996; Grabow, 2001).** Somatic coliphages occur in large numbers in sewage and polluted water environments and are easy to detect **(Grabow, 2001).** Male-specific (F-RNA) coliphages are highly specific for sewage pollution and cannot be replicated in water environments, as their detection methods are more complicated **(DWAF, 2004a, 2004b).** The presence of somatic coliphages and F-specific RNA (F-RNA) bacteriophages, however, does not always correlate with human enteric viruses ***(*Hot *et al.,* 2003; Jiang and Chu 2004).** Besides, it is difficult to differentiate between human and animal fecal contamination whilst using somatic coliphages as indicators. Consequently, many human enteric viruses, enteroviruses (EVs) **(Kopecka *et al.,* 1993;**

Gantzer *et al.*, 1998), rotaviruses (RVs) **(Miagostovich *et al.*, 2008),** adenoviruses (AdVs) **(Puig *et al.*, 1994; Pina *et al.*, 1998)** and human polyomaviruses (HPyVs) **(Bofill-Mas *et al.*, 2000; Hamza *et al.*, 2009; McQuaig *et al.*, 2009)** have been proposed as indicators of wastewater contamination in aquatic environments.

The objective of this review is to determine the suitable viral indices expressing the viral pollution in the water and addresses the advantages and limitations of these indices.

Table 1. Definitions for indicator and index micro-organisms of public health concern according to **Berg (1978).**

Group	Definition
Process indicator	A group of organisms that demonstrates the efficacy of a process, such as total heterotrophic bacteria or total coliforms for chlorine disinfection.
Faecal indicator	A group of organisms that indicates the presence of faecal contamination, such as the bacterial groups thermotolerant coliforms or E. coli. Hence, they only infer that pathogens may be present.
Index and model organisms	A group/or species indicative of pathogen presence and behavior respectively, such as E. coli as an index for Salmonella and F-RNA coliphages as models of human enteric viruses.

DISCUSSION

To achieve the objective of this study, some points will be discussed including the relationship between the presence of viruses and bacterial indicators in different water types and could bacteria be a suitable indicator of water and wastewater viral pollution, main environmental viral hazards, and the concept of using microbial indicator(s), why we need to use viral index to express viral pollution of water and wastewater, the situation of water microbial quality standards worldwide at the moment, and previous reports about the different viral candidates as indices of viral pollution of water and wastewater.

a- Relationship between bacterial indicators and enteric viruses in water and wastewater

None of the bacterial indicators currently used for monitoring meet all ideal criteria established for water quality **(Ashbolt *et al.*, 2001; Stevens *et al.*, 2001; Bitton, 2005).** The concentration of fecal bacteria can provide some level of indication of enteric

viruses when the contamination originates from human sources. This relationship may not exist when the source of pollution is of animal origin **(Payment *et al.*, 2000).** Literature has shown that the presence of viruses does not always correlate with the detection of bacterial indicators such as *E. coli,* total coliforms, and intestinal enterococci **(Baggi *et al.*, 2001; Skraber *et al.*, 2004a, 2004b; Hauri *et al.*, 2005; Miagostovich *et al.*, 2008; Espinosa *et al.*, 2009; Jurzik *et al.*, 2010). Espinosa *et al.* (2009)** reported that Bacteria used as indicators for pathogenic microorganisms in the water are not considered adequate as enteric virus indicators in different water types (surface water from a tropical high-altitude system located in Mexico City that receives rainwater, treated and non-treated wastewater used for irrigation, and groundwater used for drinking). In another study, over 20 months, the abundance of human adenovirus, human polyomavirus, enterovirus, group A rotavirus, and norovirus was determined in Ruhr and Rhine rivers, Germany. Additionally, the prevalence of different possible indicators such as somatic coliphages, *E. coli,* intestinal enterococci, and total coliforms was also considered. Moreover, the chemical parameter TCPP (tris-(2-chloro-, 1-methyl-ethyl)-phosphate), characterized by environmental stability and human origin, was included. Furthermore, chemical parameters (fluoride, chloride, nitrate, nitrite, bromide, phosphate, and sulfate) which may influence the stability and subsequently the detection rates of viruses in an aquatic environment were measured. Neither TCPP nor any other chemical or microbiological parameter can be used as a reliable indicator for the presence of enteric viruses in river water **(Jurzik *et al.*, 2010).** The most used fecal indicators worldwide (Escherichia coli and enterococci) are less resistant to environmental stresses than viruses such as temperature, pH, and sun irradiation and behave differently in the environment **(Baggi *et al.*, 2001; Skraber *et al.*, 2004b; Harwood *et al.*, 2005; Costán-Longares *et al.*, 2008). Miagostovich *et al.* (2008)** reported that the presence of viral genomes in areas where fecal contamination was not demonstrated by bacterial indicators suggests prolonged virus persistence in aquatic environments and emphasizes the enteric virus group as the most reliable for environmental monitoring. **Hauri *et al.* (2005)** reported that indicator bacteria are useful markers of faecal contamination only if the faecal contamination results of water influx into a body of water with the water influx containing an average load of pathogens (e.g. from depuration plants, agricultural land wash). If water is contaminated directly by a bather, the relation between pathogens and faecal indicators is much higher and faecal indicators fail. Furthermore, studies have revealed that enteric viruses were detected in raw, surface water, ground water, and treated drinking water despite meeting quality standards for coliform bacteria **(Gerba and Rose, 1990; Cho *et al.*, 2000; Pusch *et al.*, 2005).**

b- Main environmental virus hazards:

Food and environmental virology mostly study viruses that can be transmitted through water, sewage, soil, air, fomites (objects capable of transmitting microbial

pathogens), or food. Most such viruses are enteric viruses transmitted via the fecal-oral route. Human enteric viruses, which by definition are transmitted via the fecal-oral route, are the main waterborne group of viruses that pose a real public health hazard **(Estes *et al.*, 2006).** Thus far, enteric viruses have been divided into eight families. The most relevant ones are hepatitis A (HAV) and E (HEV), (EVs), (RVs), noroviruses (NoV), astroviruses (AstVs), and enteric AdVs, which may cause the respective severe diseases: hepatitis, paralysis, meningitis, myocarditis and heart anomalies, fever, gastroenteritis, conjunctivitis, and respiratory disease (Table 2). Infected humans can excrete large amounts of human pathogenic viruses(10^5 to 10^{11} virus particles per gram of stool); animal and plant material, as well as other excreta, can also carry high viral loads. Viruses transmitted via the fecal-oral route are generally nonenveloped and thus very stable in the environment and include major aetiological agents, some of which are thought to be emerging zoonotic pathogens. These viruses cannot always be effectively eliminated by current methods of sewage treatment and consequently cause viral contamination of the environment from treated as well as untreated wastewater. Other examples of indirect routes are run-off from manure used in agriculture. There is also direct fecal contamination of the environment from humans and animals, for example by bathers or by defecation of free-range or wild animals onto soil or surface waters. The resulting viral contamination of sea and coastal water, rivers and other surface waters, groundwater, and irrigated vegetables and fruits are associated with subsequent risks of reintroduction of the viral pathogens into human and animal populations **(Emerson *et al.*, 2005; King *et al.*, 2009).**

Human exposure to even low levels of these pathogenic viruses in the environment can cause infection and disease. Individuals with an impaired immune system, including children, the elderly, pregnant women, and people with HIV/AIDS, are more susceptible to such infections, and the disease outcome may be more severe. This is the case, for example, for RV, which is a more serious problem for young children in developing than in developed countries. Genetic susceptibility may also play a role in the susceptibility to infection, as in the case of NoV and the ABO histo-blood group receptor genotype **(Rodriguez lazaro *et al.*, 2012).**

c- Bacteria as indicators of water quality

The indicator organisms presently used for the monitoring of drinking water in both developed and developing countries are total coliforms, fecal coliforms, and/or *E. coli*. Many other organisms such as enterococci and fecal streptococci that are present in

Table 2. Properties and clinical characteristics of major human waterborne and food enteric viruses according to **Emerson *et al.*, (2005) and King *et al.*, (2009).**

Family (genus)	Common name	Size (genome)	Envelope	Incubation/illness duration (days)	Contaminated source	Season	Symptoms
Picornaviridae (enterovirus)	Polio, Coxsachie A & B, echo, human enterovirus (types 68 to 71)	28 nm (ssRNA)	No	4–35/7–14	Water and shellfish	Mostly winter	Cardiomyopathy, meningitis, CNS motor paralysis, gastroenteritis
Picornaviridae (kobuviris)	Aichivirus	28 nm (ssRNA)	No	39 h/2–4	Shellfish	All seasons	Gastroenteritis
Picornaviridae (hepatovirus)	Hepatitis A virus	28 nm (ssRNA)	No	15–50/7–14 (to several months in serious cases)	Food-borne	Late spring to early summer (in Hong-Kong)	Hepatitis
Hepeviridae (hepevirus)	Hepatitis E virus (formerly non- A non-B hepatitis)	34 nm (ssRNA)	No	21–56/7–28	Mainly water	Winter	Hepatitis
Rotaviridae	Rotavirus	70 nm (dsRNA)	No	2–6/5–7	Mainly water	Autumn and winter	Gastroenteritis (diarrhea)
Adenoviridae (mastadenovirus)	Adenovirus group F, types 40 and 41	100 nm (dsDNA)	No	8–10/5–12	Water and fecal-oral route	No certain seasonality	Mild diarrhea
Caliciviridae (saprovirus)	Saprovirus	34 nm (ssRNA)	No	1–4/6	Shellfish	Mostly winter	Gastroenteritis
Caliciviridae (norovirus)	Norovirus (Norwalk-like viruses)	34 nm (ssRNA)	No	1–3/4	Water and food-borne	Mostly winter	Explosive projectile vomiting
Astroviridae (mamastovirus)	Human astrovirus	28 nm (ssRNA)	No	1.5–2/1–4	Water and shellfish	Mostly winter	Mild gastroenteritis
Parvoviridae	Wollan, ditchling, Paramatta, and cockle agents	25 nm (ssDNA)	No	4–14/7	Food-borne (shellfish)	Late summer to late spring	Gastroenteritis
Coronaviridae	Coronavirus and Torovirus	spherical, 120–160 nm diameter (ssRNA)	Yes	2–5/7	Fecal-oral route or by aerosols of respiratory secretions	Winter and early spring	Severe acute respiratory syndrome (SARS) in humans

high numbers in the environment have been used as alternate water quality indicators. Total coliforms are typically described as "All facultative anaerobic, Gram-negative, non-spore-forming, oxidase-negative, rod-shaped bacteria that ferment lactose to acid and gas within 48 h at 35 °C or members of Enterobacteriaceae which are β-galactosidase positive" **(APHA, 1998).** The total coliform group of bacteria was originally used as a surrogate for *E. coli* which, in turn, was considered to demonstrate fecal pollution, until more specific and rapid methods became available **(Kornacki and Johnson, 2001).** Detection of total and fecal coliforms in raw water can provide authorities with an indication of any changes in water quality **(WHO, 1997).** Classical methods for the detection of total and fecal coliforms in natural waters include the most probable numbers (MPN) and the membrane filtration (MF) techniques on selective agar **(APHA, 1998).** Although the tests are simple to perform, they are time-consuming, requiring 48 h for the presumptive results which do not allow the detection of all the target bacteria in natural environments. Since the late nineteenth century, bacteria have been used as water quality indicators **(Medema *et al.*, 2003).** The use of coliforms as bacterial indicators of microbial water quality is based on the hypothesis that coliforms are present in elevated numbers in the feces of humans and other warm-blooded animals. If feces or its leachates have entered into drinking water, probably, these bacteria will also be present, even after significant dilution. It is generally accepted that the total coliform group of bacteria is diverse and can be considered as typical inhabitants of many soil and water environments which have not been impacted by fecal pollution. With a few exceptions, the coliform group of bacteria is not considered to be a health risk, but their presence indicates that fecal pollution may have occurred and pathogens might be present in the water environment as a result. Total coliforms signify only about 1% of the total population of bacteria in human feces in concentrations of about 10^9 bacteria per gram **(Brenner *et al.*, 1982).**

d- The concept of using indicator microorganisms

From an epidemiological point of view, the most relevant viral pathogens found in water are the HAV, HEV, and the gastroenteritis viruses, which include RVs, NVs, AstVs, and enteric AdVs. Due to technical difficulties, tests for most of these viruses remain restricted to laboratories with sophisticated facilities and well-trained personnel. On the other hand, it is impracticable to monitor the presence of all viral pathogens. This lead to the origin of the concept of indicator microorganisms. Since we are concerned with viruses transmitted through the fecal-oral route, microorganisms present in the fecal microbiota were immediately proposed. A good indicator should fulfill the following requirements: (i) should be associated with the source of the pathogen and should be absent in unpolluted areas, (ii) should occur in greater numbers than the pathogen, (iii) should not multiply out of the host, (iv) should be at least equally resistant to natural and artificial inactivation as the viral pathogen, (v) should be detectable using easy, rapid and inexpensive procedures, and (vi) should not be pathogenic **(Bosch, 1998).** Human enteric

viruses can tolerate fluctuating environmental conditions and survive in the environment for long periods becoming causal agents of diarrhoeal diseases. Therefore, the potential of human pathogenic viruses as significant indicators of water quality is emerging. **(Lin and Ganesh, 2013).** Viruses cannot replicate outside their host's tissues and therefore cannot multiply in the environment. They can, however, survive in the environment for extended periods, longer than most intestinal bacteria, making it unsafe to rely solely on bacteriological water quality standards. Viruses have been reported to survive and remain infective for up to 130 days in seawater, for up to 120 days in freshwater, and up to 100 days in the soil at 20-30 °C. Comparisons between enteric viruses also show variability, with AdVs potentially surviving longer in water than other enteric viruses, such as HAV virus and poliovirus. Despite the relatively low concentration of viruses in water, these micro-organisms carry health risks, since they have very low infectious doses (10-100 virions) so that even a few viral particles in water can pose health risks **(La Rosa *et al.*, 2012).**

e- The situation of water microbial quality standards worldwide at the moment

Currently, there are quite a few different types of drinking water quality standards being used in many individual countries. The three are most commonly suggested as global standards include a) USEPA National Drinking Water Criterion, b) EC Drinking Water Quality Directive, and c) WHO Drinking Water Quality Criterion, which has served as a foundation for other national water quality standards. In 2011, WHO issued a fourth Drinking Water Quality Standard, which includes the 28 microbiological indicators that are acknowledged waterborne pathogens. It contains 12 kinds of bacteria, 8 kinds of viruses, 6 kinds of protozoa, and 2 kinds of parasites **(WHO, 2011).** The presently used USEPA National Drinking Water Quality Standard, which was issued in 2009, is divided into two parts: the National Primary Drinking Water Regulation (NPDWRs) and National Secondary Drinking Water Regulation (NSDWRs). The NPDWRs belong to the compulsory standard for the public water supply system, with a total of seven microbial indicators used. In contrast, The EU Drinking Water Directive 98/83/EC, passed in 1998, contains only two microbial indicators. It is necessary to urge the standard to raise the requirements regarding the frequency of the indicators. For instance, several easily detectable indicators, such as *E. coli*, need to be tested more frequently and full-scale detection needs to be done once these indicators exceed the standard **(Cui *et al.*, 2015). Wen *et al.* (2020)** reported the situation of microbial quality standards of water samples worldwide (Table 3).

f- Why a viral index or indices for viral pollution must be used?

As mentioned above the false relationship between bacterial indicators and the presence/absence of viruses may lead to a big threat to human health. Based on the bacterial indicator, sometimes the judgment about the validity of water for drinking or irrigation is wrong. So, viruses must be examined in treated effluents or drinking water

samples to have a correct judgment on the validity of water for drinking or irrigation. But, there will be a problem of how to examine more than 100 viruses that may be present in water samples. It will be very fatigued, expensive, and need a very long time. So, the logical alternative is to have a viral index to express the presence/absence of viruses in water samples. This index must have all the conditions of any indicator except for the condition saying that it should not be pathogenic because it is a virus itself and

Table 3. The situation of microbial quality standards of water samples worldwide.

Country	Microbial quality standards of water
China	In the year 1959, the provisional act was renamed The Drinking Water Quality Standard by the Ministry of Health, which contained two bacterial indicators: aerobic bacterial count and total coliform **(Bai and Yin, 2007).** With an increasing incidence of illness caused by protozoa, Giardia and Cryptosporidium were then added to the standard in 2006. Enteric viruses, still, have not been used as a water quality indicator in China **(Wen *et al.*, 2020).**
Japan	The new revision of water quality criteria was carried out on April 1st, 2015. The revised standard states that total coliform, thermotolerant coliform bacteria, and *E. coli* cannot be detected in water and general bacteria cannot exceed ≥ 100 per mL **(Gruber *et al.*, 2014).** Enteric viruses, still, have not been used as a water quality indicator in Japan **(Wen *et al.*, 2020).**
Singapore	It has been using the water quality indicator standards issued by the WHO for drinking water. the latest guideline issued in 2011 contained 28 waterborne pathogen indicators, including 12 bacteria and 8 enteric viruses **(Wen *et al.*, 2020).**
Malaysia	It was dependent on the standards recommended by the WHO and by Australia. According to the standard issued in 1990, the microbial indicators in Malaysia contained seven types. Total coliform and enteric viruses were chosen from the WHO's Drinking Water Quality Standards issued in 1984 **(Wen *et al.*, 2020).**
European Union	Currently, in European Union, two important instructions for water protection are (1) The European Union Water Quality Directive (98/83/EC) **(European Communities, 1998)** and (2) The Water Framework Directive (2006/60/EC) **(European Communities, 2006; European Communities 2014).** The directive was suggested for every member country, and each member country can amend indicators based on their situation. The majority of countries in Europe have adopted the above instructions; but there are several countries, such as the United Kingdom, France, and Germany who have established standards based on European Union Water Quality Instructions (EUWOI). Russia, however, has established its standards different from EUWOI and WHO. The UK possesses a developed economy and the most abundant water sources, predominantly rivers, among the EU. In 1996, The Surface Water (Abstraction for Drinking Water; Classification) Regulations of 1996 was issued in England and Wales. The regulations required bacterial indicators stipulated, like Enterococci and *E. coli*, to be 0 per 1 mL in faucet water and coliform and *E. coli* to be 0 per 1 mL in reservoir

	water and water from water treatment plants **(WHO/UNICEF, 2000).** This standard was consistent with European Union Water Quality Directive. France refers to EU 80/778/EC, with the addition of three new amendments made in 1990, 1991, and 1995. Most index values used were the European highest tolerant level, especially the comprehensive microbiological standards. The standard contained seven microbiological pathogens, five bacterial indicators, and two viruses. For bacterial indicators, both *E. coli* and fecal enterococci were required to be 0 per 100 mL, sulfite-reducing Clostridia was required to be < 1/20 mL, Salmonella was required to be 0/5 L, and Staphylococcus was 0/100 mL. For viral requirements, a phage was to be 0/50 mL and no detection of enteric viruses per 10 L **(The European Commission, 2008).** Germany established several standards of its own for the international rivers. Germany began enforcing France's Drinking Water Law on May 22, 1986. What is more, Germany's standard in 2000 required drinking water to meet the following requirements: (1) it must be without pathogens, (2) without risk to human bodies, and (3) without the majority of microorganisms. The standard for E. coli was 0 per 100 mL for pipeline water and 100/1 mL for treated water **(Wen *et al.*, 2020).**
USA	The concern now in the US focuses on the study of enteric virus and other indicators that can reflect fecal contamination. US indicators rely solely on bacterial indicators such as total coliforms, fecal coliforms, and enterococci for inferring water microbiological quality, however, bacteria indicators do not always indicate the quality of water in a situation where the water meets the bacterial standards but contains a low number of viruses **(Wen *et al.*, 2020).**
Australia	It established comparatively perfect indicator standards based on the three authoritative standards spelled out by the WHO, EU, and USEPA. The currently used drinking water standard was revised in 1996. It is worth pointing out that the standard not only contained microbiological indicators but also listed irregular testing microbiological indicators. There were a total of 22 microbiological indicators including bacteria, virus, protozoa, toxic algae, *E. coli*, and total coliform all of which cannot be detected per 100 mL of water, including two microbiological indicators (*E. coli* and total coliform), and twenty irregular testing microbiological indicators, which contain several microbiological indicators that cannot be tested in water, otherwise it is necessary to adopt corresponding measures for the government and several microbiological items that have not been used as indicators due to inadequate data. Furthermore, the regulation requires a microbial test to be performed once a week for surface water and once for 2 weeks for groundwater. Enteric virus: adenovirus, enterovirus, hepatitis virus, norovirus, and rotavirus, all can cause gastroenteritis through contaminated drinking water **(National Health and Medical Research Council, 2017).** Although those indicators are not given limiting values, they are listed in the standard to protect public health. The Drinking-Water Standards for New Zealand (DWSNZ) are an important resource for monitoring whether water is safe to drink. The indicator organism *E. coli* is used in the DWSNZ to assess the bacterial quality of water. The bacterial quality of treated water is satisfactory if the *E. coli* concentration is less than 1 organism per 100 mL. When *E. coli* is detected in water it shows that the water has been in contact with feces: this means that pathogens may also be present **(Environmental Science and Research Limited,**

	2008).
African Countries	Fecal coliform has been the only indicator in the water quality criteria (WQC) in South Africa since 1984: microbial levels cannot exceed 100 CFU per 100 mL for direct contact recreational water and must be < 15 per 100 mL for drinking water **(Wen *et al.*, 2020).** Enteric viruses prevalence and resistance to treatment processes are in the phase of studies in some African countries like Egypt **(Villena *et al.*, 2003; El-Senousy, *et al.*, 2004; El-Senousy *et al.*, 2013a; El-Senousy *et al.*, 2013b; El-Senousy *et al.*, 2013c; El-Senousy *et al.*, 2015; El-Senousy and Abou-Elela, 2017; El-Senousy *et al.*, 2020).** In a recent study, adenoviruses genome and infectious units and phiX174 bacteriophage infectious units showed high efficiency to express viral pollution in Egyptian treated wastewater and drinking water **(El-Senousy *et al.*, Manuscript in preparation).** The viral genome may express viral pollution but it does not necessarily express the recent pollution. The advantage of using infectious viruses as an index for viral pollution is that they express not only the viral pollution but also they express the recent contamination of water with viruses. Till now, Egyptian water quality criteria like the majority of countries worldwide do not have any of the viruses to express the pollution of water and wastewater with enteric viruses.

this is necessary to express the viral pollution of water as a viral index. According to the reports published by several groups of authors, it may be one viral index will be used worldwide, or more than one viral index or the third possibility is to let each country or group of countries determine the suitable viral index or viral indices which is/are suitable for its/their viral situation in the aquatic environment. It depends on the prevalence of this virus or these viruses in different water types in the country which may not be the same in the other countries. This high frequency in different water types depends on some factors such as high prevalence in the infected and carrier persons in the community, survivability of genome and infectious units of these viruses, resistance to natural conditions such as temperature, humidity, pH, etc, resistance to treatment processes in water and wastewater treatment plants, periodicity of infection including the peak of frequency of different viruses if present, detection methods of genome copies and infectious units of these viruses, and capability to differentiate between human and animal viral contamination. Based on these factors, discussion of previous reports concerned with different viral candidates to show the best index or indices for viral pollution of water and wastewater will be done in the following items as follows:

1-Bacteriophages

Coliphages, which have been investigated as possible viral indicator organisms since the 1980s, may be more appropriate for monitoring the fate of viruses in water **(Furuse, 1987).** Certain strains of coliphages are small, icosahedral, and nonenveloped viruses, making them structurally similar to many human enteric viruses. They also exhibit similarities to enteric viruses about environmental transport and survival; however, coliphage survival characteristics vary by season and by coliphage group. Male-

specific coliphages, also known as F-specific or F+ coliphages, infect *E. coli* through the F pilli on the host. Male-specific coliphages appear to be present in feces and sewage and also seem to be present at low concentrations in uncontaminated environmental settings **(Cole *et al.*, 2003).** Somatic coliphages, which infect *E. coli* through the host cell wall, are the most abundant group of bacteriophages and are likely to be more persistent in water than male-specific ribonucleic acid (RNA) coliphages **(Lee and Sobsey, 2011).** Relationships among coliphages, coliforms, and pathogens vary under different conditions. **Borrego *et al.* (1990)** found that coliphages were good indicators of fecal pollution in both river and marine waters. **Ogorzaly *et al.* (2009)** studied a river in an urbanized watershed with recognized anthropogenic influences and found that bacterial indicators were correlated with somatic coliphages. Likewise, **Jiang *et al.* (2007)** investigated the occurrence and distribution of fecal indicator bacteria, male-specific coliphages, and polymerase chain reaction (PCR)–detectable human adenoviruses (HAdVs) and EVs at 15 locations around the Newport Bay, Calif., watershed. Among 206 samples, fecal indicator bacteria and coliphages had similar seasonal and freshwater-to-saltwater distribution patterns, suggesting they share similar environmental sources. Besides, coliphages were correlated with PCR-detectable human viral genomes. **Borrego *et al.* (1987)** evaluated the relationship between *E. coli* and its parasitic phages in the vicinity of sewage outfalls, river water contaminated by domestic and industrial sewage discharges, and estuarine waters. Coliphages were a good indicator of the presence of the pathogenic microorganisms studied, and coliphages may be better indicators of fecal pollution than classical bacterial indicator systems. However, bacteriophages may continue to replicate in surviving bacterial hosts after being shed in feces, and male-specific coliphages are present in human feces infrequently, are relatively scarce, and have die-off rates that vary with water temperature **(Lee and Sobsey, 2011). Payment and Locas (2011)** observed non-enteric indicators (total coliforms and aerobic endospores) more frequently than *E. coli,* male-specific coliphages, or somatic coliphages in virus-positive groundwater samples. In a study of groundwater in Massachusetts, the presence of coliforms and coliphages was uncorrelated **(Long and Dewar, 2008).** A year-long sampling campaign at nine water resource recovery facilities in different US regions (west, south, and northeast) was conducted to assess the treatability and fate of bacterial indicators, viral indicators, and viruses. Viral reduction (AdV type 41, NoV genogroup I (GGI), and NoV genogroup II (GGII) was more similar to viral indicator (male-specific and somatic coliphages) reduction than bacterial indicator (*E. coli* and enterococci) reduction. **Carducci *et al.* (1999)** found no relationship between coliphages and viral contamination in sewage treatment plants. Also, somatic coliphages have been found to have higher concentrations than male-specific phages in wastewater and raw water sources **(Stewart-Pullaro *et al.*, 2006). Payment and Locas (2011)** found that coliphages were not good predictors of the presence or absence of viruses because coliphages occurred in low numbers and less frequently than bacterial indicators. In a

study of four French rivers, **Hot *et al.* (2003)** found no statistical correlation between somatic coliphages and EVs, HAdVs, or Norwalk I and II viruses. Because of these shortcomings, male-specific coliphages have most often been used as source-tracking indicators rather than generic indicators of fecal contamination **(Cole *et al.*, 2003).** As for disadvantages, the presence of somatic coliphages and F-specific RNA (F-RNA) bacteriophages does not always correlate with human enteric viruses. It is also difficult to differentiate between human and animal fecal contamination **(Hot *et al.*, 2003; Jiang and Chu, 2004).**

McMinn *et al.* (2017) reported that bacteriophages may be adequate viral surrogates, especially in built systems, such as wastewater treatment plants. Recently, crAssphage (cross-assembly phage) was detected widely in wastewater and the environment **(Farkas *et al.*, 2020).** crAssphage is a bacteriophage that was discovered in 2014 by computational analysis of publicly accessible scientific data on human fecal metagenomes **(Dutilh *et al.*, 2014).** Its circular DNA genome is around 97 kbp in size and contains 80 predicted open reading frames, and the sequence is commonly found in human fecal samples. At its time of discovery, the virus was predicted to infect bacteria of the phylum Bacteroidetes that are common in the intestinal tract of many animals including humans **(Dutilh *et al.*, 2014).** Since then, the bacteriophage has been isolated in vitro and confirmed to infect Bacteroides intestinalis **(Shkoporov *et al.*, 2018).** Based on analysis of metagenomics data, crAssphage sequences have been identified in about half of all sampled humans **(Dutilh *et al.*, 2014).**

2-Human adenoviruses

HAdVs are members of the genus Mastadenovirus in the Adenoviridae family **(Okoh *et al.*, 2010).** They possess double-stranded linear DNA and a nonenveloped icosahedral shell that has fiber-like projections from each of its 12 vertices **(Stewart *et al.*, 1993).** Identification of AdVs generally starts with virus isolation using cell culture, followed by antibody or antigen detection and visualization by electron microscopy **(Fong and Lipp, 2005).** The progression of molecular technologies, such as PCR methods for detection and real-time PCR (qPCR) methods for quantification, has enhanced the speed and sensitivity of AdVs detection in water samples drastically **(van Heerden *et al.*, 2003, 2004, 2005).** Current adenoviruses comprise 51 serotypes classified in six species (A–F) **(Okoh *et al.*, 2010).** HAdVs are prevalent and very stable, hence, they are considered human-specific and are not detected in animal wastewaters or slaughterhouse sewage **(Girones, 2005).** Globally, HAdV is estimated to be the causal agent in 8% of viral illnesses (including~10% of childhood pneumonia, 7% of childhood upper respiratory infections, and 5% of childhood gastroenteritis) **(Wold and Ison, 2013).**

HAdVs have been shown to frequently occur in raw water sources, treated drinking water supplies, urban rivers, and polluted coastal waters **(Puig *et al.*, 1994; Tani**

***et al.*, 1995; Pina *et al.*, 1998; Jiang *et al.*, 2001; Dongdem *et al.*, 2009; Jurzik *et al.*, 2010; El-Senousy *et al.*, 2013b).** The incidence of HAdVs in such waters was surpassed only by the group EV among viruses detectable by PCR-based techniques **(Chapron *et al.*, 2000; Grabow, 2001).** HAdV infections have been reported to occur worldwide and throughout the year **(Flomenberg, 2005; Bofill-Mas *et al.*, 2006; El-Senousy *et al.*, 2013b)** and approximately 90% of the human population is seropositive for one or more serotypes of AdVs **(Fong *et al.*, 2010)** suggesting that there are no seasonal variations in the prevalence of these viruses. Given their pervasiveness and detection as enteric pathogens, their detection in water (contaminated, drinking, or recreational) represents a likely but unconfirmed source of HAdV infections **(Grabow, 2001).** Studies in the United States found that AdVs, among all the proposed enteric virus indicators, were found ubiquitously in raw sewage samples **(Symonds *et al.*, 2009).** HAdVs are also considered important because they are exceptionally resistant to some water treatment and disinfection processes, notably UV light irradiation. HAdVs have been detected in drinking-water supplies that met accepted specifications for treatment and disinfection with the use of conventional indicator organisms **(Chapron *et al.*, 2000; Grabow, 2001; Dongdem *et al.*, 2009).** In Cairo (Egypt), although, RV (Group A) was the most frequent RNA enteric viruses in raw sewage and Nile water and also the most resistant RNA enteric viruses to treatment processes in water and wastewater treatment plants, followed by HAV, AstV, and EV, and finally, NoVs respectively **(Villena *et al.*, 2003, El-Senousy, *et al.*, 2004, 2013b, 2013c),** AdVs was more frequent than RV in raw sewage and Nile water samples and more resistant to treatment processes in sewage and water treatment plants **(El-Senousy *et al.*, 2013b).** In Barcelona (Spain), high quantities of HAdV and polyomavirus type JC (JCPyV) were detected in sewage, effluent wastewater, sludge, and biosolid samples with a higher positivity rate than HEV. Both viruses showed high stability in urban sewage **(Bofill-Mas *et al.*, 2006).** HAdV and HPyV were frequently detected in high concentrations in wastewater and wastewater-contaminated waters in New Zealand confirming their use as potential indicators for the presence of human sewage. Overall, there was a correlation between the presence of HAdV and HPyV with NoV in estuarine waters impacted by wastewater overflows **(Hewitt *et al.*, 2013).** Fifteen samples from 5 dairy farms -located at the municipality of Tenente Portela, Northwest of Rio Grande do Sul, Brazil- were collected and analyzed for the presence of HAdV, as well as EV and RV. HAdV was present on 66.66% of the water samples and has been found in all samples from artesian wells and springs, which are used as sources of drinking water for the individuals inhabiting those farms. EV and RV were found only in one sample each **(Spilki *et al.*, 2013).** Also, in another survey of tap water samples collected in public schools located at six municipalities of Rio Grande do Sul, southern Brazil, seventy-three schools were included in the study and tap water samples were analyzed by conventional PCR for the presence of HAdV, HEV, and Group A RV genomes. HEV showed the highest detection rate (27.4%), followed by HAdV (23.3%),

and Group A RV (16.4%) **(Kluge *et al.*, 2014).** From the Manguinhos area, Rio de Janeiro, Brazil, three hundred and four drinking water samples (2L/each) were collected along the drinking water distribution-to-consumption pathway in households, as well as healthcare and school units. Only seven of the 45 virus-positive samples were considered unsatisfactory due to the presence of bacteria and/or physical-chemical parameters analyzed. Using qPCR, viruses were detected 50 times in the 45 viral positive water samples because of the mixed viral contamination in one sample. 19 of these positivity were HAdVs, 17 Group A RV, and 14 NoV GII **(Miagostovich *et al.*, 2020).** In China, a total of twenty-four water samples which were collected from six different sites along the Poyang Lake for sewage contamination, EV (58%) and AdV (67%) were more frequently detected from these six sites, followed by were NoV GGI (50%) and NoV GGII (38%) **(Zhu *et al.*, 2018).** HAdV is widely suggested as a virological water quality indicator **(Rames *et al.*, 2016; Puig *et al.*, 1994; Pina *et al.*, 1998; Fong and Lipp, 2005; Albinana-Gimenez *et al.*, 2006; Hundesa *et al.*, 2006; Bosch *et al.*, 2008; Jurzik *et al.*, 2010; Okoh *et al.*, 2010, El-Senousy *et al.*, 2013b),** since this pathogen is resistant to chemical and UV disinfection and is generally detected in untreated municipal wastewater and faecally contaminated environmental waters all year round, unlike other viruses such as NoV and EV that can display seasonal detection **(Katayama *et al.*, 2008; Nordgren *et al.*, 2009; Sedmak *et al.*, 2003; Wong *et al.*, 2012).** However, this virus did not correlate with HAV or EV in urban waterways **(Jiang, 2002). McQuaig *et al.*, (2009)** only detected sparse numbers of AdV in a faulty septic tank system in comparison with that of PyV. Besides the conventional polymerase chain reaction (PCR) for detecting AdV genome **(Puig *et al.*, 1994),** conventional real time quantitative PCR (qPCR) methods for detecting and quantifying the HAdV (dsDNA) genome are already established **(Rames et al. 2016; Bibby *et al.*, 2019),** enabling validation of novel testing methods. Infectious HAdVs could be detected and quantified using several methods such as cell culture-polymerase chain reaction (CC-PCR) **(El-Senousy *et al.*, 2013b; El-Senousy and Abou El-ela, 2017),** integrated cell culture – preceded by reverse transcriptase and qPCR (ICC-RT-qPCR) to quantify mRNA of HAdV **(Fongaro *et al.*, 2013),** and fluorescence-activated cell sorting assay **(Li *et al.*, 2010).** Detection of HAdVs infectious units has advantages in comparison with detection of HAdVs genome using PCR. Human infectious viruses which could be determined using primers specific to human strains express the recent contamination of different water types with human viruses because HAdV genome persists longer in water than HAdV infectious units **(Donia *et al.*, 2010, El-Senousy *et al.*, 2014, Prevost *et al.*, 2016).** Specific primers must be used to differentiate between enteric and non-enteric HAdVs when using CC-PCR assay **(Xu *et al.*, 2000),** however, some primers are specific for human enteric and non-enteric AdVs **(Puig *et al.*, 1994).**

3-Human Polyomaviruses

PyV is a small, non-enveloped double-stranded DNA virus that is the sole genus in the family Polyomaviridae **(ICTVdB, 2006).** The family Polyomaviridae, which originally contained only one single genus (Polyomavirus), has expanded into three genera: Orthopolyomavirus, Wukipolyomavirus, and Avipolyomavirus **(Johne *et al.*, 2011).** Different laboratories use a variety of assays, e.g. electron microscopy **(Schroedera *et al.*, 2003),** PCR **(Johne *et al.*, 2005; Lamontagne *et al.*, 2011),** to detect polyomavirus particles in clinical specimens. Five human PyV (BKV, JCV, KIV, WUV, and MCV) have been identified **(Kean *et al.*, 2009). Weitschek *et al.* (2012)** used the data mining techniques, by considering the complete sequences of the viruses and the sequences of the different gene regions separately, to effectively characterize the different five studied PyVs. These viruses are known for producing lifelong, asymptomatic viruria in immunocompetent individuals **(Polo *et al.*, 2004).** Over 70% of adults harbor antibodies to BKV or JCV HPyVs **(Meng and Gerba, 1996; Lukasik *et al.*, 2000).** The obligate host specificity and abundance of BKV and JCV in municipal sewage have led to the successful use of these viruses to indicate human fecal pollution in environmental water samples **(Albinana-Gimenez *et al.*, 2006; McQuaig *et al.*, 2006; Brownell *et al.*, 2007). Bofill-Mas *et al.* (2000)** suggested that JCV would be a useful indicator of human sewage in the water. The obligate host specificity of viruses such as HPyVs is advantageous for the specific identification of human sources. JCV or BKV have been detected by using conventional PCR in raw sewage from all over the globe **(Bofill-Mas *et al.*, 2000, 2001; McQuaig *et al.*, 2009; Kokkinos *et al.*, 2011).** Lower concentrations of HAdV and JCV were also found using qPCR in different treatment steps of the plants in absence of bacterial standards **(Albinana-Gimenez *et al.*, 2009).** Therefore, PyV has been proposed to be a suitable indicator of fecal contamination in river water by the detection of nucleic acids of these viruses **(Bofill-Mas *et al.*, 2006).**

Finally, HPyVs are a good candidate since they are routinely found in environmental water samples from different geographical areas with relatively high abundance. HPyVs are highly human-specific, having been detected in human waste from all age ranges and undetected in animal waste samples. Besides, HPyVs show a certain degree of resistance to high temperature, chlorine, UV, and low pH, with molecular signals (i.e., DNA) persisting in water for several months. Recently, various concentration methods (electronegative/positive filtration, ultrafiltration, skim-milk flocculation) and detection methods (immunofluorescence assay, cell culture, PCR, integrated cell culture PCR (ICC-PCR), and qPCR) have been developed and demonstrated for HPyV, which has enabled the identification and quantification of HPyV in various environmental samples, such as sewage, surface water, seawater, drinking water, and shellfish **(Rachmadi *et al.*, 2016; Farkas *et al.*, 2020).** Efficient propagation of the archetype forms of BKPyV and JCPyV was observed in 293TT cells **(Broekima**

and Imperiale, 2012; Barth *et al.*, 2016). This may open the door to the study of infectious polyomaviruses in water and wastewater.

4- Pepper mild mottle virus (PMMoV)

PMMoV belongs to the *Tobamovirus* genus which causes significant economic losses on infected pepper worldwide **(Fauquet *et al.*, 2005).** The virus consists of a rod-shaped particle in which a positive sense linear single-stranded RNA is encapsidated. Since viruses of dietary origin presumably do not depend on human infection, their detection in surface water might be less dependent on seasonality and other temporal changes in viral circulation levels. Though PMMoV infects pepper, it does not affect tomato, eggplant, and tobacco **(Elizabeth *et al.*, 2001),** which are in the same family (*Solanaceae*). Many pepper-based foods tested positive for PMMoV suggesting that food is a main source of PMMoV in human feces **(Zhang *et al.*, 2006).** This was supported by **Colson *et al.* (2010),** who recovered PMMoV RNA sequences from 57% of food products containing pepper or spice, whereas the virus was not detected in food products free from *Capsicum* spp or spice. PMMoV was suggested before as an indicator of fecal pollution in marine water **(Rosario *et al.*, 2009).**

Identification of PMMoV in feces was first achieved through viral metagenomics **(Zhang *et al.*, 2006).** They reported that the most abundant RNA virus in three fecal samples from healthy adults in the United States was PMMoV, comprising 75.7–99.4% of all sequences identified in the fecal RNA viral community. Phylogenetic analysis of PMMoV strains identified in the fecal samples indicated that the PMMoV strains were very different even in two fecal samples collected from the same individual, which implies that the PMMoV circulation in humans populations is dynamic **(Zhang *et al.*, 2006).** PMMoV was subsequently detected in feces by regular RT-PCR or RT-qPCR in six (67%) of nine samples in the United States, six (67%) of nine samples in Singapore **(Zhang *et al.*, 2006),** 19 (95%) of 20 samples in Germany **(Hamza *et al.*, 2011),** and in the specimens of one (0.48%) of 208 hospitalized children and 22 (7.2%) of 304 adult patients in France **(Colson *et al.*, 2010).** Although the detection rate varies between studies, likely due to differences in detection methods or exposure to PMMoV **(Colson *et al.*, 2010),** these studies have demonstrated that the presence of PMMoV in feces is geographically widespread. PMMoV concentrations in feces are high, ranging from 10^5 to 10^{10} copies/g-feces (dry weight) **(Zhang *et al.*, 2006).** PMMoV can be found with greater frequency in healthy human feces than pathogenic viruses **(Rosario *et al.*, 2009).** Strains isolated in human feces are genetically diverse with dynamic fecal populations within an individual and notably remain viable and infectious to host plants **(Zhang *et al.*, 2006).** One previous study documented interactions of PMMoV with the human immune system and suggested that the virus may cause clinical symptoms in humans, such as fever, abdominal pains, and pruritus; however, these symptoms may have been

confounded by spicy food rich in peppers or pepper-based products **(Colson *et al.*, 2010).** PMMoV has not been detected in fecal samples or intestinal homogenates of most animals, such as turkeys, horses, coyotes, raccoon, sheep, ducks, pigs, and dogs **(Rosario *et al.*, 2009; Hamza *et al.*, 2011).** Although fecal samples from cows, geese, seagulls, and chickens were sometimes positive for PMMoV, virus concentrations in these samples were much lower (3–4 $\log_{10}$) than those in human feces; the originating source of PMMoV in these animals is unclear **(Rosario *et al.*, 2009; Hamza *et al.*, 2011).**

PMMoV has been detected in rivers in Germany, Vietnam, the USA, and Singapore. Related viruses belonging to tobamoviruses were detected in river waters as early as 1989, in Germany which the Ruhr and Rhine rivers were sampled and analyzed for multiple viruses, including PMMoV, torque teno virus (TTV), HAdVs, human picobirnaviruses (HPBVs), and HPyVs. PMMoV was found in 100% (n = 108) of samples analyzed. It was the only virus that was consistently detected throughout all samples. Concentrations for these samples ranged from 3.0×10^3 to 1.1×10^6 genome copies/L **(Hamza *et al.*, 2011).** This range is similar to that of river water samples from Vietnam (n = 3), where **Kuroda *et al.* (2015)** reported a range of 3.0×10^4–1.8×10^6 genome copies/L. The samples in this study were taken at varying points in river systems: upstream from an urban area, 500 m downstream of a wastewater treatment plant, and from a point that receives wastewater from a different area **(Kuroda *et al.*, 2015).** This study also evaluated the presence of PMMoV in pond and irrigation waters. In pond waters, PMMoV was detected in 10/11 (91%) samples, with concentrations ranging from non-quantifiable to 1.2×10^5 genome copies/L. In irrigation waters, 3/3 (100%) samples were positive for PMMoV; however, only one sample had a quantifiable concentration of 1.0×10^4 genome copies/L **(Kuroda *et al.*, 2015).** Another study in 2017 looked at irrigation waters from the Kathmandu Valley from a variety of sources used for irrigating fresh produce **(Shrestha *et al.*, 2018).** Thirty-five surface water and six groundwater samples were collected. Sampling sites included six rivers, two ponds, one canal, and six groundwater wells. PMMoV was detected in 96% of river samples (27/28); 100% of canal samples (2/2); 60% of pond samples (3/5); and 83% of groundwater samples (5/6) **(Shrestha *et al.*, 2018).**

Betancourt *et al.* (2014) reported that PMMoV was more commonly detected than human enteric viruses (HAdV, enterovirus, and Aichi virus in groundwater samples collected from managed aquifer recharge sites in Colorado, California, and Arizona in the United States. In Mexico, groundwater samples showed the fluctuating presence of PMMoV between the rainy and dry seasons. qPCR concentrations for PMMoV in freshwater during the "rainy season" samples ranged from 1.79×10^1 to 1.04×10^4 genome copies/L. The corresponding *E. coli* counts were <1 MPN/100 mL in 7/8 (87.5%) samples, with the remaining sample yielding a count of 8 MPN/100 mL. During the "dry season", PMMoV concentrations ranged from 5.91×10^1 to 4.67×10^3

genome copies/L. Again, *E. coli* MPN did not reflect these concentrations; in 6/8 (75%) samples, <1 MPN/100 mL was reported. In brackish waters, PMMoV was only quantifiable in the "rainy season", with qPCR concentrations of 5.35×10^3 and 4.05×10^1 genome copies/L; in the same samples, *E. coli* was undetectable. This study showed that there was no correlation between PMMoV concentrations and *E. coli* or coliform concentrations, or physicochemical water parameters. Another study in which groundwaters were sampled from Vietnam showed only 3/8 (37.5%) positives for PMMoV. Two of the samples were not quantifiable, while the third had a concentration of only 19 genome copies/L **(Kuroda *et al.*, 2015).** Varying circumstances surrounding PMMoV presence in these water types have also been evaluated, such as rainy versus dry seasons, effluent influence, and correlation with other water pollution and quality indicators **(Symonds *et al.*, 2016; Rosario *et al.*, 2009; Kuroda *et al.*, 2015; Rosiles-González *et al.*, 2017).**

5-Other viruses

Some researchers have suggested EVs, NoVs, RVs, and reoviruses as indicators of other enteric viruses **(Kopecka *et al.*, 1993; Metcalf *et al.*, 1995; El-Senousy *et al.*, 2013b; Betancourt and Gerba, 2016).** However, these viruses exhibit seasonal fluctuations and epidemic spikes **(Skraber *et al.*, 2004a; Haramoto *et al.*, 2006).** Besides, quantification of infectious human noroviruses (HuNoV) in vitro has been accomplished using 3-D cell culture **(Straub *et al.*, 2007)** and successful cultivation of multiple HuNoV strains in enterocytes in stem cell-derived, nontransformed human intestinal enteroid monolayer cultures **(Ettayebi *et al.*, 2016)** which are well beyond the analytical capabilities of typical water testing laboratories.

TTV was another candidate indicator of enteric viruses compared to traditional bacterial indicators and proposed viral indicators. TTV is an enterically transmitted human virus, but it exhibits characteristics that distinguish it from other enteric viruses. Some studies toward understanding the biology and occurrence of TTV provide preliminary support for this hypothesis. TTV is a non-enveloped virus with a single-stranded, circular DNA genome **(Nishizawa *et al.*, 1997; Okamoto *et al.*, 1998; Miyata *et al.*, 1999).** TTV isolates are remarkably variable with 47–70% divergence at the amino acid level **(Biagini *et al.*, 1999; Luo *et al.*, 2002).** TTV divergence is unevenly distributed across the genome; hypervariable regions exist within the coding region **(Nishizawa *et al.*, 1999),** and the untranslated region contains conserved regulatory sequences **(Leary *et al.*, 1999).**

Initially, TTV was described as a novel hepatitis virus **(Nishizawa *et al.*, 1997),** but it was later determined that TTV circulates in a large proportion of healthy individuals **(Vaidya *et al.*, 2002; Haramoto *et al.*, 2005; Diniz-Mendes *et al.*, 2008)** with an average worldwide prevalence estimated at 80% **(Springfeld *et al.*,**

2000; Bendinelli *et al.*, 2001). The virus appears to elicit both persistent and transient infections **(Nishizawa *et al.*, 1997).** Transmission of TTV is primarily by the fecal-oral route **(Bendinelli *et al.*, 2001),** but it is detected in a variety of human tissues and fluids, including plasma and serum **(Ross *et al.*, 1999; Okamoto *et al.*, 2000; Okamoto *et al.*, 2001; Pollicino *et al.*, 2003).** Many attempts have been made to assign a pathology to TTV, but none have been substantiated. **Griffiths (Griffiths, 1999) and Simmonds *et al.* (1999)** have suggested that TTV may constitute the first known commensal human virus.

A few investigators have tracked TTV in the environment or treatment systems. Their results suggest that TTV may co-locate with various enteric viruses. **Takayama *et al.* (1999)** demonstrated that TTV infectivity was not lost after 95 hours of dry heat treatment. Investigators suspect that TTV particles are highly resistant to environmental stressors **(Verani *et al.*, 2006).**

In polluted streams of Brazil, TTV was found to be spatially and temporally constant **(Diniz-Mendes *et al.*, 2008),** and the TTV positivity rate of 92.3% paralleled the positivity rate reported by de Paula *et al.* **(de Paula *et al.*, 2007)** for HAV in the same geographic region. In Italy, river water samples receiving waste treatment effluent were found to contain TTV and other enteric viruses **(Verani *et al.*, 2006).** TTV and RV occurred either simultaneously or within 1 month's sampling period of each other. Besides, TTV occurred 1–2 months after EV was detected and simultaneously or within 2 months of NoVs GGI and GGII in all but one case. **Vaidya *et al.* (2002)** compared sewage treatment plant influent and effluent concentrations of TTV, HAV, and HEVvia PCR and observed that raw sewage prevalence of TTV DNA was statistically similar to the prevalence of hepatitis E virus RNA and HAV RNA. Following treatment, HAV RNA was significantly reduced, but the reductions in TTV and HEV genetic material were not statistically significant. When TTV was monitored through activated sludge wastewater treatment plants in Japan, researchers reported that the TTV genome was detected with 97% frequency in influent, 18% in secondary effluent after activated sludge but before chlorination, 24% in final effluent after chlorination, and 0% in the effluent for reuse following filtration and ozonation **(Haramoto *et al.*, 2005).** In contrast, coliforms decreased sequentially with each step in the treatment process, and the concentration of coliforms did not correlate with the number of positive TTV samples collected at any step.

Poor sanitation may increase TTV transmission by the fecal-oral route, as the countries of Bolivia and Burma – both with high risks of waterborne disease – have TTV incidences of 82% and 96%, respectively, among otherwise healthy individuals **(Abe *et al.*, 1999).** In contrast, TTV prevalence in the United States is estimated to be 10% **(Desai**

***et al.,* 1999).** It is hypothesized that at this prevalence, TTV would be present in most environmental samples at levels high enough to be detected using PCR **(Bendinelli *et al.,* 2001)** except for contamination resulting from single septic systems.

HPBV had been detected at substantial levels in human feces **(Symonds *et al.,* 2009).** The researchers also proposed that the detection of nucleic acids of these viruses might be a suitable indicator of fecal contamination in river water. HPBVs are fairly small (35 nm), non-enveloped, spherical viruses. They have been found in a wide range of hosts, including humans, with or without gastroenteritis. These viruses have not been successfully cultured in the laboratory. High levels of occurrence in wastewater samples have been reported. ***Symonds et al.* (2009)** detected HPBV in 100% of raw sewage samples and 33% of final effluent samples. Whether the presence of HPBV in aquatic environments is specific to human fecal contamination or whether animals may act as a source of contamination is unclear. They suggested that these viruses could be used, along with AdVs as potential markers of fecal contamination. However, in the study of ***Hamza et al.* (2011),** HPBVs were detected in only 25% of wastewater samples, casting doubt on their suitability as indicators of fecal contamination in water.

FUTURE VISION AND CONCLUSION

Bacterial indicators could not express the pollution of water and wastewater samples with enteric viruses. On the other hand, there is more than one group of viral candidates that could be used as indices for pollution of water and wastewater with enteric viruses such as HAdVs, HPyVs, TTV, and bacteriophages. HAdVs and PMMoV are more frequent than other viral candidates in different water types. So, they represent strong candidate indices for pollution of treated wastewater and drinking water with human enteric viruses, however, more studies are still needed to compare them with HPyVs, TTV, and bacteriophages in different types of water samples. Although RVs have a high frequency in autumn and winter months, the low frequency in spring and summer represents a big disadvantage when using these viruses as indices of viral pollution in water and wastewater. Also, EVs, NoVs, and reoviruses exhibit seasonal fluctuations and epidemic spikes. The frequency all over the year of HAdVs and PMMoV represents a strong advantage for these viruses. Additionally, although, some types of infectious bacteriophages are easy to be quantified very fast, the presence of somatic coliphages and F-specific RNA (F-RNA) bacteriophages does not always correlate with human enteric viruses. It is also difficult to differentiate between human and animal fecal contamination. The viral genome may express viral pollution but it does not necessarily express recent pollution. The advantage of using infectious viruses as an index for viral pollution is that they express not only the viral pollution but also they express the recent contamination of water with viruses. Infectious HAdVs have some advantages as viral

index expresses the contamination of different water types with viruses such as determination of viral contamination source (human). Infectious PMMoV may be an effective viral index, however, it does not determine the source of pollution (human or animals). Difficulties in the propagation of PyVs in cell lines may represent a disadvantage of using these infectious viruses as viral indices, however, trials to improve the propagation of these viruses in cell lines are running. Water quality criteria must have a viral index to express the pollution of water and wastewater with enteric viruses through a limited number of years. Within this time, scientific research must try to determine which virus should be used as human enteric viruses index in water and wastewater between AdVs, PMMoV, PyVs, TTV, and bacteriophages. Infectious viruses are strongly recommended than the viral genome to express the recent contamination of different water types with viruses.

REFERENCES

Abe, K.; Inami, T.; Asano, K.; Miyoshi, C.; Masaki, N.; Hayashi, S.; Ishikawa, K.; Takebe, Y.; Win, K.M.; El-Zayadi, A.R.; Han, K. and Zhang, D.Y. (1999). TT virus infection is widespread in the general populations from different geographic regions. J Clin Microbiol, 37(8): 2703-5.

Albinana-Gimenez, N.; Clemente-Casares, P.; Bofill-Mas, S.; Hundesa, A.; Ribas, F. and Girones, R. (2006). Distribution of human polyomaviruses, adenoviruses, and hepatitis E virus in the environment and in a drinking-water treatment plant. Environ Sci Technol, 40: 7416–7422.

Albinana-Gimenez, N.; Miagostovich, M.P.; Calgua, B.; Huguet, J.M.; Matia, L. and Girones R. (2009). Analysis of adenoviruses and polyomaviruses quantified by qPCR as indicators of water quality in source and drinking-water treatment plants. Water Res, 43: 2011–2019.

[APHA] American Public Health Association (1998). Standard method for the examination of water and wastewater, 20th ed. Washington (DC): APHA, AWWA, pp. 924-947.

Ashbolt, N.J.; Grabow, W.O. and Snozzi, M. (2001). Indicators of microbial water quality. In: Fewtrell L, Bartram J, editors. Water quality – guidelines, standards and health: assessment of risk and risk management for water-related infectious disease. Geneva: World Health Organization, pp. 257-288.

Baggi, F.; Demarta, A. and Peduzzi, R. (2001). Persistence of viral pathogens and bacteriophages during sewage treatment: lack of correlation with indicator bacteria. Res Microbiol, 152: 743–751.

Bai, F.L. and Yin, Y.J. (2007). Comparative Study on Hygienic Microbiological Criteria of Drinking Water in China and Developed Countries. Chin. J. Food Hyg, 6: 26–32.

Barth, H.; Solis, M.; Kack-Kack, W.; Soulier, E.; Velay, A. and Fafi-Kremer, S. (2016). In vitro and in vivo models for the study of human polyomavirus infection. Viruses, 8: 292-294.

Bendinelli, M.; Pistello, M.; Maggi, F.; Fornai, C.; Freer, G. et al. (2001). Molecular properties, biology, and clinical implications of TT virus, a recently identified widespread infectious agent of humans. Clin Microbiol Rev, 14: 98-113.

Berg, G. (1978). The indicator system. In Indictors of Viruses in Water and Food (ed. G. Berg), Ann Arbor Science Publishers, Ann Arbor, MI, pp. 1–13.

Betancourt, W. Q. and Gerba, C. P. (2016). Rethinking the significance of reovirus in water and wastewater. Food and Environmental Virology, 8(3): 161–173.

Betancourt, W. Q.; Kitajima, M.; Wing, A. D.; Regnery, J.; Drewes, J. E.; Pepper, I. L. and Gerba, C. P. (2014). Assessment of Virus Removal by Managed Aquifer Recharge at Three Full-Scale Operations." Journal of Environmental Science and Health, Part A. Toxic/Hazardous Substances & Environment Engineering, 49: 1685–92.

Biagini, P.; Gallian, P.; Attoui, H.; Cantaloube, J.F.; de Micco, P. and de Lamballerie, X. (1999). Determination and phylogenetic analysis of partial sequences from TT virus isolates. J Gen Virol, 80: 419-424.

Bibby, K.; Crank, K.; Greaves, J.; Li, X.; Wu, Z.; Hamza, I.A. and Stachler, E. (2019). Metagenomics and the development of viral water quality tools. npj Clean Water, 2: 9-21.

Bitton, G. (2005). Wastewater microbiology, 3rd ed. Hoboken (NJ): John Wiley, pp. 17-31.

Bofill-Mas, S.; Albinana-Gimenez, N.; Clemente-Casares, P.; Hundesa, A.; Rodriguez-Manzano, J. and Allard, A. (2006). Quantification and stability of human adenoviruses and polyomavirus JCPyV in wastewater matrices. Appl Environ Microbiol, 72: 7894–7896.

Bofill-Mas, S.; Formiga-Cruz, M.; Clemente-Casares, P.; Calafell, F. and Girones, R. (2001). Potential transmission of human polyomaviruses through the gastrointestinal tract after exposure to virions or viral DNA. Virol J, 75: 10290–10299.

Bofill-Mas, S.; Pina, S. and Girones, R. (2000). Documenting the epidemiologic patterns of Polyomaviruses in human populations by studying their presence in urban sewage. Appl Environ Microbiol, 66: 238–245.

Borrego, J.J.; Cornax, R.; Morinigo, M.A.; Martinez-Manzanares, E. and Romero, P. (1990). Coliphages as an Indicator of Faecal Pollution in Water. Their Survival and Productive Infectivity in Natural Aquatic Environments. Water Res, 24: 111-112.

Borrego, J.J.; Morinigo, M.A.; de Vicente, A.; Cornax, R. and Romero, P. (1987). Coliphages as an Indicator of Faecal Pollution in Water. Its Relationship with Indicator and Pathogenic Microorganisms. Water Res, 21: 1473-1485.

Bosch, A. (1998). Human enteric viruses in the water environment: a minireview. Internatl. Microbiol, 1: 191–196.

Bosch, A.; Guix, S.; Sano, D. and Pinto, R.M. (2008). New tools for the study and direct surveillance of viral pathogens in water. Curr Opin Biotechnol, 19: 295–301.

Brenner, D.J.; David, B.R. and Steigerwalt, A.G. (1982). Atypical biogroups of Escherichia coli found in clinical specimens and description of Escherichia hermanii sp. nov. J Clin Microbiol, 15: 703–713.

Broekema, N.M. and Imperiale, M.J. (2012). Efficient propagation of archetype BK and JC polyomaviruses. Virology, 422: 235–241.

Brownell, M.J.; Harwood, V.J.; Kurz, R.C.; McQuaig, S.M.; Lukasik, J. and Scott, T.M. (2007). Confirmation of putative stormwater impact on water quality at a Florida beach by microbial source tracking methods and structure of indicator organism populations. Water Res, 41: 3747–3757.

Carducci, A.; Gemelli, C.; Cantiani, L.; Casini, B. and Rovini, E. (1999). Assessment of Microbial Parameters as Indicators of Viral Contamination of Aerosol From Urban Sewage Treatment Plants. Letters in Applied Microbiology, 28: 207-210.

Chapron, C.D.; Ballester, N.A.; Fontaine, J.H.; Frades, C.N. and Margolin, A.B. (2000). Detection of Astroviruses, Enteroviruses, and Adenovirus types 40 and 41 in surface water collected and evaluated by the information collection rule and an integrated cell culture-nested PCR procedure. Appl Environ Microbiol, 66: 2520–2525.

Chen, H.L.; Yang, H.; Chen, Y.J.; Wang, D.L.; Shu, B.H. and He, Y. (2012). Environmental surveillance of human enterovirus in rivers and wastewater in Shenzhen. Chin. J Health Lab Tec, 12: 2948–2950.

Cho, H.B.; Lee, S.H.; Cho, J.C. and Kim, S.J. (2000). Detection of adenoviruses and enteroviruses in tap water and river water by reverse transcription multiplex PCR. Can J Microbiol, 46: 417–424.

Cole, D.; Long, S.C. and Sobsey, M.D. (2003). Evaluation of F+ RNA and DNA Coliphages as Source-Specific Indicators of Fecal Contamination in Surface Waters. Appl Environ Microbiol, 69: 6507-6518.

Colford, J.M.; Roy, S.; Beach, M.J.; Hightower, A.; Shaw, S.E. and Wade, T.J. (2006). A review of household drinking water intervention trials and an approach to the estimation of endemic waterborne gastroenteritis in the United States. J Water Health, 4: 71–88.

Colson, P.; Richet, H.; Desnues, C.; Balique, F.; Moal, V.; Grob, J.; Berbis, P.; Lecoq, H.; Harlé, J.R.; Berland, Y. and Raoult, D. (2010). Pepper mild mottle virus, a plant virus associated with specific immune responses, fever, abdominal pains, and pruritus in humans. PLoS One, 5: 1-12.

Costán-Longares, A.; Montemayor, M.; Payán, A.; Méndez, J.; Jofre, J.; Mujeriego, R. et al. (2008). Microbial indicators and pathogens: removal, relationships and predictive capabilities in water reclamation facilities. Water Res, 42: 4439–4448.

Cui, Y.; Liu, Y. and Tian, Z. (2015). Comparative review of microbial indexes of Chinese and foreign water quality standards. J Food Saf Qual, 7: 105–116.

de Paula, V.S.; Diniz- Mendes, L.; Villar, L.M.; Luz, S.L.; Silva, L.A.; Jesus, M.S.; Da Silva, N.M and Gaspar, A.M. (2007). Hepatitis A virus in environmental water samples from the Amazon Basin. Water Res, 41: 1169– 1176.

Desai, S.M.; Muerhoff, A.S.; Leary, T.P.; Erker, J.C.; Simons, J.N.; Chalmers, M.L.; Birkenmeyer, L.G.; Pilot-Matias, T.J. and Mushahwar, I.K. (1999). Prevalence of TT virus infection in US blood donors and populations at risk for acquiring parenterally transmitted viruses. J Infect Dis, 179: 1242–1244.

Diniz-Mendes, L.; Paula, V.S.; Luz, S.L. and Niel, C. (2008). High prevalence of human Torque teno virus in streams crossing the city of Manaus, Brazilian Amazon. J Appl Microbiol, 105: 51-58.

Dongdem, J.T.; Soyiri, I. and Ocloo, A. (2009). Public health significance of viral contamination of drinking water. Afr J Microbiol, 3: 856–861.

Donia, D.; Bonanni, E.; Diaco, L. and Divizia, M. (2010). Statistical correlation between enterovirus genome copy numbers and infectious viral particles in wastewater samples. Lett Appl Microbiol, 50: 237–240.

Dutilh, B.E.; Cassman, N.; McNair, K.; Sanchez, S.E.; Silva, G.G.Z.; Boling, L.; Barr, J.J.; Speth, D.R.; Seguritan, V.; Aziz, R.K.; Felts, B.; Dinsdale, E.A.; Mokili, J.L. and Edwards, R.A. (2014). A highly abundant bacteriophage discovered in the unknown sequences of human faecal metagenomes. Nature Communications, 5: 4498-4502.

[DWAF] Department of Water Affairs and Forestry. (1996a). South African water quality guidelines – recreational use, 2nd ed. Pretoria (SA): Department of Water Affairs and Forestry, pp. 345-363.

[DWAF] Department of Water Affairs and Forestry. (2004a). National water resource strategy (NWRS), 1st ed. Pretoria (SA): Department of Water Affairs and Forestry, pp. 288-311.

[DWAF] Department of Water Affairs and Forestry. (2004b). Water quality management series subseries no. MS 13.3. Operational policy for the disposal of land-derived water containing waste to the marine environment of South Africa – guidance on implementation, 1st ed. Pretoria (SA): Department of Water Affairs and Forestry, pp. 412-433.

Edberg, S.C.; Rice, E.W.; Karlin, R.J. and Allen, M.J. (2000). Escherichia coli: the best biological drinking water indicator for public health protection. J Appl Microbiol, 105: 106–116.

Elizabeth, M.L.; Scott, A.; Kenneth, D.S. and Pamela, D.R. (2001). Pepper Mild Mottle Virus. University of Florida Institute of Food and Agricultural Sciences, Gainesville, pp. 154-171.

El-Senousy, W. M. and Abou-Elela, S. I. (2017). Assessment and evaluation of an integrated hybrid anaerobic–aerobic sewage treatment system for the removal of enteric viruses. Food and Environmental Virology, 9(3): 287–303.

El-Senousy, W.M.; Abu Senna, A.S.M.; Mohsen, N.A.; Hasan, S.F. and Sidkey, N.M. (2020). Clinical and environmental surveillance of rotavirus common genotypes showed high prevalence of common P genotypes in Egypt. Food and Environmental Virology, 12(2): 99–117.

El-Senousy, W. M.; Barakat, A. B.; Ghanem, H. E. and Kamel, M. A. (2013a). Molecular epidemiology of human adenoviruses and rotaviruses as candidate viral indicators in the Egyptian sewage and water samples. World Applied Sciences Journal, 27(10): 1235–1247.

El-Senousy, W. M.; Costafreda, M. I.; Pintó, R. M. and Bosch, A. (2013b). Method validation for norovirus detection in naturally contaminated irrigation water and fresh produce. International Journal of Food Microbiology, 167: 74–79.

El-Senousy, W. M.; Osman, G. A. and Melegy, A. A. (2014). Survival of adenovirus, rotavirus, hepatitis A virus, pathogenic bacteria and bacterial indicators in ground water. World Applied Sciences Journal, 29(3): 337–348.

El-Senousy, W. M.; Pintó, R. M. and Bosch, A. (2004). Epidemiology of human enteric viruses in the Cairo water environment. Paper presented at the 1st International Conference of Environmental Research Division on Sustainable Development Environmental Challenges Facing Egypt. National Research Centre, Cairo, Egypt, pp. 63-68.

El-Senousy, W. M.; Ragab, A. M. E. and Handak, E. M. A. (2013c). Rotaviruses group A and C in clinical samples. The New Egyptian Journal of Medicine, 49(1): 1–6.

El-Senousy, W. M.; Ragab, A. M. E. and Handak, E. M. A. (2015). Prevalence of rotaviruses groups A and C in Egyptian children and aquatic environment. Food and Environmental Virology, 7(2): 132–141.

Emerson, S.U. et al. Hepevirus. In: Fauquet, C.M. et al. (2005). Virus taxonomy, VIIth report of the ICTV. San Diego: Elsevier Academic Press; 853 pp.

Environmental Science and Research Limited (2008). A Guide to the Ministry of Health Drinking-Water Standards for New Zealand; Environmental Science and Research Limited: Wellington, New Zealand, 43 pp.

Espinosa, A.C.; Arias, C.F.; Sánchez-Colón, S. and Mazari-Hiriart, M. (2009). Comparative study of enteric viruses, coliphages and indicator bacteria for evaluating water quality in a tropical high-altitude system. Environ Health, 8: 49-52.

Estes, M.K.; Prasad, B.V. and Atmar, R.L. (2006). Noroviruses everywhere: has something changed? Curr Opin Infect Dis, 19(5): 467–474.

Ettayebi, K.; Crawford, S.E.; Murakami, K.; Broughman, J.R.; Karandikar, U.; Tenge, V.R.; Neill, F.H.; Blutt, S.E.; Zeng, X.L.; Qu, L.; Kou, B.; Opekun, A.R.; Burrin, D.; Graham, D.Y.; Ramani, S.; Atmar, R.L. and Estes, M.K. (2016). Replication of human noroviruses in stem cell-derived human enteroids. Science, 353: 1387-1393.

European Communities (2006). Common implementation strategy for the water framework directive(2006/60/EC). Off J Eur Communities, pp 24-41.

European Communities Council (1998). directive 98/83/EC of 3 November on the quality of water intended for human consumption. Off J Eur Communities, pp 32–54.

European Communities Council (2014). directive 2014/101/EU of 30 October amending Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for Community action in the field of water policy. Off J Eur Communities, pp 32–35.

Farkas, K.; Walker, D.I.; Adriaenssens, E.M.; McDonald, J.E.; Hillary, L.S.; Malham, S.K. and Jones, D.L. (2020). Viral indicators for tracking domestic wastewater contamination in the aquatic environment. Water Res, 181: 1-20.

Fauquet, C.M.; Mayo, M.A.; Maniloff, J.; Desselberger, U. and Ball, L.A. (2005). Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, Amsterdam, The Netherlands, pp. 344-383.

Flomenberg, P. (2005). Adenovirus infections. Medicine, 33: 128–130.

Fong, T.T. and Lipp, E.K. (2005). Enteric viruses of humans and animals in aquatic environments: healthrisks, detection, and potential water quality assessment tools. Microbiol Mol Biol Rev, 69: 357–371.

Fong, T.T.; Phanikumar, M.S.; Xagorraki, I. and Rose, J.B. (2010). Quantitative detection of human adenoviruses in wastewater and combined sewer overflows influencing a Michigan river. Appl Environ Microbiol, 76: 715–723.

Fongaro, G.; Nascimento, M.A.; Rigotto, C.; Ritterbusch, G.; da Silva, A.; Esteves, P. and Barardi, C. R. M. (2013). Evaluation and molecular characterization of human adenovirus in drinking water supplies: viral integrity and viability assays. Virol J, 10: 166-174.

Furuse, K. (1987). Distribution of Coliphages in the Environment: General Considerations. Phage Ecology (S.M. Goyal, C.P. Gerba, & G. Bitton, editors). Wiley Interscience, New York, pp. 77-96.

Gantzer, C.; Maul, A.; Audic, J.M. and Schwartzbrod, L. (1998). Detection of infectious enteroviruses, enterovirus genomes, somatic coliphages, and Bacteroides fragilis phages in treated wastewater. Appl Environ Microbiol, 64: 4307–4312.

Gerba, C.P. and Rose J.B. (1990). Viruses in source and drinking water. In: McFeters GA, editor. Drinking-water microbiology: progress and recent developments. New York (NY): Springer-Verlag; pp. 380–396.

Girones, R. (2005). Nested multiplex PCR assay for detection of human enteric viruses in shellfish and sewage. J Virol Methods, 125: 111–118.

Grabow, W.O.K. (1996). Waterborne diseases: Update on water quality assessment and control. Water SA, 22: 193–202.

Grabow W.O.K. (2001). Bacteriophages: update on application as models for viruses in water. Water SA, 27: 251–268.

Griffiths, P. (1999). Time to consider the concept of a commensal virus? Rev Med Virol, 9: 73-74.

Gruber, J.S.; Ercumen, A. and Colford, J.M. (2014). Coliform Bacteria as Indicators of Diarrheal Risk in Household Drinking Water: Systematic Review and Meta-Analysis. PLoS ONE, 9: 429-440.

Hamza, I.A.; Jurzik, L.; Stang, A.; Sure, K.; Uberla, K. and Wilhelm, M. (2009). Detection of human viruses in rivers of a densely-populated area in Germany using a virus adsorption elution method optimized for PCR analyses. Water Res, 43: 2657–2668.

Hamza, I. A.; Jurzik, L.; Überla, K. and Wilhelm, M. (2011). Evaluation of pepper mild mottle virus, human picobirnavirus and Torque teno virus as indicators of fecal contamination in river water. Water Res, 45: 1358–1368.

Haramoto, E.; Katayama, H.; Oguma, K.; Yamashita, H.; Nakajima, E. et al. (2005). One-year monthly monitoring of Torque teno virus (TTV) in wastewater treatment plants in Japan. Water Res, 39: 2008-2013.

Haramoto, E.; Katayama, H.; Oguma, K.; Yamashita, H.; Tajima, A.; Nakajima, H. and Ohgaki, S. (2006). Seasonal profiles of human noroviruses and indicator bacteria in a wastewater treatment plant in Tokyo, Japan. Water Sci Technol, 54: 301-308.

Harwood, V. J.; Levine, A. D.; Scott, T. M.; Chivukula, V.; Lukasik, J.; Farrah, S. R. et al. (2005). Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. Appl Environ Microbiol, 71: 3163-3173,

Hauri, A.M.; Schimmelpfennig, M.; Walter-Domes, M.; Letz, A.; Diedrich, S.; Lopez-Pila, J. and Schreier, E. (2005). An outbreak of viral meningitis associated with a public swimming pond. Epidemiol Infect, 133: 291–298.

Hewitt, J.; Bell, D.; Simmons, G.C.; Rivera-Aban, M.; Wolf, S. and Greening, G.E. (2007). Gastroenteritis outbreak caused by waterborne norovirus at a New Zealand ski resort. Appl Environ Microbiol, 73: 7853–7857.

Hewitt, J.; Greening, G.E.; Leonard, M. and Lewis, G.D. (2013). Evaluation of human adenovirus and human polyomavirus as indicators of human sewage contamination in the aquatic environment. Water Res, 47: 6750-6761.

Hot, D.; Legeay, O.; Jacques, J.; Gantzer, C.; Caudrelier, Y.; Guyard, K.; Lange, M. and Andreoletti, L. (2003). Detection of somatic phages, infectious enteroviruses and enterovirus genomes as indicators of human enteric viral pollution in surface water. Water Res, 37: 4703–4710.

Hundesa, A.; Maluquer de Motes, C.; Bofill-Mas, S.; Albinana-Gimenez, N. and Girones, R. (2006). Identification of human and animal adenoviruses and polyomaviruses for determination of sources of fecal contamination in the environment. Appl Environ Microbiol, 72: 7886–7893.

ICTVdB Management (2006). Polyomaviridae, entry 00.047. In: Buchen- Osmond C, editor. ICTVdB: the universal virus database, version 4. New York (NY): Columbia University, pp. 73-91.

Jiang, S.C. (2002). Adenovirus as an index of human viral contamination. US EPA Workshop on Microbial Source Tracking; 5 February; Irvine, pp. 75–78.

Jiang, S.C. and Chu, W. (2004). PCR detection of pathogenic viruses in southern California urban rivers. J Appl Microbiol, 97: 17–28.

Jiang, S.C.; Chu, W. and He, J.W. (2007). Seasonal Detection of Human Viruses and Coliphage in Newport Bay, California. Appl Environ Microbiol, 73: 6468-6474.

Jiang, S.; Noble, R. and Chu, W. (2001). Human adenoviruses and coliphages in urban runoff-impacted coastal waters of southern California. Appl Environ Microbiol, 67: 179–184.

Johne, R.; Buck, C.B.; Allander, T.; Atwood, W.J.; Garcea, R.L.; Imperiale, M.J.; Major, E.O.; Ramqvist, T. and Norkin, L.C. (2011). Taxonomical developments in the family Polyomaviridae. Arch Virol, 156: 1627–1634.

Johne, R.; Enderlein, D.; Nieper, H. and Muller, H. (2005). Novel polyomavirus detected in the faeces of a chimpanzee by nested broad-spectrum PCR. J Virol 79: 3883–3887.

Jurzik, L.; Hamza, I.A.; Puchert, W.; Überla, K. and Wilhelm, M. (2010). Chemical and microbiological parameters as possible indicators for human enteric viruses in surface water. Int J Hyg Environ Health, 213: 210–216.

Katayama, H.; Haramoto, E.; Oguma, K.; Yamashita, H.; Tajima, A.; Nakajima, H. and Ohgaki, S. (2008). One-year monthly quantitative survey of noroviruses, enteroviruses, and adenoviruses in wastewater collected from six plants in Japan. Water Res, 42: 1441–1448.

Kean, J.M.; Rao, S.; Wang, M. and Garcea, R.L. (2009). Seroepidemiology of human polyomaviruses. PLoS Pathog, 5: 363-368.

King, A.M.Q.; Adams, M.J.; Carstens, E.B. and Lefkowitz, E.J. (2009). Virus taxinomy: classification and nomenclature of viruses. King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), Ninth Report of the International Committee on Taxonomy of Viruses, Elsevier Academic Press, pp. 202-234.

Kluge, M.; Fleck, J.D.; Soliman, M.C.; Luz, R.B.; Fabres, R.B.; Comerlato, J.; Silva, J.V.S.; Staggemeier, R.; Vecchia, A.D.; Capalonga, R.; Oliveira, A.B.; Henzel, A.; Rigotto, C. and Spilki, F.R. (2014). Human adenovirus (HAdV), human enterovirus (HEV), and genogroup a rotavirus (GARV) in tap water in southern Brazil. J Water Health, 12: 526–532.

Kokkinos, P.A; Ziros, P.G.; Mpalasopoulou, A.; Galanis, A. and Vantarakis, A. (2011). Molecular detection of multiple viral targets in untreated urban sewage from Greece. Virol J, 8: 195-199.

Kopecka, H.; Dubrou, S.; Prevot, J.; Marechal, J. and Lopez-Pila, J.M. (1993). Detection of naturally occurring enteroviruses in waters by reverse transcription, polymerase chain reaction, and hybridization. Appl Environ Microbiol, 59: 1213–1219.

Kornacki, J.L. and Johnson, J.L. (2001). Enterobacteriaceae, coliforms and Escherichia coli as quality and safety indicators. In: Downs F, editor. Compendium of methods for the microbiological examination of foods. Washington (DC): APHA; pp. 69–82.

Kuroda, K.; Nakada, N.; Hanamoto, S.; Inaba, M.; Katayama, H.; Do, A.T.; Nga, T.T.V.; Oguma, K.; Hayashi, T. and Takizawa, S. (2015). Pepper mild mottle virus as an indicator and a tracer of fecal pollution in water environments: Comparative evaluation with wastewater-tracer pharmaceuticals in Hanoi, Vietnam. *Sci Total Environ*, 506–507, 287–298.

Lamontagne, B.; Girard, N.; Boucher, A. and Labbé, A.C. (2011). Improved detection and quantitation of human BK polyomavirus by PCR assay. *J Clin Microbiol*, 49, 2778–2779.

La Rosa, G.; Fratini, M.; della Libera, S.; Iaconelli, M. and Muscillo, M. (2012). Emerging and potentially emerging viruses in water environments. *Ann Ist Super Sanità*, 48(4), 397-406.

Leary, T.P.; Erker, J.C.; Chalmers, M.L.; Desai, S.M. and Mushahwar, I.K. (1999). Improved detection systems for TT virus reveal high prevalence in humans, non-human primates and farm animals. *J Gen Virol*, 80, 2115-2120.

Lee, H.S. and Sobsey, M.D. (2011). Survival of Prototype Strains of Somatic Coliphage Families in Environmental Waters and When Exposed to UV Low- Pressure Monochromatic Radiation or Heat. *Water Res*, 45, 3723-3729.

Li, D.; He, M. and Jiang, S.C. (2010). Detection of infectious adenoviruses in environmental waters by fluorescence-activated cell sorting assay. *Appl Environ Microbiol*, 76, 1442–1448.

Liang, J.L.; Dziuban, E.J.; Craun, G.F.; Hill, V.; Moore, M.R.; Gelting, R.J.; Calderon, R.L.; Beach, M.J. and Roy, S.L. (2006). Centers for disease control and prevention (CDC): surveillance for waterborne disease and outbreaks associated with drinking water and water not intended for drinking–United States, 2003–2004. *MMWR Surveillance Summary*, 55, 31–65.

Lin, J. and Ganesh, A. (2013). Water quality indicators, bacteria, coliphage, enteric viruses. *International Journal of Environmental Health Research*, 3, 1-23.

Long, S.C. and Dewar, K.G. (2008). Coliform and Coliphage Monitoring for Groundwater Wells in Massachusetts. *Journal of the New England Water Works Association*, 122, 1:12.

Lukasik, J.; Scott, T.M.; Andryshak, D. and Farrah, S.R. (2000). Influence of salts on virus adsorption to microporous filters. *Appl Environ Microbiol,* 66, 2914–2920.

Luo, K.; He, H.; Liu, Z.; Liu, D.; Xiao, H.; Jiang, X.; Liang, W. and Zhang, L. (2002). Novel variants related to TT virus distributed widely in China. *J Med Virol,* 67, 118-126.

Maunula, L.; Klemola, P.; Kauppinen, A.; Soderberg, K.; Nguyen, T.; Pitkanen, T.; Kaijalainen, S.; Simonen, M.L.; Miettinen, I.T.; Lappalainen, M. et al. (2009). Enteric viruses in a large waterborne outbreak of acute gastroenteritis in Finland. *Food and Environmental Virology,* 1, 31–36.

McMinn, B.R.; Ashbolt, N.J. and Korajkic, A. (2017). Bacteriophages as indicators of faecal pollution and enteric virus removal. *Lett Appl Microbiol,* 65, 11-26.

McQuaig, S.M.; Scott, T.M.; Harwood, V.J.; Farrah, S.R. and Lukasik, J.O. (2006). Detection of human derived fecal pollution in environmental waters using a PCR-based human polyomavirus assay. *Appl Environ Microbiol,* 72, 7567–7574.

McQuaig, S.M.; Scott, T.M.; Lukasik, J.O.; Paul, J.H. and Harwood, V.J. (2009). Quantification of human polyomaviruses JC virus and BK virus by TaqMan quantitative PCR and comparison to other water quality indicators in water and fecal samples. *Appl Environ Microbiol,* 75, 3379–3388.

Meays, C.L.; Broersma, K.; Nordin, R. and Mazumder, A. (2004). Source tracking fecal bacteria in water: A criticalreview of current methods. *J Environ Manag,* 73, 71–79.

Medema, G.J.; Payment, P.; Dufour, A.; Robertson, W.; Waite, M.; Hunter, P.; Kirby, R. and Andersson, Y. (2003). Safe drinking water: an ongoing challenge. In Assessing microbial safety of drinking water. Improving approaches and methods OCDE, WHO. Cornwall: IWA; p. 12–47.

Meng, Q.S. and Gerba C.P. (1996). Comparative inactivation of enteric adenoviruses, polioviruses and coliphages by ultraviolet irradiation. *Water Res,* 30, 2665–2668.

Metcalf, T.G.; Melnick, J.L. and Estes, M.K. (1995). Environmental virology: from detection of virus in sewage and water by isolation to identificaton by molecular biology. A trip of over 50 years. *Annual Reviews in Microbiology,* 49, 461–487.

Miagostovich, P.M.; Ferreira, F.F.M.; Guimaraes, F.R.; Fumian, T.M.; Diniz-Mendes, L.; Luz, S.L.B.; Silva, L.A. and Leite, J.P.G. (2008). Molecular detection and characterization of gastroenteritis viruses occurring naturally in stream waters of Manaus, Central Amazonia, Brazil. *Appl Environ Microbiol,* 74, 375–382.

Miagostovich, M.P.; Rocha, M.S.; dos Reis, F.B.; Sampaio, M.S.; Carrijo, R.S.; Malta, F.C.; Rodrigues, J.; Genuino, A.; da Silva-Assis, M.R.; Fumian, T.M. and Barrocas, P.R. (2020). Gastroenteric Viruses Detection in a Drinking Water Distribution-to-Consumption System in a Low-Income Community in Rio de Janeiro. *Food and Environmental Virology,* 12, 130–136.

Miyata, H.; Tsunoda, H.; Kazi, A.; Yamada, A.; Khan, M.A.; Murakami, J.; Kamahora, T.; Shiraki, K. and Hino, S. (1999). Identification of a novel GC-rich 113-

nucleotide region to complete the circular, singlestranded DNA genome of TT virus, the first human circovirus. *J Virol*, 73, 3582-3586.

National Health and Medical Research Council (2017). Australian Drinking Water Guidelines 6 National Water Quality Management Strategy; National Health and Medical Research Council: Canberra, Australia, pp. 38-62.

Nishizawa, T.; Okamoto, H.; Konishi, K.; Yoshizawa, H.; Miyakawa, Y. and Mayumi, M. (1997). A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. *Biochem Biophys Res Commun*, 241, 92-97.

Nishizawa, T.; Okamoto, H.; Tsuda, F.; Aikawa, T.; Sugai, Y.; Konishi, K.; Akahane, Y.; Ukita, M.; Tanaka, T.; Miyakawa, T. and Mayumi, M. (1999). Quasispecies of TT virus (TTV) with sequence divergence in hypervariable regions of the capsid protein in chronic TTV infection. *J Virol*, 73, 9604-9608.

Nordgren, J.; Matussek, A.; Mattsson, A.; Svensson, L. and Lindgren, P.E. (2009). Prevalence of norovirus and factors influencing virus concentrations during one year in a full- scale wastewater treatment plant. *Water Res*, 43, 1117– 1125.

Ogorzaly, L.; Tissier, A.; Bertrand, I.; Maul, A. and Gantzer, C. (2009). Relationship between F-specific RNA phage genogroups, faecal pollution indicators and human adenoviruses in river water. *Water Res*, 43, 1257–1264.

Okamoto, H.; Nishizawa, T.; Kato, N.; Ukita, M.; Ikeda, H.; Iizuka, H.; Miyakawa, Y. and Mayumi, M. (1998). Molecular cloning and characterization of a novel DNA virus (TTV) associated with posttransfusion hepatitis of unknown etiology. *Hepatol Res*, 10, 1-16.

Okamoto, H.; Nishizawa, T.; Takahashi, M. et al. (2001) Genomic and evolutionary characterization of TT virus (TTV) in tupaias and comparison with species-specific TTVs in humans and non-human primates. *J Gen Virol*, 82, 2041–2050.

Okamoto, H.; Nishizawa, T.; Tawara, A.; Peng, Y.; Takahashi, M. et al. (2000). Speciesspecific TT viruses in humans and nonhuman primates and their phylogenetic relatedness. *Virology*, 277, 368-378.

Okoh, A.I.; Sibanda, T. and Gusha, S.S. (2010). Inadequately treated wastewater as a source of human enteric viruses in the environment. *Int J Environ Res Public Health*, 7, 2620–2637.

Payment, P.; Berte, A.; Prevost, M.; Menard, B. and Barbeau, B. (2000). Occurrence of pathogenic microorganisms in the Saint Lawrence River (Canada) and comparison of health risks for populations using it as their source of drinking water. *Can J Microbiol*, 46, 565–576.

Payment, P. and Locas, A. (2011). Pathogens in Water: Value and Limits of Correlation with Microbial Indicators. *Ground Water*, 49, 1-4.

Pina, S.; Puig, M.; Lucena, F.; Jofre, J. and Girones R. (1998). Viral pollution in the environment and in shellfish: human adenovirus detection by PCR as an index of human viruses. *Appl Environ Microbiol*, 64, 3376–3382.

Pollicino, T.; Raffa, G.; Squadrito, G.; Costantino, L.; Cacciola, I.; Brancatelli, S.; Alafaci, C.; Florio, M.G. and Raimondo, G. (2003). TT virus has ubiquitous diffusion in human body tissues: analyses of paired serum and tissue samples. *Journal of Viral Hepatitis*, 10, 95-102.

Polo, C.; Perez, J.L.; Mielnichuck, A.; Fedele, C.G.; Niubo, J. and Tenorio, A. (2004). Prevalence and patterns of polyomavirus urinary excretion in immunocompetent adults and children. *J Clin Microbiol Infect*, 10, 640–644.

Prevost, B.; Goulet, M.; Lucas, F.S.; Joyeux, M.; Moulin, L. and Wurtzer, S. (2016). Viral persistence in surface and drinking water: suitability of PCR pre-treatment with intercalating dyes. *Water Res*, 91, 68–76.

Puig, M.; Jofre, J.; Lucena, F.; Allard, A.; Wadell, G. and Girones, R. (1994). Detection of adenoviruses and enteroviruses in polluted waters by nested PCR amplification. *Appl Environ Microbiol*, 60, 2963–2970.

Pusch, D.; Oh, D.Y.; Wolf, S.; Dumke, R.; Schröter-Bobsin, U.; Höhne, M.; Röske, I. and Schreier, E. (2005). Detection of enteric viruses and bacterial indicators in German environmental waters. *Arch Virol*, 150, 929–947.

Rachmadi, A.T.; Torrey, J.R. and Kitajima, M. (2016). Human polyomavirus: advantages and lim-itations as a human-specific viral marker in aquatic environments. *Water Res*, 105, 456-469.

Rames, E.; Roiko, A.; Stratton, H. and Macdonald, J. (2016). Technical aspects of using human adenovirus as a viral water quality indicator. Water Res, 96, 308–326.

Rodríguez-Lázaro, D.; Cook, N.; Ruggeri, F.M.; Sellwood, J.; Nasser, A.; Nascimento, M.S.; D'Agostino, M.; Santos, R.; Saiz, J.C.; Rzeżutka, A.; Bosch, A.; Gironés, R.; Carducci, A.; Muscillo, M.; Kovač, K.; Diez-Valcarce, M.; Vantarakis, A.; von Bonsdorff, C.H.; de Roda Husman, A. M.; Hernández, M. and van der Poel, W.H. (2012). Virus hazards from food, water and other contaminated environments. *FEMS Microbiology Reviews*, 36, 786–814.

Rosario, K.; Nilsson, C.; Lim, Y.W.; Ruan, Y. and Breitbart, M. (2009). Metagenomic analysis of viruses in reclaimed water. *Environ Microbiol*, 11, 2806–2820.

Rosiles-González, G.; Ávila-Torres, G.; Moreno-Valenzuela, O.A.; Acosta-González, G.; Leal-Bautista, R.M.; Grimaldo-Hernández, C.D.; Brown, J.K.; Chaidez-Quiroz, C.; Betancourt, W.Q.; Gerba, C.P. and Hernández-Zepeda, C. (2017). Occurrence of pepper mild mottle virus (PMMoV) in groundwater from a karst aquifer system in the Yucatan Peninsula, Mexico. *Food and Environmental Virology*, 9, 487–497.

Ross, R.S.; Viazov, S.; Runde, V.; Schaefer, U.W. and Roggendorf, M. (1999). Detection of TT virus DNA in specimens other than blood. *J Clin Virol,* 13, 181-184.

Schroedera, J.A.; Krämerb, B.K. and Hofstaedter, F. (2003). Non-invasive electron microscopic rapid virus diagnosis of negative-stained urine samples can be useful in the diagnosis and monitoring of polyomavirus infections in renal transplant recipients. *Microsc Microanal,* 9(3), 520–521.

Sedmak, G.; Bina, D. and MacDonald, J. (2003). Assessment of an enterovirus sewage surveillance system by comparison of clinical isolates with sewage isolates from milwaukee, wisconsin, collected August 1994 to December 2002. *Appl Environ Microbiol,* 69, 7181– 7187.

Shkoporov, A.N.; Khokhlova, E.V.; Fitzgerald, C.B.; Stockdale, S.R.; Draper, L.A.; Ross, P. and Hill, C. (2018). "ΦCrAss001 represents the most abundant bacteriophage family in the human gut and infects Bacteroides intestinalis". *Nature Communications,* 9 (1), 4781-4789.

Shrestha, S.; Shrestha, S.; Shindo, J.; Sherchand, J. B. and Haramoto, E. (2018). Virological quality of irrigation water sources and pepper mild mottle virus and tobacco mosaic virus as index of pathogenic virus contamination level. *Food and Environmental Virology,* 10, 107–120.

Simmonds, P.; Prescott, L.E.; Logue, C.; Davidson, F.; Thomas, A.E. et al. (1999). TT virus--part of the normal human flora? *J Infect Dis,* 180, 1748-1750.

Skraber, S.; Gassilloud, B. and Gantzer, C. (2004a). Comparison of coliforms and coliphages as tools for assessment of viral contamination in river water. *Appl Environ Microbiol,* 70, 3644–3649.

Skraber, S.; Gassilloud, B.; Schwartzbrod, L. and Gantzer, C. (2004b). Survival of infectious Poliovirus-1 in river water compared to the persistence of somatic coliphages, thermotolerant coliforms and Poliovirus-1 genome. *Water Res,* 38, 2927–2933.

Spilki, F.R.; da Luz, R.B.; Fabres, R.B.; Soliman, M.C.; Kluge, M.; Fleck, J.D.; Rodrigues, M.T.; Comerlato, J.; Cenci, A.; Cerva, C. et al. (2013). Detection of Human Adenovirus, Rotavirus and Enterovirus in Water Samples Collected on Dairy Farms from Tenente Portela, Northwest of Rio Grande Do Sul, Brazil. *Braz J Microbiol,* 44, 953–957.

Springfeld, C.; Bugert, J.J.; Schnitzler, P.; Tobiasch, E.; Kehm, R. and Darai, G. (2000). TT virus as a human pathogen: significance and problems. *Virus Genes,* 20, 35-45.

Stevens, M.; Ashbolt, N. and Cunliffe, D. (2001). Microbial indicators of water quality. *NHMRC,* 411-420.

Stewart-Pullaro, J.; Daugomah, J.W.; Chestnut, D.E.; Graves, D.A.; Sobsey, M.D. and Scott, G.I. (2006). F+ RNA Coliphage Typing for Microbial Source Tracking in Surface Waters. *Journal of Applied Microbiology,* 101, 1015-1021.

Stewart, P.L.; Fuller, S.D. and Burnett, R.M. (1993). Difference imaging of adenovirus: bridging the resolution gap between X-ray crystallography and electron microscopy. *EMBO,* 12, 2589–2599.

Straub, T. M.; Honer zu Bentrup, K.; Orosz-Coghlan, P.; Dohnalkova, A.; Mayer, B. K.; Bartholomew, R. A. et al. (2007). In vitro cell culture infectivity assay for human noroviruses. *Emerging Infectious Diseases,* 13, 396–403.

Symonds, E.; Griffin, D. and Breitbart, M. (2009). Eukaryotic viruses in wastewater samples from the United States. *Appl Environ Microbiol,* 75, 1402–1409.

Symonds, E.; Sinigalliano, C.; Gidley, M.L.; Ahmed, W.; McQuaig, S. and Breitbart, M. (2016). Faecal pollution along the southeastern coast of Florida and insight into the use of pepper mild mottle virus as an indicator. *J Appl Microbiol,* 121, 1469–1481.

Tani, N.; Dohi, Y.; Kurumatani, N. and Yonemasu, K. (1995). Seasonal distribution of adenoviruses, enteroviruses and reoviruses in urban river water. *Microbiol Immunol,* 39, 577–580.

The European Commission (2008). The Quality of Drinking Water in the European Union, Available online: https://circabc.europa.eu/sd/a/58220131-ecc4-49f9-9ad1-bbb6e9e79578/report1999-2001.pdf (accessed on 6 December 2019), pp. 113-137.

Vaidya, S.R.; Chitambar, S.D. and Arankalle, V.A. (2002). Polymerase chain reactionbased prevalence of hepatitis A, hepatitis E and TT viruses in sewage from an endemic area. *J Hepatol,* 37, 131-136.

Van Heerden, J.; Ehlers, M.M.; van Zyl, W.B. and Grabow, W.O.K. (2003). Incidence of adenoviruses in raw and treated water. *Water Res,* 37, 3704–3708.

Van Heerden, J.; Ehlers, M.M.; van Zyl, W.B. and Grabow, W.O.K. (2004). Prevalence of human adenoviruses in raw and treated water. *Water Sci Technol,* 50, 39–43.

Van Heerden, J.; Ehlers, M.M.; Vivier, J.C. and Grabow, W.O.K. (2005). Risk assessment of adenoviruses detected in treated drinking water and recreational water. *J Appl Microbiol,* 99, 926–933.

Vantarakis, A. and Papapetropoulou, M. (1999). Detection of enteroviruses, adenoviruses and hepatitis A viruses in raw sewage and treated effluents by nested-PCR. *Water Air Soil Pollut,* 114, 85–93.

Verani, M.; Casini, B.; Battistini, R.; Pizzi, F.; Rovini, E. and Carducci, A. (2006). One- year monthly monitoring of Torque teno virus (TTV) in river water in Italy. *Water Sci Technol,* 54, 191– 195.

Villena, C.; El-Senousy, W. M.; Abad, F. X.; Pintó, R. M. and Bosch, A. (2003). Group A rotavirus in sewage samples from Barcelona and Cairo: Emergence of unusual genotypes. *Appl Environ Microbiol,* 69, 3919–3923.

Weitschek, E.; Presti, A.O.; Drovandi, G.; Felici, G.; Ciccozzi, M.; Ciotti, M. and Bertolazzi, P. (2012). Human polyomaviruses identification by logic mining techniques. *Virol J*, 9, 58-65.

Wen, X.; Chen, F.; Lin, Y.; Zhu, H.; Yuan, F.; Kuang, D.; Jia, Z. and Yuan, Z. (2020). "Microbial Indicators and Their Use for Monitoring Drinking Water Quality—A Review. *Sustainability*, *MDPI*, 12(6), 1-14.

[WHO] World Health Organization (1997). Guidelines for drinking water quality, 2nd ed. Volume 3: surveillance and control of community supplies. Geneva: World Health Organization, pp. 51-66.

[WHO] World Health Organization (2011). Guidelines for Drinking-Water Quality, 4th ed.; World Health Organization: Geneva, Switzerland, pp. 171-198.

WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation (2000) Global water supply and sanitation assessment 2000 report. Geneva, World Health Organization, Water Supply and Sanitation Collaborative Council and United Nations Children Fund, pp. 88-109.

Wilkes, G.; Edge, T.; Gannon V; Jokinen, C.; Lyautey, E.; Medeiros, D.; Neumann, N.; Ruecker, N.; Topp, E. and Lapen, D.R. (2009). Seasonal relationships among indicator bacteria, pathogenic bacteria, Cryptosporidium oocysts, Giardia cysts, and hydrological indices for surface waters within an agricultural landscape. *Water Res*, 43, 2209–2223.

Wold, W.S.M. and Ison, M.G. (2013). Adenoviruses, *In* Knipe DM, Howley PM, Cohen JI, Griffin DE, Lamb RA, Martin MA, Racaniello VR, Roizman B. (ed), Fields virology, 6th ed. Lippincott Williams & Wilkins, Philadelphia, PA, pp. 1732–1767.

Wong, K.; Mukherjee, B.; Kahler, A. M.; Zepp, R. and Molina, M. (2012). Influence of inorganic ions on aggregation and adsorption behaviors of human adenovirus. *Environmental Science and Technology*, *46*(20), 11145–11153.

Xu, W.; McDonough, M.C. and Erdman, D.D. (2000). Species-specific identification of human adenoviruses by a multiplex PCR assay. *J Clin Microbiol*, 38, 4114–4120.

Zhang, T.; Breitbart, M.; Lee, W.H.; Run, J.Q.; Wei, C.L.; Soh, S.W.; Hibberd, M.L.; Liu, E.T.; Rohwer, F. and Ruan, Y. (2006). RNA viral community in human feces: prevalence of plant pathogenic viruses. *PLoS Biol*, 4, 3-7.

Zhu, H.; Yuan, F.; Yuan, Z.; Liu, R.; Xie, F.; Huang, L.; Liu, X.; Jiang, X.; Wang, J.; Xu, Y.; Shen, Z.; Liu, D.; Zhang, R. and Lu, Y. (2018). Monitoring of Poyang lake water for sewage contamination using human enteric viruses as an indicator. *Virol J*, 15(3), 624-636.