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Review

Human polyomavirus: Advantages and limitations as a human-specific viral marker in aquatic environments

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ABSTRACT

Human polyomaviruses (HPyVs) cause persistent infections in organs such as kidney, brain, skin, liver, respiratory tract, etc., and some types of HPyV are constantly excreted in the urine and/or feces of infected and healthy individuals. The use of an enteric virus as an indicator for human sewage/waste contamination in aquatic environments has been proposed; 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. In addition, 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, polymerase chain reaction (PCR), integrated cell culture PCR (ICC-PCR), and quantitative PCR) 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. In this paper, we summarize these recent advancements in detection methods and the accumulation of environmental surveillance and laboratory-scale experiment data, and discuss the potential advantages as well as limitations of HPyV as a human-specific viral marker in aquatic environments.

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1. Introduction

Microbial source tracking (MST) is an effort to track the dominant sources of fecal contamination (human or animal) in environmental waters for the purpose of human health risk assessment (Harwood et al., 2014). Until now, *Bacteroides* has been used as a primary target in MST studies, although enteric viruses, which are the primary etiological agent of waterborne disease (Reynolds et al., 2008), should also be considered as targets. The use of *Bacteroides* in MST has recently been called into question because these organisms are also excreted by other warm-blooded animals and are therefore not human specific, and are at times not directly correlated with the presence of human enteric viruses (McQuaig et al., 2009; Harwood et al., 2014; Havelaar et al., 1990). In addition, it has been reported that fecal indicator bacteria (FIB) are capable of multiplying in environmental waters (especially in tropical regions), which is an undesirable feature for indicators of fecal pollution (Winfield and Groisman, 2003; Rochelle-Newall et al., 2015). Recently, researchers have become increasingly aware of the presence of human enteric viruses in the environment, and their use as an indicator of human waste contamination has been frequently discussed. Seasonality precludes the use of some of these viruses as year-round indicators; for example, previous research found that norovirus (NoV), one of the most significant waterborne gastroenteritis viruses, shows clear seasonality, with peak virus concentrations occurring during the winter in wastewater (Kitajima et al., 2012, 2014). On the other hand, some enteric viruses such as adenovirus (AdV), Aichivirus (AiV), and human polyomavirus (HPyV) are detected in all seasons with relatively high abundance in wastewater (influent and effluent) (Kitajima et al., 2014), as well as sewage-impacted rivers and coastal areas (McQuaig et al., 2012); this prevalence, along with the host-specific nature of these viruses makes them suitable tools for MST and they have been proposed as indicators for human enteric virus contamination (Harwood et al., 2005; Kitajima and Gerba, 2015).

HPyVs have recently drawn considerable attention in this research area. HPyVs are members of the genus *Orthopolyomavirus* in the family *Polyomaviridae*, and are non enveloped viruses that have a virion with a diameter of 40–50 nm and a circular, double-stranded DNA genome of approximately 5.13 kb (Johne et al., 2011; Bofill-Mas et al., 2001). HPyVs were first recognized in 1971 when JC virus (JCV or JCPyV) and BK virus (BKV or BKPyV) were isolated from a brain tissue sample and a urine sample, respectively (Gardner et al., 2010; Padgett et al., 1971). In the last decade, several new species of HPyV were identified, including Merkel cell (MC) PyV, KIPyV, and WUPyV, among others; at the time of this review, at least 13 distinct human polyomaviruses has been described (Table 1 and Table S1 in the supplementary material).

Recent environmental studies found that HPyV could be detected in almost all types of environmental water, including wastewater (Calgua et al., 2011; Kitajima et al., 2014; Hamza et al., 2014), coastal seawater (Moresco et al., 2012), stormwater (Sidhu et al., 2012), river water (Calgua et al., 2013; Hamza et al., 2014; Haramoto et al., 2010), and drinking water sources (Albinana-Gimenez et al., 2006); in addition, it has been suggested that the inhalation and/or ingestion of contaminated water may be one of the possible pathways of HPyV infections considering the high environmental occurrence of HPyV (Fratini et al., 2013). On the basis of this background, this review paper summarizes specific detection efforts as well as studies on the occurrence and fate of HPyV in environmental waters; we present recent advancements in concentration/detection methods, the occurrence of HPyVs in various water matrices, and the persistence of HPyVs in both natural environments and through water treatment processes.

2. Human specificity

Among HPyV species identified thus far, BKV, KIPyV, MWPyV, MxPyV, HPyV-6, HPyV-10, HPyV12, and WUPyV have been detected in human stool (Vanchiere et al., 2009; Neske et al., 2009; Yu et al., 2012; Allander et al., 2007) and BKV, JCV, HPyV-9, STLPyV and TSPyV have been detected in human urine, leading to the likely association of many of these viruses with sewage and fecal contamination in general (Table 1 and Table S1 in the supplementary material). Several newly discovered HPyVs (HPyV-7, TSPyV, HPyV-13, and HPyV-10) were also found in human skin, liver, pancreatic and respiratory cells (Table S1 in the supplementary material) although there is currently limited information regarding their presence in water environments. With respect to host age dependency, JCV was detected in human urine sample from all age range of healthy individuals (McQuaig et al., 2009); this is further supported by studies where antigens and DNA from MWPyV and HPyV-7 were found in clinical samples from both children and adults (Berrios et al., 2015; Rockett et al., 2013).

HPyVs in particular have shown high specificity to human excreta when compared to fecal indicator bacteria. JCV and BKV showed higher detection in human contaminated environmental samples with higher specificity than *Bacteroidetes* (HF183) or *Methanobrebacter smithii* (McQuaig et al., 2009). In addition, the specificity of HPyVs to the origin/source of fecal samples was evaluated for a wide variety of potential hosts (i.e., dogs, chickens, ducks, cow, sheep, pigs, birds, and humans); HPyVs (specifically JCV and BKV) were only detected from human sources, further demonstrating their high host specificity (Staley et al., 2012; McQuaig et al., 2009). Similar results were shown by Ahmed et al. (2010) where BKV and JCV markers were detected in domestic/human wastewater (by qPCR) but not detected from 80 farm/animal wastewater and animal fecal samples in Australia. Although BKV and JCV have been confirmed to be human specific, there remains a large data gap regarding the specificity of other newly discovered polyomaviruses; this information will likely play a role in assessing the environmental occurrence and MST potential of these emerging viruses and represents an opportunity for further scientific investigation.

3. Methods for concentration and detection of human polyomaviruses in environmental water

3.1. Concentration methods

Enteric viruses are usually present at a low concentration in

Table 1
Human polyomavirus types and their symptoms, transmission, and environmental occurrence.

Virus	Proposed transmission	Discovery year	Isolated from	Symptoms/Disease	Excretion in		Environmental occurrence	References
					Feces	Urine		
B.K. virus (BKV)	Respiratory and fecal route proposed	1971	Urine from kidney allograft recipient with advanced renal failure	BKPyV-associated nephropathy (BKVAN)	Yes	Yes	+[a]	Bofill-Mas et al., 2010, Fratini et al., 2013, Torres et al., 2016
John Cunningham virus JCV	Respiratory and fecal route proposed	1971	Brain tissue	Respiratory system, kidney, brain infections. Progressive multifocal leukoencephalopathy (PML)	No	Yes	+	Bofill-Mas et al., 2010, Calgua et al., 2011, Torres et al., 2016
Karolinska Institute virus (KI virus)	Respiratory, fecal	2007	Nasoparengeal aspirate and feces of patients	Associated with wheezing and sputum production	Yes	No	+	Bofill-Mas et al., 2010, Torres et al., 2016, Allander et al., 2007
Washington University virus (WU virus)	Respiratory	2007	Nasoparengeal aspirate patient	Associated with wheezing and sputum production	Yes	No	+	Bofill-Mas et al., 2010, Fratini et al., 2013, Torres et al., 2016, Gaynor et al., 2007, Neske et al., 2009, Kuypers et al., 2012
Merkel Cell polyomavirus (MCV)	Fecal oral route proposed	2008	Skin cancer	Associated with Merkel Cell Carcinomas	Yes	Yes	+	Bofill-Mas et al., 2010, Iaconelli et al., 2015, Rusiñol et al., 2015, Torres et al., 2016, Feng et al., 2008, Signorini et al., 2014, Li et al., 2013

[a] +, Detected in the environment.

environmental waters; thus, concentrating virus particles is an essential procedure prior to virus detection with cell culture and/or molecular biological techniques. Typically microorganisms are concentrated from water samples by means of microfiltration (e.g., ranging from 0.1 to 10 μm); however, the smaller size of enteric viruses (~45 nm for HPyV) prevents effective concentration by size exclusion alone in this range (Pinto and Dobson, 2014). Several methods have been developed and applied to concentration of viruses in environmental waters, including **vir**us **ad**sorption and **el**ution (VIRADEL) methods which rely on electrostatic binding of virus to charged microfiltration membranes (pore size of 0.1–10 μm), including electronegative filters (Katayama et al., 2002; Haramoto et al., 2010) (e.g., HA) and electropositive filters such as 1MDS (Karim et al., 2009) and NanoCeram (Tepper and Kaledin, 2006). Other methods such as skim milk flocculation (Calgua et al., 2013), and ultrafiltration (Liang et al., 2015) have also been developed for virus concentration in water samples. Virus concentration methods that have been evaluated for HPyV specific recovery efficiencies are summarized in Table 2.

Electronegative filters such as HA membranes require the binding of multivalent cations to their surface to facilitate the binding of negatively charged virus particles at neutral pH. One commonly used method involves the addition of $MgCl_2$ to freshwater samples prior to filtration to capture virus particles, followed by an acid rinse (0.5 mM H_2SO_4) to remove $MgCl_2$ and finally an alkaline elution (1 mM NaOH) in a smaller volume to recover the virus particles from the filter (Katayama et al., 2002). HA membrane filtration/elution followed by secondary concentration by centrifugal filtration with Centriprep filters was shown to have 62% and >61% average recovery yields for poliovirus spiked in pure water and seawater, respectively, as determined by plaque assay (Katayama et al., 2002). The HPyV specific recovery efficiency of this method was evaluated by Ahmed et al. (2015) under a variety of conditions for tap and river water matrices spiked with raw sewage. In this study, HPyV recovery from HA membranes (0.45 μm pore size, 90 mm diameter) following the same procedure above was lower (6% and 7% on average by qPCR for tap water and river water, respectively) than for poliovirus reported in Katayama et al. (2002). These recoveries were improved slightly to ~7% and 12% (tap water and river water, respectively) when the HA filter was transferred to a 50 mL tube for the final NaOH elution step. In addition, qPCR amplification inhibition was observed for river water samples but not from tap water samples, most likely due to co-concentrated inhibitory organic substances. In the same study, direct filtration of 40 L water samples through HA membranes with sample pre-acidification using HCl (pH 3.5) but no $MgCl_2$ addition followed by direct extraction of viral DNA from the membrane resulted in higher recovery efficiencies of 40% and 78% from tap and river water, respectively; additionally, no PCR inhibition was observed under these concentration conditions (Ahmed et al., 2015). It is unclear to what degree these improvements are due to the omission of $MgCl_2$, the lack of rapid pH changes (3.5–11

Table 2
Virus concentration methods that have been tested for recovery efficiencies of polyomaviruses.

Methods	Key procedure	Matrix	HPyV strain used	Percent recovery [a]	References
HA filter	Direct filtration of acidified sample	Tap water	Sewage-origin HPyV	40%	Ahmed et al., 2015
		River water	Sewage-origin HPyV	78%	
	Filtration with $MgCl_2$ followed by acid rinse and elution by NaOH	Tap water	Sewage-origin HPyV	6%	
		River water	Sewage-origin HPyV	7%	
	Filtration with $MgCl_2$ followed by acid rinse and vortex the filter in NaOH	Tap water	Sewage-origin HPyV	7%	
		River water	Sewage-origin HPyV	12%	
Glass wool column	Filtration of pre-acidified sample through glass wool column with peristaltic pump	River water	JCV Mad 4	4.36%	Albinana-Gimenez et al., 2009a
	Filtration of sample without pre-acidification through glass wool with peristaltic pump	River water	JCV Mad 4	2.82%	Albinana-Gimenez et al., 2009a
Skimmed milk flocculation	Mix the acidified sample with 1% pre-flocculated skimmed milk	River water	JCV Mad 4	48%	Calgua et al., 2013
Ultrafiltration cartridges	Filtration of sample using ultrafiltration cartridges	River water	JCV Mad 4	18.70%	Albinana-Gimenez et al., 2009a
Zeta Plus MK Electropositive cartridges	Filtration of sample using electropositive cartridges	River water	JCV Mad 4	0.37%	Albinana-Gimenez et al., 2009a
1MDS cartridges filter (electropositive membrane)	Filtration with 1MDS followed by virus elution with 1.5% beef extract	Treated wastewater	JCV	0.15%	Bofill-Mas et al., 2006

[a] Average value; all recovery efficiency values were determined by qPCR.

during rinse/elution steps), or the direct extraction of DNA from the filter. Elution with a high salt alkaline phosphate buffer has also been found to improve recovery efficiencies from HA membranes for several viruses including HPyVs (Hamza et al., 2009). Methods utilizing HA electronegative membranes can be used to concentrate viruses from various volumes of water samples (a few liters to >1000 L) and have been used to evaluate a number of different water matrices including surface water (Haramoto et al., 2010), seawater (Katayama et al., 2002), raw sewage (Hata et al., 2013; Kitajima et al., 2014), treated wastewater (Rachmadi et al., 2016) and drinking water (Haramoto et al., 2012). For seawater samples, HA membrane methods offer the advantage of not requiring any pretreatment due to high cation concentrations in the sample matrix. Samples that have high turbidity or contain larger debris or particles can potentially clog membranes; however, this can be mitigated by pre-filtration using regular glass fiber filters with a larger pore size (Rachmadi et al., 2016). In addition, the chemicals used for elution of HA membranes do not inhibit downstream PCR reactions, although for larger volumes there may be significant co-concentration of humic acids and other organic PCR inhibitors.

Electropositive media such as 1MDS, Zeta plus MK and NanoCeram have the benefit of binding negatively charged viruses directly without the need for sample pretreatment or acidification in the case of freshwater samples. After filtration, solutions such as beef extract, glycine buffer, and urea-arginine phosphate buffer are frequently used to elute the virus (Karim et al., 2009). While collection of larger volume water samples is possible, in highly turbid waters (90 NTU), NanoCeram filters and 1MDS filters tend to clog rapidly and the use of a prefilter is advised (Karim et al., 2009; Cashdollar and Wymer, 2013).

The NanoCeram filter, an electropositive filter consisting of nanoalumina fibers with a diameter of 2 nm and pore size of 2–3 μm, has shown promise for concentration of viruses from water samples; however, to date methods utilizing this filter have not been evaluated for their performance in concentrating HPyVs. In a study by Tepper and Kaledin (2006), NanoCeram filters were shown to have reasonably high virus recovery efficiencies (enterovirus and NoV) for large volume water samples compared to other methods, with comparable performance to 1MDS filters when concentrating large volumes (15–100 L) of high-turbidity water samples such as river water (Karim et al., 2009). Recovery efficiencies for NanoCeram filters have been reported for poliovirus (66%), echovirus (1.83%), coxsackievirus (1.77%), adenovirus (14%), and MS2 coliphage (56%) determined by qPCR (Ikner et al., 2011). Although this method is typically more expensive than those utilizing electronegative membranes such as HA, NanoCeram is less expensive than 1MDS cartridges and, depending on the elution medium used, is compatible with both cell culture and PCR detection methods (Ikner et al., 2011).

Virus concentration by glass wool was introduced to eliminate the need for acidification of water samples, which can lead to virus inactivation (Vilagines et al., 1993). The recovery of poliovirus from glass wool was found to be 57–63% in small and large volume samples (30–100 L) by cell culture method (Vilagines et al., 1993). In contrast, Albinana-Gimenez et al. (2009a) found recovery rates for JCV to be much lower as observed by qPCR; JCV seeded in 10 L of river water was evaluated for glass wool columns with and without pre-acidification to pH 3.5 utilizing glycine containing 3% beef extract as an elution buffer, resulting in 4.36% and 2.82% recovery efficiencies, respectively. When sample volumes were increased to 50 L, the recovery efficiency from non-acidified samples in glass wool rose to 13.7%. In the same study, glass wool columns were found to be more efficient than electropositive filter in terms of virus recovery; Zeta plus MK electropositive cartridges used to concentrate 50 L samples followed by elution with alkaline glycine buffer and 1% beef extract yielded 0.37% virus recovery (Albinana-Gimenez et al., 2009a). The 1MDS cartridge filter, which has also been evaluated for JCV recovery from wastewater effluent, had a similarly low recovery efficiency of 0.15%–0.16% as determined by qPCR, though this may have been due to inhibition caused by the complexity of this matrix (Bofill-Mas et al., 2006).

Mechanical filtration using ultrafiltration has been developed to concentrate protozoa, bacteria, and viruses in 2–100 L volumes of water (Smith and Hill, 2009; Olszewski et al., 2005; Kearns et al., 2008). To elute the viruses from the filter, a backwashing procedure using phosphate buffer or phosphate buffer saline is conducted

(Kearns et al., 2008). In a comparative study by Albinana-Gimenez et al. (2009a), ultrafiltration and NaOH elution of a 10 L volume of river water spiked with JCV showed a higher recovery (18.7%) compared to glass wool concentration methods with and without pre-acidification (4.36% and 2.82%, respectively) determined by qPCR. Ultrafiltration was also successfully applied by Liang et al. (2015) using a tangential-flow-ultrafiltration method to concentrate HPyV in urban catchment samples collected in Singapore, although exact recovery efficiencies were not reported. Rajal et al. (2007) reported that ultrafiltration methods might be more reliable and consistent than electrostatic microfiltration for recovery of viruses. As an additional benefit, because ultrafiltration is a size exclusion method, several microorganisms (viruses, bacteria and parasites) can be simultaneously concentrated from large volume samples allowing for efficient microbial assessment (Morales-Morales et al., 2003; Smith and Hill, 2009; Olszewski et al., 2005; Kearns et al., 2008). Ultrafiltration methods are typically more expensive than others and samples with higher organic contents and higher turbidity due to higher suspended solids may limit sample sizes that can be processed due to clogging.

A low cost and simple alternative to filter-based concentration methods is the skimmed milk flocculation method. This process is performed by mixing 1–10 L of acidified sample with 1% (w/v) pre-flocculated skimmed milk proteins at room temperature for 8 h (Calgua et al., 2013). Under these conditions, viruses from the sample become associated with the flocculated milk proteins and are concentrated by separating the gravity-sedimented floc from the supernatant. The recovery efficiency of HPyV by skimmed milk flocculation method was 45–51% determined by qPCR, which was higher than the recovery efficiencies of rotavirus (RV) (32–41%) by this method (Calgua et al., 2013) and also higher than many of the previously described filter-based methods. The ease of this method makes it possible to process a larger number of water samples simultaneously (Calgua et al., 2014); however, an extended time period is required for stirring the samples (8 h to overnight) thus increasing overall processing time. In addition, the method has only been demonstrated for samples up to 10 L and may not be practical for larger volume samples.

As described above, several well-tested options for HPyV concentration methods for environmental sampling are commercially available. While these studies have contributed greatly to our understanding of the efficiency of virus concentration methods for recovering HPyVs from environmental water samples, research gaps that need to be addressed still remain. First, there is a lack of efficiency studies for NanoCeram filters, which are of great interest as this filtration media has been shown to be cheaper and effective in situations where other positively charge filter media are insufficient (Karim et al., 2009). Second, while previous studies have reported recovery efficiencies of HPyV for freshwater resources such as river water and tap water (Table 2), efficiencies for other environmental sample matrices such as seawater, raw sewage, and wastewater effluent have not been thoroughly evaluated. Finally, further comparative studies between multiple concentration methods (including newer methods such as skimmed-milk flocculation) would allow for more detailed cost-benefit analysis and aid in selection of appropriate methods for a given matrix.

3.2. Detection methods

After concentrating virus particles in environmental water samples, HPyVs can be detected using various methods, including cell culture using primary human fetal glial (PHFG) cells for JCV or an NCI-60 panel of human tumor cells for MCPyV and BKV (Schowalter et al., 2012; Padgett et al., 1971), immunofluorescence (Calgua et al., 2011), microarray (Gardner et al., 2010), and polymerase chain reaction (PCR) (Sidhu et al., 2012). Research by Foundation et al. (1990) led to development of the shell vial cell culture assay using a cross-reactive monoclonal antibody to the antigen of simian virus 40 to confirm infections faster; infections in culture can be confirmed within 36 h post inoculation. Calgua et al. (2011) compared a cell culture-based immunofluorescence assay and qPCR; after UV disinfection both AdV and HPyV were not detected with the culture-based assay but their viral genomes were detected by qPCR.

Since its invention in the 1980s, PCR has been widely used for detection of enteric viruses in environmental samples (Rodriguez et al., 2009). Conventional PCR offers qualitative detection of viral genomes, whereas real-time quantitative PCR (qPCR) provides quantitative data (i.e., genome copy numbers). PCR-based techniques are the most frequently used for HPyV detection in environmental samples; several examples of target regions of PCR and qPCR on the HPyV genome are described in Fig. 1. The qPCR assay developed by McQuaig et al. (2009), which targets the conserved T antigen of JCV and BKV, has been most widely used for detection of HPyV in environmental samples (Wong et al., 2012; Liang et al., 2015). The sensitivity (limit of detection) of the qPCR assay for JCV and BKV was $\geq$10 gene copies per reaction using JCV and BKV standard plasmid DNA spiked as the qPCR control (McQuaig et al., 2009). In addition, the use of HPyV as a human viral marker would be slightly less costly compared to RNA viruses as reverse transcription is not required for HPyV detection.

Recently, a multiplex Luminex® xMAP® PCR assay, which can detect multiple targets simultaneously, has been developed for rapid detection of human AdV, HPyV, enterovirus, RV, and NoV (Hamza et al., 2014). This assay offers more rapid detection and lower running cost than regular qPCR but the sensitivity can be reduced due to the potential primer and/or enzyme competitions between multiple assays within a single reaction; therefore, primer design is critical for the success of this method (Hamza et al., 2014). Regardless of primer design, this multiplex assay is still considered less sensitive than regular qPCR for detecting virus genomes in environmental samples (Hamza et al., 2014). In addition, multiplex PCR for simultaneous detection of JCV, BKV, and SV40 has also been developed (Fedele et al., 1999; Pal et al., 2006). Recently, a specific rolling circle amplification method was also developed to amplify low copy numbers of HPyVs (including several newly discovered HPyVs) in clinical sample, which could potentially be used to increase the detection rate of HPyVs in the environment (Rockett et al., 2015).

It should be noted that, while qPCR quantification determines the numbers of genome copies of a target enteric virus present in the environment, the detected quantity does not necessarily represent the number of infectious virus particles; cell culture-based methods have long been known as the gold standard for determining viral infectivity. However, since the cell line and cell culture assays are not available yet many enteric viruses including the newly discovered HPyVs, molecular detection methods are currently the most widely used for environmental detection of viruses. HPyVs are no exception to this trend, and with the discovery of new HPyVs it may be the case that the development of cell culture methods for these emerging viruses may lag behind that of PCR based detection methods.

4. Environmental occurrence of human polyomaviruses

Fecal contamination in the environment can cause a significant impact to public health, therefore the relative occurrence of any potential MST marker with respect to other enteric pathogens must be taken into account. Previous studies have reported that HPyV showed higher abundance than fecal indicator bacteria (e.g.,

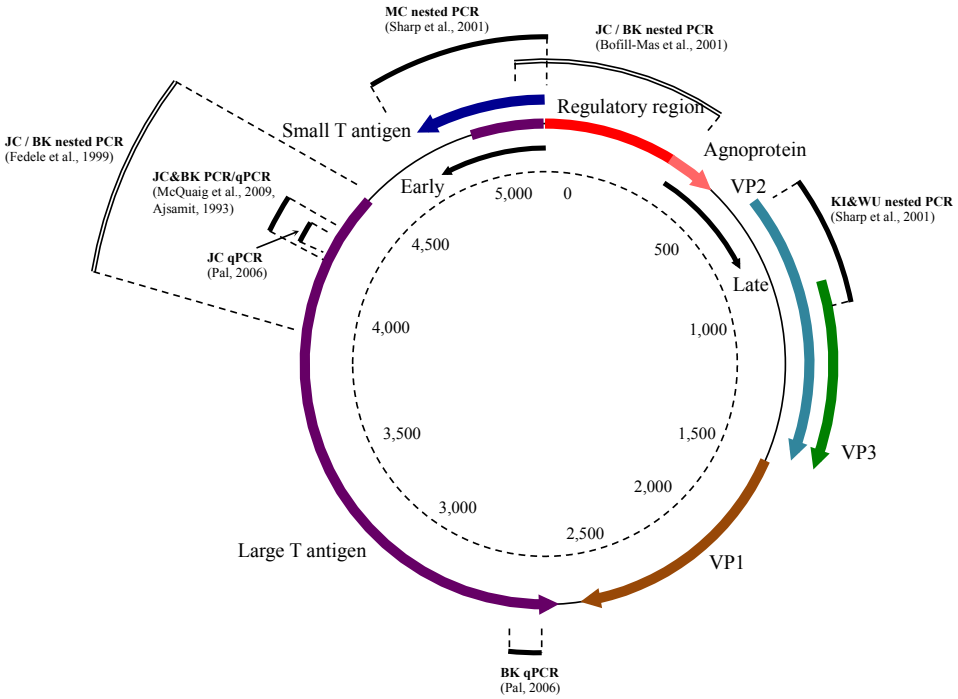

Fig. 1. Polyomavirus genome organization and locations of PCR target regions.

coliforms and *E. coli*) in certain environments (Staley et al., 2013; Sidhu et al., 2012, 2013). HPyVs have been found in environmental water samples in Australia (Sidhu et al., 2012), Japan (Haramoto et al., 2010), United States (Kitajima et al., 2014), Spain (Calgua et al., 2011; Albinana-Gimenez et al., 2006), Germany (Hamza et al., 2014), and Brazil (Calgua et al., 2013), suggesting their prevalence in environmental waters worldwide. Table 3 summarizes the occurrence of HPyV in the environment reported in recent literature. It has been indicated by several studies that JCV is frequently detected at higher concentrations than BKV in wastewater treatment plants (WWTPs) (Bofill-Mas and Girones, 2003; Kitajima et al., 2014; Hata et al., 2013; Schmitz et al., 2016). This is likely due to the fact that JCV is excreted more often in urine and mainly by immunocompetent individuals, is present in wider types of cells and organs (e.g., brain cells, tonsil, human lower and upper gastrointestinal tract, and immune cells), and has higher resistance to pH and temperature compared to BKV in the environment (Bofill-Mas and Girones, 2003). The recent discovery of several new HPyVs in clinical and environmental settings has opened the door to a new era for future research regarding the prevalence and diversity of HPyV in the environment and their potential usage as MST markers.

4.1. Sewage

In general, virus occurrence in raw sewage reflects the actual prevalence of viruses among human populations in a given service area, regardless of symptoms (Sinclair et al., 2008). JCV and BKV were first identified in sewage samples in 2000 (Bofill-Mas et al., 2000); this was the first documentation of the presence of HPyVs in environmental samples. Since then, a number of studies have investigated HPyV occurrence in municipal sewage. Multiple studies investigating the occurrence of PyV in sewage over 6 months or longer have observed no evident seasonal dependence (Di Bonito et al., 2014; Jurzik et al., 2015). Kitajima et al. (2014) reported that JCV and BKV were detected in 91.7% (22/24) and 87.5% (21/24), respectively, of raw sewage samples and 54.2% (13/24) and 79.2% (19/24) of treated sewage samples over the course of one year (two samples per month) from two WWTPs in Arizona, United States using qPCR methods. There was little difference in concentrations and positive rate observed over one year of sampling, implying a lack of seasonality for JCV and BKV (Kitajima et al., 2014). On the other hand, within the same study other enteric viruses such as enterovirus and NoV showed clear seasonal trends; for example, NoV was detected at high concentration during the winter than during other seasons. The same trend was confirmed by Schmitz et al. (2016) in a follow-up study carried out by the same research group in Arizona two years later; similar incidence rates of both JCV and BKV in raw sewage were found, confirming that these viruses are consistently found in raw sewage with little seasonal variation (Schmitz et al., 2016). Furthermore, JCV and BKV were also found in a wetland receiving the effluent water from one of the WWTPs studied in Kitajima et al. (2014); 75% (18/24) of these wetland samples were positive for HPyVs with a concentration of 10^2–10^3 gene copies (GC)/L (Rachmadi et al., 2016). In a WWTP in Japan using activated sludge followed by chlorination and sand filtration processes, the geometric mean of JCV in the influent was 2.1×10^5 copies/L, which was 1 $\log_{10}$ lower than that of AdV, although the removal of JCV was higher than that of AdV (Hata et al., 2013). Similar results were reported by Calgua et al. (2011), Albinana-Gimenez et al. (2006), and McQuaig et al. (2009) in Spain and the United States. These research groups detected JCV and BKV in 100% of sewage samples with high concentrations ranging from 10^4 to 10^5 GC/L. Other HPyVs such as KIPyV, WUPyV, and MCPyV were also detected in raw sewage in Barcelona, Spain using a conventional PCR method (Bofill-Mas et al., 2010). Lymphotropic polyomavirus (LyPyV), which was found in simian species and has been suggested to infect humans was not detected in sewage, sludge, or biosolid samples with qPCR and nested PCR methods (Bofill-Mas et al., 2010).

Since discharged water from WWTP can potentially impact recreational or irrigation waters, pathogenic viruses in treated

Table 3
Detection of HPyV in water, biosolids, and shellfish.

Samples		Detection methods	Positive ratio	Detection target	Concentration[a]	Country	Reference
Water	Raw Sewage	Immunofluorescence assay	100% (7/7)	JCV strain Mad-4	1.58×10^7–3.50×10^7 FFU/L	Spain	Calgua et al., 2011
		qPCR	100% (7/7)	JCV strain Mad-4	5×10^8 GC/L	Spain	Calgua et al., 2011
		qPCR	100% (5/5)	JCV	1.83×10^5 –8.9×10^6 GC/L	Spain	Albinana-Gimenez et al., 2006
		qPCR	74% (29/39) [b]	HPyV (JCV + BKV)	9.6×10^3 GC/L (mean value)	Germany	Jurzik et al., 2015
		qPCR	50.3% (66/131) [c]	MCPyV	3.96×10^4 –4.79×10^5 GC/L	Italy	Di Bonito et al., 2014
		qPCR	100% (39/39)	HPyV (JCV + BKV)	$(3.0 \pm 1.7) \times 10^4$ GC/L	United States	McQuaig et al., 2009
		Nested PCR	95.8% (23/24)	JCV	10^4 - 10^5 virus particle/L	Spain	Bofill-Mas et al., 2001
		qPCR	100% (6/6) [d]	JCV	3.16×10^4 –9.70×10^5 copies/L	Japan	Hata et al., 2013
		qPCR	100% (6/6) [d]	BKV	2.49×10^3 –8.29×10^4 copies/L	Japan	Hata et al., 2013
		qPCR	91.7% (22/24) [e]	JCV	Up to 1.49×10^6 copies/L	United States	Kitajima et al., 2014
		qPCR	87.5% (21/24) [e]	BKV	Up to 4.79×10^5 copies/L	United States	Kitajima et al., 2014
		qPCR	91.7% (22/24) [f]	JCV	Up to 5.95×10^5 copies/L	United States	Schmitz et al., 2016
		qPCR	87.5% (21/24) [f]	BKV	Up to 1.61×10^6 copies/L	United States	Schmitz et al., 2016
	Treated Sewage	qPCR	22.2% (2/9)	HPyV (JCV + BKV)	$(1.2–2.5) \times 10^1$ GC/L	United States	McQuaig et al., 2009
		qPCR	79% (31/39)	HPyV (JCV + BKV)	4.1×10^3 GC/L (mean value)	Germany	Jurzik et al., 2015
		qPCR	100% (6/6)	JCV	4.42×10^1 –2.31×10^2 copies/L	Japan	Hata et al., 2013
		qPCR	83.3% (5/6)	BKV	Up to 9.12×10^1 copies/L	Japan	Hata et al., 2013
		qPCR	54.2% (13/24)	JCV	Up to 5.26×10^4 copies/L	United States	Kitajima et al., 2014
		qPCR	79.2% (19/24)	BKV	Up to 1.03×10^5 copies/L	United States	Kitajima et al., 2014
		qPCR	33.3% (8/24)	JCV	Up to 1.41×10^4 copies/L	United States	Schmitz et al., 2016
		qPCR	50% (12/24)	BKV	Up to 1.29×10^5 copies/L	United States	Schmitz et al., 2016
	Drinking Water (GAC treated water)	qPCR	56% (5/9)	JCV	0.46–5.47 copies/L	Spain	Albinana-Gimenez et al., 2006
	Drinking Water Resources	qPCR	3% (2/64)	JCV	2.3×10^2 –1.3×10^3 copies/L	Japan	Haramoto et al., 2012
			2% (1/64)	BKV	2.5×10^2 copies/L		
	River	Immunofluorescence assay	100% (5/5)	JCV strain Mad-4	1.00×10^7–1.90×10^7 FFU/L	Spain	Calgua et al., 2011
		qPCR	100% (5/5)	JCV strain Mad-4	2.35×10^8 GC/L	Spain	Calgua et al., 2011
		qPCR	11% (2/18)	JCV	7.91×10^2 –3.42×10^3 GC/L	Japan	Haramoto et al., 2010
		qPCR	100% (12/12)	JCV	1.58×10^2 –2.97×10^4 GC/L	Brazil	Calgua et al., 2013
		Nested PCR	40% (10/25)	JCV, BKV, MCPyV	NA	Italy	Iaconelli et al., 2015
		Nested PCR	84% (21/25) in 2006 85% (17/20) in 2012	HPyV (JCV + BKV + MCPyV)	NA	Argentina	Torres et al., 2016
		qPCR	100% (14/14)	JCV	2.6×10^1 GC/L	Spain	Albinana-Gimenez et al., 2006
		qPCR	97.5% (40/41)	HPyV (JCV + BKV)	1.4×10^2 –1.91×10^4 GC/L	Germany	Hamza et al., 2009
	Undeveloped Lakes	Regular PCR	14% (2/14)	HPyV (JCV + BKV)	NA	United States	Staley et al., 2013
	Coastal Water	qPCR	3% (4/132)	HPyV (JCV + BKV)	10^2 GC/L (mean value)	Brazil	Moresco et al., 2012
	Stormwater	Regular PCR	50% (12/24)	HPyV (JCV + BKV)	NA	Australia	Sidhu et al., 2012
	Polishing Ponds	qPCR	69% (18/29)	HPyV (JCV + BKV)	1.1×10^4 GC/L (mean value)	Germany	Jurzik et al., 2015
	Swimming Pools	Nested PCR	28% (4/14)	JCV + MCPyV	NA	Italy	La Rosa et al., 2015
	Urban Catchment	qPCR	46% (64/138)	HPyV (JCV + BKV)	Up to 6.31×10^3 GC/L	Singapore	Liang et al., 2015
Sludge/ Biosolids	Sludge	qPCR	20% (1/5)	JCV	1.2×10^4 GC/L	Spain	Albinana-Gimenez et al., 2006
	Sludge	qPCR	100% (8/8)	JCV	2.4×10^1 –3.95×10^4 GC/L	Spain	Bofill-Mas et al., 2006
	Biosolid	qPCR	100% (6/6)	JCV		Spain	

Table 3 (*continued*)

Samples	Detection methods	Positive ratio	Detection target	Concentration[a]	Country	Reference
				3.42×10^2 -7.71×10^3 GC/L		Bofill-Mas et al., 2006
Shellfish	Regular PCR	50%	HPyV (JCV + BKV)	NA	Spain	Bofill-Mas et al., 2001

[a] FFU, focus forming unit; GC, genome copies; NA, not available.
[b] Samples were collected monthly for 8 months (January–August 2010).
[c] Samples were collected monthly for one year (January–December 2013).
[d] Samples were collected monthly for 6 months (October 2007–March 2008).
[e] Samples were collected monthly for one year (August 2011–July 2012).
[f] Samples were collected monthly for one year (June 2014–May 2015).

wastewater poses a risk of infection to the public. In addition, with the recent discovery of many new HPyVs, WWTPs represent a key potential point for controlling the release of these emerging viruses to open water bodies. Employment of highly efficient treatment systems is crucial to achieve adequate reductions of viruses that may pose a health hazard in aquatic environments.

4.2. Surface and recreational water

HPyV contamination in surface water manifests due to leakage from urban sewers, discharge of treated sewage, and/or direct urination into water bodies. JCV and BKV have been detected in rivers worldwide (Japan, Spain, Brazil) at high concentrations. Haramoto et al. (2010) reported JCV concentrations of 7.91×10^2–3.42×10^3 GC/L in the Tama River (Tokyo, Japan) as determined by qPCR. Calgua et al. (2011 & 2013) reported 100% detection of JCV in more than 20 samples collected from rivers in Spain and Brazil by qPCR and immunofluorescence assay. The occurrence of HPyV was also reported by Rusiñol et al. (2015), who showed high abundance (up to 10^4 GC/L with 50–80% of positive rates) of HPyV throughout the year in different river catchments in Europe and Brazil. The more recently discovered MCPyV was detected in 50% of river water samples collected in Spain and Brazil, which was the first documented detection of MCPyV in environmental samples (Calgua et al., 2013). This virus has also been found through the analysis of skin microbiome, therefore transmission through skin contact and bathing has also been suggested (Calgua et al., 2013). These findings are important because river water can be used for recreational purposes and drinking water production, thus representing potential routes of infection in humans.

Staley et al. (2013) attempted to investigate the abundance of JCV and BKV in a variety of lakes, including urban lakes, lakes near cattle grazing, and undeveloped lakes (i.e., no human impact other than fishing and no livestock in the surrounding catchment) in Florida, United States using PCR methods; interestingly, JCV and BKV were only detected in undeveloped lakes. The detection of JCV in the undeveloped lake was considered background level since it only received minimal impact from non-natural sources, but it was suspected that a nearby housing development was the source of JCV and BKV (Staley et al., 2013). This difference between the prevalence in lakes and river may suggest that the persistence of HPyVs in water bodies with differing residence times may vary and warrants further research.

Recent research has also reported the presence of JCV and MCPyV in swimming pools, which likely originated from human sources due to the fact that viruses are not able to multiply outside their host (La Rosa et al., 2015). In addition, JCV and MCPyV have been detected in 28% (4/14) of samples taken from indoor/outdoor swimming pools in Italy (La Rosa et al., 2015). Due to the presence of HPyV in recreational waters and swimming pools, this could be considered as a potential source of infection.

At recreational beaches in Brazil, JCV was detected in 3% of water samples, however human AdV and hepatitis A virus were found to be more abundant and persistent in coastal seawater (Moresco et al., 2012). In a similar study, only 2% of the water samples were positive for JCV from coastal area used for shellfish farming (Moresco et al., 2012). Abdelzaher et al. (2010) also reported the presence of JCV in recreational coastal water possibly due to discharge of untreated human excreta from the household septic systems or outfalls. The lower detection frequencies of HPyVs in coastal waters may indicate that these viruses are less persistent in marine aquatic environments compared to other enteric viruses; another potential explanation is that concentration recovery efficiencies for HPyVs in marine waters may be lower than for other viruses. Both of these points require further investigation to confirm the prevalence of HPyVs in marine waters.

The detection of JCV in coastal areas is important not only because of the usage of these waters for recreational purpose but also for shellfish farming operations. Bofill-Mas et al. (2001) found 50% (3/6) of samples from shellfish farming area were positive for JCV in the Ribro delta, Spain. Shellfish filter large amounts of water during feeding, and viruses ingested by the shellfish tend to remain in its digestive system; direct consumption of these contaminated shellfish may cause infections in humans, particularly of lymphoid cells in the human intestinal epithelium. The transmissibility of HPyVs from contaminated shellfish to human lymphoid cells, including the resistance of HPyVs to human digestive proteolytic enzymes, is not fully understood and warrants further investigation.

4.3. Stormwater

Urban stormwater runoff from the developed/built environment can lead to pollution of water environments. Human enteric pathogens can be present in stormwater due to leaks from sewer systems, sewer pumping station, sewer overflows, individual septic systems and surface runoff containing human fecal material, as well as the discharge of treated wastewater (Sidhu et al., 2012). Sidhu et al. (2012, 2013) found 50% (12/24) and 56% (12/23) occurrence of JCV and BKV, respectively, in stormwater in Australia during dry and wet seasons using a hollow fiber ultrafiltration concentration method followed by PCR. These results suggest that urban stormwater runoff can transport HPyVs and other enteric viruses to surface water bodies, such as rivers, lakes, and creeks (Noble et al., 2006).

In addition, sediments during dry season can also act as a reservoir of enteric viruses before getting flushed by rainwater into larger water bodies during the wet season (Sidhu et al., 2012). For example, poliovirus has been found to survive longer in sediments due to absorption (Labelle and Gerba, 1980). The time needed to inactivate more than 99% of poliovirus increased from 1.4 days in seawater to 6 days in estuarine sediments and from 1 h in polluted water to 4.25 days in sediments due to virus adsorption (Labelle

and Gerba, 1980). Although the survival and transmissibility of HPyVs in soil has not been evaluated, JCV and BKV were detected in groundwater in Ghana (Gibson et al., 2011) and Spain (Albinana-Gimenez et al., 2009b), which indicates that HPyVs may be able to pass through soil and enter the aquifer, although further research is needed to substantiate this point.

4.4. Drinking water systems

Due to the fact that HPyVs have been frequently detected in surface waters, it is possible that the use of these waters as a source for drinking water could potentially act as a route for HPyV infection. In a study by Asami et al. (2016), JCV and BKV were detected in raw source waters for a drinking water treatment plant (DWTPs) in Bangkok, Thailand (Table 3). JCV was detected more frequently (11 of 11 samples) than BKV (2 of 11 samples), with average concentrations of 1.16×10^3 and 3.3×10^2 copies/L, respectively.

Albinana-Gimenez et al. (2006) found JCV and BKV in a number of drinking water sources in Spain. Samples collected from the Llobregat and Ter River in northern Spain and concentrated by using a Zeta Plus MK electropositive filter with peristaltic pump were 100% positive for JCV (5/5, 2.6×10^1 GC/L and 9/9, 2.7×10^1 GC/L, respectively). In a more recent study, JCV concentrations in the Llobregat River have been reported to be 4.62×10^3 GC/L, two orders of magnitude higher than were previously measured (Albinana-Gimenez et al., 2009b); this increase may be due to difference in sampling location along the river or the use of a different ultracentrifugation method in the latter sampling. BKV was also detected in several samples from the Llobregat River (4/6, 2.1×10^1 GC/L) using an ultrafiltration method (Albinana-Gimenez et al., 2006). Another similar study in Spain found 48% (16/33) positive rate for JCV in various water sources used by DWTPs, including two separate rivers (4/9, 7.40×10^2; 6/12, 1.3×10^3 GC/L) and two parallel processing lines of well water (3/6, 2.46×10^0, 1.84×10^0 GC/L) (Albinana-Gimenez et al., 2009b).

In Japan, HPyV has been detected on several occasions in drinking water sources. Haramoto et al. (2012) found HPyVs in samples from rivers used for drinking water production at 16 DWTPs representing all six regions of Japan, including JCV (2/64, 2.90×10^2–1.3×10^3 GC/L) and BKV (1/64, 2.50×10^2 GC/L). In addition, Asami et al. (2015) detected JCV in raw water from western Japan (15/46 samples) at similar average concentrations as the previous studies (6.58×10^2 GC/L).

4.5. HPyV as a human genetic marker for MST target in the environment

To design an effective method for human waste tracking in the aquatic environment several factors should be considered for the indicator chosen including the specificity to humans, high environmental abundance, low seasonality, ease and rapidity of measurement, and cost effectiveness. The sampling regimen, location, frequency of sampling, and number of samples taken should be designed in advance to allow reliable interpretation of fecal contamination in water bodies (Stoeckel and Harwood, 2007). Since HPyVs are detected in almost all water environments without seasonal variation, they have the potential to be used as a human contamination marker. In addition, an accurate, transferable and reproducible method of concentration that is appropriate for the chosen water matrix (discussed in Section 3 of this paper) should be applied in order to maximize the chance of detecting the target (Stoeckel and Harwood, 2007).

Molecular-based detection (qPCR) can be applied for HPyV detection with the benefits that it is both more rapid and cost effective compared to cell culture assays; however, since quantities determined by qPCR may not necessarily represent infectious virus, confirmation by cell culture assay is desirable if within the scope of the study. Since it is too costly and time consuming to detect multiple enteric viruses as human contamination markers in the environments, HPyV could be a useful marker to track contaminants originated from human excreta in aquatic environments. Another advantage is that HPyVs do not require reverse transcription for molecular detection (viral genome is DNA unlike many other enteric viruses that poses RNA as viral genome) and their free DNA can persist within sewage matrices for more than 110 days, which demonstrates the usefulness of HPyVs as a conservative/persistent genetic marker (Bofill-Mas et al., 2006).

Nevertheless, several limitations of HPyVs as a genetic marker can also be pointed out. For example, the dsDNA structure of HPyVs does not represent the majority of enteric viruses (mostly RNA). In addition, the higher persistence of HPyV genomes in the environment may make HPyV too conservative and therefore, the incidence of HPyV in a given environmental sample may be significantly different from that of other enteric viruses.

5. Persistence of human polyomaviruses

Enteric viruses are highly persistent in the environment due to their resistance to environmental (physical and chemical) stresses. This is of great importance for HPyVs in terms of the potential for the virus to spread via water to new hosts as well as for determining their usefulness in MST of fecal contamination. It is known that viruses are likely to adsorb onto the surface of particles such as organic matter and that such particle associated viruses may remain viable for longer than dispersed (free floating) viruses due to the particles providing some degree of shielding from harsh environmental conditions and protection from disinfectants (Templeton et al., 2005, 2008). This suggests that viruses shed in fecal material may be less susceptible to degradation and inactivation compared to dispersed virus particles in liquid, which may be of importance for certain HPyVs such as JCV which are primarily excreted in urine rather than feces. Most studies on HPyV persistence have been performed using JCV and BKV to date, presumably due to the availability of virus and cell culture methods for these viruses (Albinana-Gimenez et al., 2006; McQuaig et al., 2009); JCV is targeted more frequently because it is typically more prevalent than BKV in wastewater (Table 3). In the future, comparisons between various HPyVs in persistence studies may be of value for determining their relative value as MST markers.

5.1. Effect of temperature and pH

The survival of HPyVs at different temperatures has been evaluated for several water matrices. Atwood (2001) reported the persistence of HPyV in water at 55 °C for up to 1 h. In a sewage matrix, the survival of JCV at 25 °C and 35 °C was found to be slightly higher than for FIB, with greater reductions of FIB observed after 7 and 14 days than for JCV (McQuaig et al., 2009). The persistence of JCV was similar to that of AdV at both 25 °C and 35 °C temperatures in the environment, suggesting that JCV and BKV may be good indicators for the fate and persistence of other pathogenic viruses (McQuaig et al., 2009). The stability of JCV in sewage water at 20 °C determined by qPCR showed a t_{90} (time required to observe a reduction of 90% in the initial viral concentration) of 63.9 days with structured viral particles still found after 73 days under the same environmental condition (Bofill-Mas et al., 2001, 2006). Simian PyV SV40 (strain 777), which infects monkeys and has been used as a surrogate for HPyV, was found to be reduced by >5 $\log_{10}$ after 1 h of dry or moist heat treatment at 95 °C (Sauerbrei and Wutzler, 2009).

HPyVs have also been found to be resistant to acidic conditions. JCV subjected to low pH with 10 μg/L trypsin was able to infect SVG cells even after treatment at pH 3 for 30 min, supporting the assumption that JCV could survive and be transported through the human gastrointestinal tract; furthermore, JCV DNA was still detected after 30 min of treatment at pH 1 (Bofill-Mas and Girones, 2003), although this does not necessarily indicate the persistence of infectious virus.

5.2. Removal by wastewater and drinking water treatment

Treated wastewater after conventional treatment could contain pathogenic microorganisms which may pose a health hazard to humans from direct or indirect contact, therefore reducing their number is important for water reuse purposes and general environmental health (Kitajima et al., 2014). UV (23 mW cm^{-2}) and UF treatment of secondary effluent (after sedimentation and activated sludge processes) in a WWTP in Canada showed 2.28 ± 0.52 and 5.88 ± 0.48 log_{10} removal, respectively, of JCV where similar result (2.02 ± 0.36 and 6.96 ± 0.49 log_{10} removal, respectively) was observed for AdV (Qiu et al., 2015). A WWTP in Spain, which used activated sludge as the secondary treatment showed 2.61 log_{10} reduction of JCV; furthermore, tertiary treatment using chlorination, Actiflo® filtration and/or flocculation and UV disinfection contributed to an additional 1.58 log_{10} reduction where 2 log_{10} and 1.44 log_{10} reduction were observed for AdV in secondary and tertiary treatment respectively (Rusiñol et al., 2015). In a study by Kitajima et al. (2014), two WWTPs using either activated sludge or a biological trickling filter showed 1.64 and 2.56 log_{10} removal of JCV and 1.11 and 1.60 log_{10} removal of BKV, respectively, determined by qPCR where AdV reduction is almost similar (0.68 and 1.34 log_{10} removal) although PMMoV removal was found to be lower (0.76 and 0.99 log_{10} removal). Similar results were reported by Hata et al. (2013), where a 2.18 ± 0.51 log_{10} reduction of BKV and 3.19 ± 0.63 log_{10} reduction of JCV were observed in a WWTP that utilized an activated sludge process with chlorination followed by sand filtration; the reduction of AdV in this system was found to be 2.69 ± 0.65 log_{10}, which was again quite similar to that of the two HPyVs. In all of these studies JCV appeared to be more efficiently removed by wastewater treatment than BKV (Hata et al., 2013; Kitajima et al., 2014; Schmitz et al., 2016), indicating that not all polyomaviruses are removed with equivalent efficiencies. These studies also indicate that HPyVs may be more sensitive to biological treatment such as activated sludge when compared to AdV and PMMoV (Hata et al., 2013; Kitajima et al., 2014; Rusiñol et al., 2015). Given the discovery of several new polyomaviruses, this warrants further investigation of the removal efficiencies of multiple HPyVs at WWTPs with different treatment technologies.

Constructed wetlands have been proposed as a passive method of wastewater treatment and have been evaluated for HPyV removal. In a wetland receiving effluent water from a WWTP, which was previously studied by Kitajima et al. (2014), reductions of 0.76 and 1.43 log_{10} on average were observed for JCV and BKV, respectively, after 7 days of water retention time. Lower reductions were observed for PMMoV (<1 log_{10} reduction) but higher reductions (2 log_{10} reduction) were observed for AdV (Rachmadi et al., 2016). It has been suggested that physicochemical processes (adsorption, sedimentation, and reactive oxygen species) and biological processes in wetland are responsible for the reduction of enteric viruses (Kaldec and Wallace, 2009; Rachmadi et al., 2016).

Drinking water treatment processes have also been investigated for HPyV removal. Asami et al. (2016) investigated the removal of JCV and BKV at a DWTP in Bangkok utilizing coagulation/sedimentation (CS) and rapid sand filtration (RSF) in series. Both viruses were detected in several samples (5/10 and 2/10 positive samples for JCV and BKV, respectively) after treatment processes but before chlorination. JCV in particular was detected at high enough concentrations, which allowed determination of the virus removal efficiencies of individual treatment processes (1.88 and 0.52 log_{10} for CS and RSF, respectively). In comparison, PMMoV was more prevalent (10/10 positive samples) and different removal efficiencies (0.40 and 1.26 log_{10} reduction for CS and RSF, respectively). This suggests that JCV is more susceptible to CS, but on the other hand, it is less sensitive to RSF method compared to PMMoV (Asami et al., 2016). Differences in virus removal efficiencies might be due to different water quality and/or shape and surface properties of virus particles. For coagulation/flocculation treatment, isoelectric value of viruses has been found to be an important parameter affecting their removal (Abbaszadegan et al., 2008). Albinana-Gimenez et al. (2009b) also determined JCV removal at a DWTP utilizing flocculation, sedimentation, and GAC filtration followed by chlorination. Flocculation/sedimentation in this study reduced JCV by 0.87 log_{10}, while GAC filtration achieved additional 1.9 log_{10} removal; when compared to AdV (1.98 log_{10} removal by flocculation/sedimentation and 0.64 log_{10} removal by GAC), the removal of JCV by flocculation/sedimentation was lower, whereas the removal by GAC was higher. In both studies the coagulant utilized by the DWTPs was aluminium sulphate, however the removal observed by Asami et al. (2016) was notably higher. It is possible that different coagulant dosage or raw water quality could have resulted in this observed difference in removal efficiencies. Several other DWTPs utilizing CS followed by RSF have been evaluated in the western region of Japan which were able to successfully remove JCV to below detection limits (Plant 1, >2.49 log_{10}; Plant 2, >1.76 log_{10}); the average initial concentrations of JCV were an order of magnitude higher in Plant 1 than for Plant 2 which may explain the difference between the apparent removal rates of both plants. Within the same study, two other treatment systems utilizing only slow sand filtration (SSF) or RSF and SSF in series were found to reduce JCV below detection in most cases (>2.38 log_{10} for SSF only; >1.91 log_{10} for RSF-SSF); however, JCV was detected on two occasions after these SF processes, indicating that SF may not be able to remove JCV under certain circumstances (changes in raw water quality, etc.) (Asami et al., 2016). Although subsequent disinfection treatments should be able to inactivate remaining JCV before distribution, this finding emphasizes the importance of combining multiple treatment steps with different mechanisms of particle removal to ensure high HPyV removal efficiencies.

HPyV removal has been evaluated in DWTPs utilizing advanced treatment systems, generally including three or more in-series treatment processes. One study compared JCV and AdV removal (by qPCR as well as infectivity assay) in a DWTP using two parallel systems consisting of in series filtration cartridges, UV disinfection, and either nanofiltration (NF) in line 1 or reverse osmosis (RO) in line 2 for treatment before post-chlorination; JCV removal in both of these process lines was negligible (0 and 0.09 log_{10}, filter cartridge; 0.38 log_{10}, UV-NF; 0.56 log_{10}, UV-RO), however post-chlorination reduced JCV to below the detection limit for a total reduction of >2 log_{10}. Higher removal was shown for AdV in filter cartridges from one line (1.78 log_{10}) while in the other parallel line no reduction was observed from filtration (0 log_{10}); similar removal compared to JCV was reported for AdV by UV-NF (0.3 log_{10}) and UV-RO (0.44 log_{10}). The concentration of JCV and AdV in the source well water were notably lower than in other raw water sources, therefore it is possible that their removal by these treatment processes could be higher in more contaminated water (Albinana-Gimenez et al., 2009b). The greater reduction of AdV compared to HPyV by filtration in one line may have been due to the larger virus size or differing surface characteristics of AdV; a simpler explanation is that the low concentrations of both viruses in source waters may

have contributed to the highly variable results between AdV reductions in the parallel filtration systems.

Another advanced treatment plant using flocculation, sedimentation, sand filtration, ozonation, and GAC filtration could remove up to 2 $\log_{10}$ of JCV and AdV with an initial concentration of 2.6×10^2 GC/L and 4.2×10^2 GC/L in the raw water, respectively; this system was also found to remove BKV to below detection with an initial concentration of 2×10^1 GC/L (Albinana-Gimenez et al., 2006). In a different study, a DWTP with a similar treatment processes but with an input of groundwater between the sand filtration and ozonation steps removed 3.51 $\log_{10}$ of JCV ($7.4 \times 10^2 \pm 1.1 \times 10^3$ GC/L initial concentration); a higher reduction was observed for AdV (5.13 $\log_{10}$ reduction with $1.24 \times 10^4 \pm 1.6 \times 10^4$ GC/L initial concentration) (Albinana-Gimenez et al., 2009b).

Collectively, these studies indicate that, while both AdV and HPyV reduction is possible by physical removal processes in DWTPs, reductions can vary based on the processes used and the differences in physical and chemical characteristics of both the target viruses and the raw water; therefore, they do not guarantee HPyV and AdV removal. Even in the case of advanced treatment in DWTPs (e.g., additional disinfection and/or filtration), HPyV can still on occasion be detected in treated pre-chlorinated water; therefore, additional disinfection such as final chlorination appears to be essential to achieve HPyV reductions below limits of detection. It should be noted that molecular signals are less sensitive to common disinfection practices than are infectious virus particles, meaning that the reductions in infectious virus are likely larger than reductions observed in molecular signals in these studies.

5.3. Inactivation by disinfection processes

Disinfection processes are widely used for water and wastewater treatment as well as for sanitizing purposes; the efficacy of several of these processes has been evaluated for HPyVs. Perhaps the most commonly used disinfectant in water treatment is chlorine. In seawater matrices, the addition of 2.5 mg/L chlorine with a contact time of 60 min showed a less than 1.5 $\log_{10}$ reduction of HPyV with qPCR and enzymatic treatment (ET)-qPCR method, suggesting that HPyV is more resistant to chlorine than human AdV type 2 and MNV-1 that showed 2.5 $\log_{10}$ and 4 $\log_{10}$ reduction, respectively (De Abreu Corrêa et al., 2012). Since molecular signals (nucleic acids) are not equally susceptible to chlorine as infectious virus particles detected by cell culture assays (Chern et al., 2014), ET-qPCR applying nucleases was used to access the integrity of the viral capsid, because liberated DNA/RNA and DNA/RNA in chlorine-damaged capsids would be digested by the enzyme. In addition, Hata et al. (2013) reported that no significant removal of BKV and JCV by chlorination after activated sludge process was observed at a WWTP in Japan. In contrast, the addition of chlorine as the final disinfection step in DWTPs was found to provide significant reductions of JCV and BKV, as described above (Albinana-Gimenez et al. (2009b); this indicates that the difference in matrices (seawater, wastewater, and drinking water) may have an effect on the inactivation of HPyV by chlorination, most likely due to high salt and organic content interfering with chlorination.

Disinfection by ultraviolet (UV) light is an increasingly popular alternative to chlorine disinfection used in many water treatment plants. A previous study reported that JCV (Mad-4 strain) exposed to a UV dose of 140 mJ/cm^2 and 180 mJ/cm^2 (253.7-nm wavelength) was inactivated by 2.27 and 3.33 $\log_{10}$, respectively, as determined by qPCR and inactivity assay where lesion rate of targeted genome region by qPCR was calibrated relatively to inactivation rate of the virus, under the assumption that the damage is evenly distributed across the genome), respectively; from these numbers, the calculated value of K ($\log_{10}$ inactivation constant per unit fluency) was 0.058 per mJ/cm^2 (Calgua et al., 2014). Lower reductions were obtained by Nims and Plavsic (2013), who reported the K values of UV inactivation of SV40 PyV in PBS as being 0.0014 ± 0.007 $\log_{10}$ reduction determined by infectivity assay (cell culture) per mJ/cm^2. It was reported that HPyVs are as resistant to UV disinfection as AdVs (Nims and Plavsic, 2013); this is likely because both of these viruses possess dsDNA genomic material and the viral genome replicates in the nucleus of their host cells. While DNA genomes can be damaged by UV, the damage can be repaired by the host cell's DNA repair machinery as the viral dsDNA is recognized by the machinery because of its structural similarity to the host cell's genomic DNA; this phenomena was not observed for single stranded DNA or RNA viruses (Eischeid et al., 2009, Eischeid and Linden, 2011). The resistance of HPyV to UV disinfection is further supported by the observance of negligible reductions of JCV in a full-scale DWTP containing UV processes combined with nanofiltration or reverse osmosis (see 5.2 above).

While ozone disinfection is utilized in many advanced DWTPs, the efficacy of ozone disinfection for HPyV treatment has not been adequately evaluated in the literature. One study by Albinana-Gimenez et al. (2009b) found negligible reductions of JCV by ozone processes in a DWTP, however as these results were obtained by qPCR it is possible that the viruses may still have been inactivated by oxidative damage to the viral capsid. In addition, the efficacy of ozone can change based on virus types, therefore this topic requires further research as many advanced treatment processes rely on ozonation as a key disinfection step.

Some studies have suggested that viruses are likely to adsorb onto particles and those particle-associated viruses may remain viable longer (especially through disinfection processes) than dispersed viruses (Templeton et al., 2008); despite this, to date no studies have evaluated the degree of HPyV attachment to suspended solids and/or the degree to which this attachment may protect HPyVs from disinfection processes. Potential adsorbents of viruses in natural waters include sand, pure clays (e.g., montmorillonite, illite, kaolinite, bentonite), bacterial cells, naturally occurring suspended colloids, and estuarine silts and sediments (Templeton et al., 2008). The adsorption of viruses onto particles may lead to reduction of disinfectant efficacy and removal in DWTP and/or WWTP processes. Sakoda et al. (1997) found that adsorption of viruses onto solid surfaces in the environment would make them more stable compared to dispersed viruses in water. Since disinfectants rely on the ability of a chemical or reactive compound to contact with the target organism, shielded virus particles may be more resistant to these treatments (Templeton et al., 2008). Even though these viruses are attached onto suspended solids, they may still be infectious and therefore monitoring of virus levels should consider not only free virus in water but also those attached to suspended solids (Moore et al., 1975). Kaneko and Igarashi (1983) reported that activated sludge, which has a porous structure, can absorb 96% of poliovirus and protect them from inactivation by chlorine. In another study, similar results were observed where bacteriophages attached to clay were more resistant to chlorine; in this case doubling of chlorine concentrations was necessary to achieve 99% removal of the virus (Stagg et al., 1977). During UV treatment, particles less than 2 μM in diameter are enough to protect viruses from UV light and those particulate chemical composition in water may be a critical point for determining the survival of particle-associated viruses during UV infection (Templeton et al., 2005). Considering the importance of these issues for determining the survival of other enteric viruses, this represents a critical data gap in the HPyV disinfection literature which deserves attention.

6. Conclusions

HPyVs have been shown to have various advantages as well as disadvantages as a human-specific viral marker in the environment. We have drawn the following conclusions based on detailed literature review:

- HPyVs are highly specific to humans with frequent excretion regardless of symptoms as well as unique excretion origins (i.e., urine).
- HPyVs are highly prevalent in various water matrices with little seasonality, and are known to be highly persistent in environmental waters.
- The presence of HPyVs in the environment tends to be positively correlated with other enteric viruses.
- The discovery of new HPyVs has opened more opportunities for their potential development as human-specific viral markers in addition to commonly used viral markers such as AdV.
- HPyVs have limitations as viral marker, including: their circular dsDNA genome does not resemble genomes of other major enteric viruses (i.e., linear ssRNA/dsRNA), their pathogenicity makes them potentially dangerous to work with, and the persistence of their viral genome can lead to overly conservative values compared to other viruses.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2016.09.010.

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