GLOBAL WATER PATHOGEN PROJECT

PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND EPIDEMIOLOGY ASPECTS

POLYOMAVIRUS

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Summary

Human Polyomaviruses (HPyVs) are small, non-enveloped double-stranded DNA viruses. They are found in urine, feces and wastewater as a result are used as sewage source tracking viruses and are emerging as potential environmentally related transmission of viral induced cancer. These viruses had previously been classified as belonging to a single family: Papovaviridae (Papillomaviruses, Polyomaviruses and Vacuolating Simian Virus 40). In 2002 however, the International Committee of Viruses Taxonomy decided that enough evidence had been published to warrant the division of the Papovaviridae family into two families: Papillomaviridae and Polyomaviridae. Since then, the classification of both families has been evolving constantly due to an explosion of newly discovered viruses by innovative molecular detection methods.

Human Polyomaviruses JC and BK were discovered in 1971. Although they persistently infect humans, they are intermittently excreted in urine and rarely cause pathology. However, under impairment of the immune system (HIV, chemotherapy, immunosuppression after transplantation, immunomodulatory therapies for autoimmune diseases, etc) they may cause serious illness. Progressive multifocal leukoencephalopathy, a fatal demyelinating disease is a consequence of JCPyV reactivation in the brain. Nephropathy and hemorrhagic cystitis are caused by BKPyV reactivation in the kidney. Since 2007 and with the advent of new molecular techniques, eleven new human polyomaviruses have been discovered. Between those, Merkel cell polyomavirus (MCPyV) has been reported=’ to be integrated into a high number of Merkel cell carcinomas, a type of neuroendocrine tumors of elderly and/or immunosuppressed people. MCPyV was detected and present in environmental samples. By 2010 this was the first cancer related virus to be described as a potential environmental contaminant.

The transmission route for JCPyV and BKPyV has still not been fully defined yet both respiratory and oral-fecal routes are plausible transmission pathways for these viruses. Exposure likely occurs during childhood, as most people are seropositive for these viruses when they reach adulthood for these new polyomaviruses as occur with JC and BK. With the advent of the discovery of these new polyomavirus it seem clear that apart from those human polyomaviruses shed in urine as JC and BK some others have been reported to occur in gastroenteritis affected children and others to be shed in the skin being the infection of epithelia a potential route of entry for them into the host.
JC and BK polyomaviruses were first detected in environmental samples in 2000. JCPyV is present in nearly 100% of sewage samples worldwide and it has been proposed as a human fecal viral indicator. It has also been detected in drinking water, seawater, river water, reclaimed water and shellfish samples growing in sewage-impacted areas.

HPyVs are not “classic” waterborne pathogens. Their presence in water environments is a relatively recent discovery and they are thus considered as emerging or potentially emerging waterborne pathogens. The present chapter describes the state of the art on human polyomaviruses and their presence in water environments. The first section of the chapter covers the epidemiology and clinical relevance of HPyV. The second section focuses on the occurrence of these viruses in different water environments. Finally, data on stability and inactivation of these viruses is addressed. Such information is essential for the improvement of wastewater management programs in terms of both sewage treatment and water quality surveillance.

Polyomavirus

The Polyomaviruses are prevalent in global populations, known to cause serious illness in immunocompromised individuals and are found at high levels in sewage. While our knowledge base is growing their importance in sewage is just now being recognized as source tracking markers and potential waterborne pathogens.

1.0 Epidemiology of the Disease and Pathogen(s)

1.1 Global Burden of Disease

1.1.1 Global distribution

The classical human polyomaviruses, JCPyV and BKPyV, are distributed worldwide as demonstrated by detectable levels of circulating antibodies in the majority of the healthy population (Moens et al., 2013). Most adult individuals show detectable levels of antibodies against the human polyomaviruses described, suggesting persistent infections (Moens et al., 2013). These viruses seem to establish persistent infections, usually early in life, after which they remain latent in the tonsils, urinary tract, lymphoid tonsils and bone marrow. Merkel Cell Polyomavirus (MCPyV) a human polyomavirus recently described, also shows evidences of being widely distributed. Viruria is observed especially in immunodeficient hosts but also in healthy individuals and these viruses are prevalently detected in sewage from different geographical areas (Bofill-Mas et al., 2000). Environmental surveillance of these viruses needs to be conducted globally.

1.1.2 Symptomatology

JCPyV and BKPyV were both discovered in 1971 (Gardner et al 1971; Padgett et al, 1971). Although they persistently infect humans, they rarely cause pathology. However, under impairment of the immune system (HIV, chemotherapy, immunosuppression after transplantation, immunomodulatory therapies for autoimmune diseases, etc) they may cause serious illness. Progressive multifocal leukoencephalopathy (PML), a fatal demyelinating disease is a consequence of JCPyV reactivation in the brain.

Nephropathy and hemorrhagic cystitis are caused by BKPyV reactivation in the kidney.

Apart from JCPyV and BKPyV, and since 2007, eleven different human polyomaviruses have been described. KIPyV and WUPyV were initially detected in 2007 in nasopharyngeal aspirates from patients with acute respiratory infections (Allander et al., 2007; Gaynor et al., 2007), but it is not clear whether these viruses are agents of human respiratory diseases. MCPyV were discovered in 2008 associated with a high number of Merkel cell carcinomas (MCC), a type of neuroendocrine tumors of elderly and/or immunosuppressed people (Feng et al., 2008) and it has been accepted as a etiologic agent of these tumors and classified as to be probably carcinogenic by the International Agency for Research in Cancer (IARC). HPyV7 was detected in skin swabs from healthy human adults and more recently have been related to pruritus rash in a lung transplanted patient (Ho et al., 2015). Trichodysplasia spinulosa-associated polyomavirus (TSPyV) has been linked to the development of a rare skin disease in immunosupressed (Kirchhof et al., 2014).

However, most of the new HPyV described to date (HPyV6, 9, 10, STLPyV and NJPyV-2013) have still not been related to any particular disease although most humans are seropositive for them at an early age (Moens et al., 2013) suggesting they might be transmitted by the respiratory or fecal-oral route. Human polyomaviruses seem to be ubiquitous to several parts of the human body. Some of the human polyomaviruses are frequently excreted in urine (JCPyV, BKPyV), others are shed in the skin (HPyV6, 7, MCPyV, TSPyV) and some others have been described to be excreted in feces (HPyV10, HPyV12). It seems plausible to think that these viruses might infect humans persistently and cause disease in immunosuppressed as it is the case of those polyomaviruses for which an etiologic role have been established. It should be remarked that, until now, all diseases linked to polyomaviruses are quite severe being some of them fatal in a high percentage of patients, nephropathy, PML, MCC (White et al., 2013).

Moreover, polyomaviruses possess a T-Antigen gene that codifies for a protein that may attach to p53 and pBR causing the cells to enter the S phase, consequently causing tumors. Despite MCPyV being classified by the IARC as a possible carcinogen, the role of JCPyV in human cancers needs further study and remains controversial. In particular, JCPyV have been reported by some research groups to be associated with Colorectal Cancer (Coelho et al., 2013).

1.2 Taxonomic Classification of the Agent (s)

Only one genus is currently recognized, the family description corresponds to the genus description. Species and genus demarcation criteria are being developed and in the interim, the list of species is provisional, and all species are assigned to a single genus.

1.2.1 Physical description of the agent

Virions are non-enveloped and approximately 40-45nm in diameter (Figure 1). The icosahedral capsid is composed of 72 capsomers in a skewed (T=7d) lattice arrangement. Buoyant density of virions in sucrose and CsCl gradients is 1.20 and 1.34 g/cm^3 respectively. Virions are resistant to ether, acid and heat treatment (50ºC, 1h).

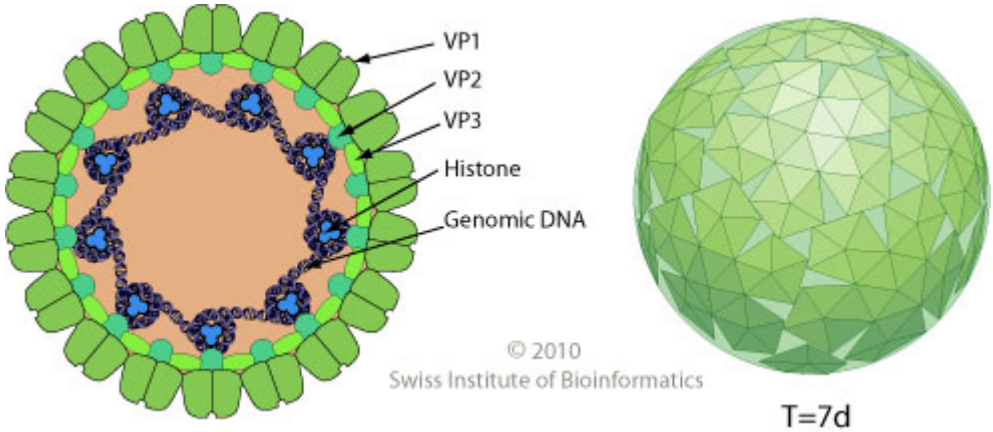

Figure 1: Polyomavirus viral particle (http://viralzone.expasy.org/ With permission of P. Le Mercier)

Virions contain a single molecule of circular dsDNA (Figure 2). The genomic size is fairly uniform within the genus, averaging approximately 5kbp. The DNA constitutes about 10-13% of virion weight. The G+C content varies between 40 and 50%. In the mature virion, DNA is associated with host cell histone proteins in a supercoiled, chromatin-like complex.

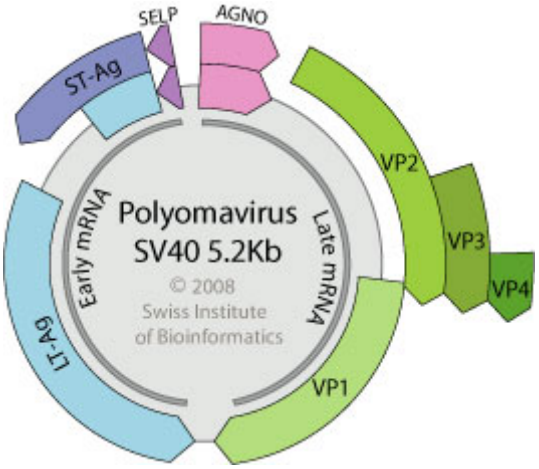

Figure 2: Polyoma virion with a single molecule of circular dsDNA (http://viralzone.expasy.org/ With permission of P. Le Mercier)

Polyomavirus genomes are known to code for 5-9 proteins. Transcription from one side of the ori results in mRNAs encoding early proteins referred to as tumor (T) antigens because they interfere with cell cycle regulation and may in some cases induce tumor formation (large T, small t and others). Alternative splicing appears to be responsible of 2-5 related proteins expressed from each polyomavirus T gene that share amino-terminal sequence. The T antigens initiate bidirectional genome replication as well as transcription of late viral mRNAs which are transcribed form the strand complementary to the one used for early transcription and initiated in the other side of the ori. Greater than 70% of the virion protein is VP1, other virion proteins are VP2 and VP3. The agnoprotein may have some role in viral capsid assembly but is not a component of the mature virion.

1.2.2 New variants

Thirteen human polyomaviruses have been described to date, including JC and BK polyomaviruses (JCPyV and BKPyV) (Gardner et al 1971; Padgett et al, 1971), Karolinska Institute polyomavirus (KIPyV) (Allander et al., 2007), Washington University polyomavirus (WUPyV) (Gaynor et al., 2007), Merkel cell polyomavirus (MCPyV) (Feng et al., 2008), human polyomaviruses 6 and 7 (HPyV6 and HPyV7) (Schowalter et al., 2010), trichodysplasia spinulosa-associated polyomavirus (TSPyV) (van der Meijden et la., 2010), human polyomavirus 9 (HPyV9) (Scuda et al., 2011, Sauvage et al., 2011), Malawi polyomavirus/human polyomavirus 10 (MWPyV/HPyV10) (Buck et al., 2012; Siebrasse et al., 2012), Saint Louis polyomavirus (STLPyV) (Lim et al., 2013), human polyomavirus 12 (HPyV12) (Korup et al., 2013) and NJPyV-2013 (Mishra et al., 2014). Among all these viruses, The International Committee on Taxonomy of Viruses (http://www.ictvonline.org/), in its ninth report, only recognize BKPyV and JCPyV as species in the genus. The other newly described polyomaviruses have been included in a list of other related viruses, which may be members of the genus but have not been approved as species because, although distinctive polyomaviral genomes have been detected by PCR-based assays, viruses have not yet been cultivated by inoculating cells in culture.

1.3 Transmission

1.3.1 Routes of transmission

HPyV have been reported to occur in several organs and tissues. It is clear that JCPyV and BKPyV are excreted in urine and for that reason are prevalently present in sewage. Some new HPyV have been detected in high concentration in skin samples (MCPyV, TSPyV, HPyV6, HPyV7) while some others (HPyV10, STLPyV and HPyV12) have been linked to children with gastroenteritis symptoms and have been described to be present in feces (Lim et al; 2013; Korup et al., 2013; Yu et al., 2012). Polyomavirus have been described as to be quite resistant to inactivation (de Abreu Correâ et al., 2012; Nims et al., 2013) which suggests a potential role for the environment in its transmission. However, the transmission route of mammalian polyomaviruses is unclear. Virus shedding in urine is thought to play a role; however, since some human polyomaviruses have been detected in tonsils the respiratory tract may also be involved in transmission. The recent detection of new human polyomaviruses in skin makes this route a potential way for transmission of some species of polyomaviruses as it happens with human papillomavirus.

1.4 Population and Individual Control Measures

1.4.1 Vaccines and therapies

Diseases caused by JCPyV and BKPyV have been recognized over 40 years in mainly immunosuppressed people, especially when there exists T-cell immunosupresion. Patients undergoing transplants or administered with any immunomodulatory treatment would be susceptible to reactivation of polyomaviral persistent infections. To avoid PML, polyomaviral nephropathy and other polyomavirus caused diseases a vaccine would be needed yet none is available at this time. Currently, there is no treatment for PML, and studies with cidofovir, with antiviral activity against cytomegalovirus; with cytosine

arabinoside, a chemotherapeutic that targets DNA replication; with mefloquine, an antimalarial drug that passes the blood brain barrier and inhibits JCV replication in vitro; and with mirtazapine, a serotonin receptor inhibitor, all failed in demonstrating efficacy against PML (Brew et al., 2010; Ferenczy et al., 2012). However, subcutaneous injections of r-hIL-7 (CYT107), aimed to restore T cell immunity and in combination with a therapeutic vaccine consisting in JCV VP1 protein and a TLR7 agonist, that aimed to boost $CD4^+$JCV-specific T cells, provided promising results (Sospedra et al., 2014). Regarding BKPyV, in recipients of kidney transplants, the emergence of BKPyV syndromes, such as viruria, viremia, and nephropathy, coincided with the advent of potent immunosuppressive therapy. There is currently no standardized protocol for the management of BK viruria or viremia, or established nephropathy.

BKV infection can occur under all combinations of immunosuppressive therapy. Although both humoral and cellular immunity may be essential, BKV-specific T-cell immunity appears to play a pivotal role in controlling BKV replication. Thus, development of T-cell or antibody-based vaccines against BKV is a subject of future research (Pham et al., 2014).

Since several human polyomaviruses have been recently described and are expected to be discovered in the future, the potential of the capsid protein VP1 to form virus-like particles for use as vaccines against polyomavirus infections should be considered (Dalianis et al., 2012).

2.0 Environmental Occurrence and Persistence

2.1 Detection Methods

JCPyV are difficult to grow in cell culture due to the very limited number of cell culture types that can support virus replication in vitro. JCPyV strains presenting rearranged regulatory regions have been described to infect SVG human glial cells however these cells do not support the growth of the ones excreted by healthy individuals and, as a consequence, ones present in environmental samples (archetypal strains with no rearrangements in the regulatory regions) (Bofill-Mas et al., 2001). By coupling infection of rearranged strains in SVG cells to fluorescent marked antibodies, immunofluorecent assays have been developed (Calgua et al., 2011) and could be used for water samples.However, BKPyV and SV40, a simian polyomavirus used as a DNA replication model, infect a wide variety of cell types in vitro (Atwood, 2001). In fact, SV40 was initially isolated from cultures of rhesus monkey kidney cells being used to propagate vaccine strains of poliovirus (Sweet and Hilleman, 1960). The main methods used to detect polyomaviruses in the environment as well as in clinical samples are PCR-based methods. JCPyV, has attracted the most effort regarding detection due to its role as the etiologic agent of PML. Although no gold PCR standard method has been developed yet for JCPyV detection several attempts to evaluate the repeatability of several assays running in different laboratories have been developed (WHO Collaborative study for the 1st WHO International standard for BKV/JCV NAT-based assays). Classical human polyomaviruses were firstly described to occur in the environment in 2000 by applying nested PCR assays (nPCR) detecting JCPyV and BKPyV separately (Bofill-Mas et al., 2000). Since then, some assay for detecting only JCPyV and others that detect JCPyV as well as BKPyV under the denomination of human polyomaviruses (HPyV) have been extensively applied for detection of these viruses in environmental samples. However, it should be taken into consideration that since 2007 human polyomaviruses comprise a group of 13 different species, which still need to be better evaluated in sewage. While classical HPyV have been detected since 2000 in several types of environmental samples by nPCR methods (Bofill-Mas et al., 2000), since 2006, quantitative studies (qPCR) have replaced nPCR based ones. Most of the PCR based assays used to detect polyomaviruses amplify the VP1 gene since variations in this region allow differentiating between different isolates. In fact, the intergenic region, between VP1 and the regulatory region, is the more hypervariable region of the JCPyV genome and has been extensively used to study different JCPyV strains that may provide information on the geographical origin of those populations excreting the virus (Agostini et al., 2001). Also, TAg (T antigen) region may be amplified when JCPyV and BKPyV are detected all together (McQuaig et al., 2009; Biel et al., 2000). Rearrangements may make strains more likely to be pathogenic. To know if the detected viruses present rearrangements of its regulatory region, as may happen with pathogenic JCPyV and BKPyV strains, primers directed to the regulatory region should be used so as not to miss new variants (Bofill-Mas et al., 2001). Recently, new human polyomaviruses, such as MCPyV and others, have been also described to occur in environmental samples by applying nPCR as well as qPCR assays. It should be emphasized that there is still very little information about the presence of these viruses in environmental samples. Better global surveillance is needed.

2.2 Data on Occurrence in the Environment

2.2.1 Excreta

Based on what has been observed for JCPyV and BKPyV, polyomaviruses produce persistent infections and are intermittently excreted in urine. About one-quarter of the human population sheds JCPyV DNA in urine in variable concentrations, referred to as the DNA of archetype virus and excretion may increase as immunosuppressed populations increase (Yogo et al., 1990; McQuaig et al., 2009). The other human polyomaviruses described to date have been described to occur in urine and feces as well as on the skin, so the origin of polyomaviruses in wastewater and water bodies impacted by sewage discharges may include urine, feces and/or skin. It is remarkable that MCPyV is the first virus detected in environmental samples that has been described as probably carcinogenic.

2.2.2 Sewage and surface waters

Presence of human polyomaviruses in sewage and environmental samples impacted by sewage (surface

waters, ground water, drinking water, seawater, sludge) have now been reported. JCPyV and BKPyV were first described to occur in sewage in 2000 (Bofill-Mas et al., 2000). Since then JCPyV and/or BKPyV has been reported in wastewater from all over the world at concentrations as high as 10E+08 GC/l (Each gene copy (GC) are presumed to equal 1 virus) as well as in water matrices impacted by sewage discharges albeit at lower concentrations due to dilution and inactivation. In sludge or/and biosolids produced in wastewater treatment plants, JCPyV is present in high concentrations up to 10^3GC/gr (Bofill-Mas et al., 2006). JCPyV has been proposed as a human fecal viral indicator due to its high prevalence in sewage from all geographical areas where it has been tested thus far and due to the persistent shedding by human population, its highly stable in the environment and because is host specific. All these features make JCPyV a potential Microbial Source Tracking (MST) tool since it could specifically indicate presence of human fecal pollution (Bofill-Mas et al., 2000; 2013). Polyomaviruses are very host specific and apart from human polyomaviruses, other polyomaviruses have been proposed as MST tools such as bovine and ovine polyomaviruses (Hundesa et al., 2010; Rusiñol et al., 2013). Other human polyomaviruses have also been recently described in wastewater samples and other environmental samples impacted by wastewater discharges. MCPyV, KIPyV and WUPyV were reported in sewage and polluted river water samples in 2010 (Bofill-Mas et al., 2010) and since then, some of them have been reported in a number of water matrices. Human polyomavirus 6 (HPyV6) another new human polyomavirus recently described in human skin sample was detected in a sewage sample by Next Generation Sequencing (NGS) in 2011 (Cantalupo et al., 2011) and in 4/12 hospital raw wastewater samples (Fernandez-Cassi et al., 2016). A summary of existing occurrence information is summarized in Tables 1 (raw sewage), 2 (treated sewage) and 3 (other water matrices).

Table 1. Summary of studies reporting detection of human polyomaviruses by PCR-based methods in raw sewage

Area	Virus	Percent positive (# of samples)	Concentration Average (range) GC/L	Reference
Brazil	JCPyV	96% (6/7)	1.2E+06	Fumian et al., 2010
Brazil	JCPyV	100% (24/24)	1E+05 to 1E+06	Fumian et al., 2013
Brazil and Spain (Catalonia)	JCPyV	100% (12/12)	1.5E+04	Calgua et al., 2013b
Germany	HPyV	100% (12/12)	1.0E+08	Hamza et al., 2011
Greece	HPyV	68.8% (33/48)	NR	Kokkinos et al., 2011
Italy	MCPyV	50% (65/131)	NR	Di Bonito et al., 2015
New Zealand	HPyV	97% (36/37)	1.5E+06	Hewitt et al., 2013
Spain	HPyV	100% (6/6)	5.4E+05	Rodríguez-Manzano et al., 2012
Spain (Catalonia)	JCPyV	96% (26/28)	NR	Bofill-Mas et al., 2000
Spain (Catalonia)	BKPyV	77.8% (22/28)	NR	Bofill-Mas et al., 2000
Spain (Catalonia)	JCPyV	100% (6/6)	6.11E+06	Bofill-Mas et al., 2006
Spain (Catalonia)	JCPyV	100% (7/7)	3E+05	Bofill-Mas et al., 2010
Spain (Catalonia)	KIPyV	12% (1/8)	NR	Bofill-Mas et al., 2010
Spain (Catalonia)	WUPyV	25% (2/8)	NR	Bofill-Mas et al., 2010
Spain (Catalonia)	MCPyV	87% (7/8)	NR	Bofill-Mas et al., 2010
Spain (Catalonia)	MCPyV	75% (31/37)	NR	Rusiñol et al., 2015

Area	Virus	Percent positive (# of samples)	Concentration Average (range) GC/L	Reference
USA	JCPyV	100% (13/13)	NR	Rafique et al., 2008
USA	HPyV	100% (39/39)	3E+07	McQuaig et al., 2009
USA	HPyV	100% (15/15)	NR	Hellein et al., 2011
USA	HPyV	100%	NR	Staley et al., 2012

NR - Not Reported

[a] GC: genome copies per liter (L) presume 1 GC= 1 virion;

Table 2. Summary of studies reporting detection of human polyomaviruses by PCR-based methods in treated sewage

Area	Virus	Matrices Analyzed	Percent Positive (# of samples)	Concentration Average (Range) GC/L	Reference
Brazil	JCPyV	Sewage (Outlet)	43% (9/23)	1.3E+05 to 3.1E+06	Fumian et al., 2010
Spain (Catalonia)	JCPyV	Effluent	100% (3/3)	6.3E+02	Bofill-Mas et al., 2006
Spain (Catalonia)	JCPyV	Sludge	100% (8/8)	2.35E+02	Bofill-Mas et al., 2006
Spain (Catalonia)	JCPyV	Biosolids	100% (8/8)	8.0E+02	Bofill-Mas et al., 2006
USA	JCPyV	Fresh to marine water (Tertiary treated wastewater)	22.2% (2/9)	1.2E+02	McQuaig et al., 2009

[a] GC: genome copies per liter (L) presume 1 GC= 1 virion

Table 3. Summary of studies reporting detection of human polyomaviruses by PCR-based methods in different water matrices

Area	Virus	Matrices analyzed	Percent positive (# of samples)	Concentration Average (range) GC/L	Reference
Australia	HPyV	Groundwater; polluted storm water	52% (12/23)	NR	Sidhu et al., 2013
Brazil	HPyV	Lagoon	21% (10/48)	NR	Fongaro et al., 2012

Area	Virus	Matrices analyzed	Percent positive (# of samples)	Concentration Average (range) GC/L	Reference
Brazil and Spain (Catalonia)	JCPyV	River water	100% (12/12)	9.38E+03	Calgua et al., 2013
	MCPyV	River Water	50% (3/6)		
Germany	HPyV	River	97.5%	2.4E+04	Hamza et al., 2009
Germany	HPyV	River	69% (129/188)	1.4E+04	Jurzik et al., 2010
Germany	HPyV	River	66% (73/111)	1.0E+03	Hamza et al., 2011
Italy	MCPyV	River	60% (3/25)	NR	Iaconelli et al., 2015
Italy	JCPyV	River	28% (7/25)	NR	Iaconelli et al., 2015
Italy	BKPyV	River	20% (5/25)	NR	Iaconelli et al., 2015
Italy	MCPyV	Swimming-pools	25% (3/12)	NR	La Rosa et al., 2015
Japan	JCPyV	River	11% (2/18) BKPyV not detected	7.91E+02 to 3.42E+03	Haramoto et al., 2010
New Zealand	HPyV	Seawater	51% (18/35)	1.0E+03	Hewitt et al., 2013
	HPyV	Riverwater	67% (7/15)		
Spain (Catalonia)	JCPyV	River	100% (9/9)	2.7E+04	Albinana-Gimenez et al., 2006
Spain (Catalonia)	JCPyV	River	75% (18/27)	7.42E+02 to 1.3E+03	Albinana-Gimenez et al., 2006
Spain (Catalonia)	JCPyV	River and drinking-water treatment plant (DWTP)	48% (different steps of the DWTP)	1.0E+01 to 1E+03	Albinana-Gimenez et al., 2006
Spain (Catalonia)	MCPyV	River	29% (2/7)	NR	Bofill-Mas et al., 2010
Spain (Catalonia)	MCPyV	River water	29%	NR	Rusiñol et al., 2015
Spain (Catalonia)	MCPyV	Seawater	18%	NR	Rusiñol et al., 2015
USA	HPyV	Seawater	3% (1/32)	NR	Hellein et al., 2011
USA	HPyV	River: near septic systems	6% (2/35)	1.0E+04	Chase et al., 2012
USA	HPyV	Seawater	20% (26/130)	5.0E+02 to 3.55E+05	McQuaig et al., 2012
USA	HPyV	River water	64%	NR	Staley et al., 2012

NR - Not Reported

[a] GC: genome copies per liter (L) presumed 1 GC = 1 virion

2.2.3 Irrigation water and shellfish

Scarce data on the presence of human polyomaviruses in irrigation water and food are available although the consumption of food irrigated or cultured with polluted water might pose a threat regarding the potential oral-fecal transmission of these viruses. Table 3 summarizes data on the occurrence of human polyomaviruses in surface water samples as well as in coastal waters.

Some studies have shown that JCPyV was present in 3/12 irrigation water obtained after lagooning of a

wastewater effluent (Fernandez-Cassi et al., 2016). In the same study, HPyV6 was also detected in one reclaimed wastewater sample. JCPyV and MCPyV have also been detected in 8/8 and 2/8 typical coagulation-filtration-chlorination-UV tertiary treated wastewater effluents used for irrigation purposes (Fernandez-Cassi et al., 2016). JCPyV was detected in 50% of shellfish samples analyzed by Bofill-Mas and coworkers in 2001 (Bofill-Mas et al., 2001) and in an oyster by Souza and coworkers (Souza et al., 2012).

2.3 Persistence

Polyomaviruses have been described as to be highly stable viruses. Polyomaviruses appear to be relatively resistant to UVC irradiation. The viruses are also stable over a wide range of pH found in natural waters.

In evaluating the stability of polyomaviruses in sewage at 20ºC, Bofill-Mas and coworkers estimated a T90 of 26.7 days and a T99 of 61.5 days for JCPyV. For BKPyV the T90 was estimated at 53.6 days and the T9996.8 days (Bofill-Mas et al., 2001). In other experiments conducted by McQuaig and coworkers, HPyV concentrations (JCPyV and BKPyV) in sewage decreased 0.73 $\log_{10}$ and 0.8 $\log_{10}$ over 28 days incubation at 25 and 35ºC, respectively (McQuaig et al., 2009).

3.0 Reductions by Sanitation Management

3.1 Wastewater Treatment

JCPyV is very prevalent in wastewater samples and diverse studies have reported reductions in the number of positive samples after secondary treatment (activated sludge) although the virus is still present in secondary treated effluent samples and typical reductions in concentration are observed to be of approximately between 1 and 2 $\log_{10}$ (Rusiñol et al., 2015; Mayer et al., submitted for publication). Table 2 summarizes data on the occurrence of human polyomaviruses in treated wastewater. A study by Fernández-Cassi and coworkers showed a reduction in the number of JCPyV positive samples before and after lagooning of a secondary wastewater urban effluent (from 9/12 to 3/12) although mean concentrations observed before and after the lagooning ($6.06x10^3$ and $1,38x10^3$ GC/l respectively) were not significantly reduced (Fernández-Cassi et al., 2016). Jurzic and coworkers (2015) have reported that HPyV concentrations did not change significantly during a treatment of secondary sewage effluents in polishing ponds and even higher mean concentrations were detected after the treatment (from $9,3x10^3$GC/l to $4,1x10^3$GC/l).

3.2 Disinfection

There are a number of studies where the stability of the viruses were examined after exposure to extreme conditions (pH, heat) as well as after disinfection with chlorine or UV. The following paragraphs provide an overview of these studies.

As mentioned Polyomaviruses are very stable. Low pH inactivation can be effective, especially at pH or below 3. Bofill-Mas and coworkers found BKPyV and JCPyV viral particles however were stable at pH 5 for 30 minutes (Bofill-Mas et al., 2001) although free DNA was detected when pH lower than 3 were applied. It should be taken into account that destruction of the oncogenic properties of the polyomaviruses may require harsher environments or higher doses of disinfectants than those required to inactivate infectivity.

Relatively high temperatures (>70ºC) are required to effect thermal inactivation of the polyomaviruses. The chemical inactivants that are effective are those that have displayed efficacy for other small non-enveloped viruses (ethanol, sodium hydroxide, formaldehyde). Nims and coworkers have extensively reviewed data on polyomavirus inactivation (Nims et al., 2012). Polyomavirus inactivation has been studied since 1950s when certain polio vaccines became contaminated with SV40. Also the high economic losses produced by avian polyomaviruses have had consequently impact on other inactivation treatments for these viruses which began to be evaluated. More recently, bovine polyomavirus was discovered as a contaminant of commercially-sourced bovine serum and thus there is a continual need to understand how to inactivate these viruses. When tertiary treatment (such as disinfection) reductions observed for JCPyV varied between 2.5 and 4 $\log_{10}$ (Rusiñol et al., 2015; McQuaig et al., 2009). More information on removal by various technologies is warranted. When present in tertiary treated wastewater (that has been disinfected with UV) it is difficult to know whether these viruses remain infectious or not due to difficulties in growing them in cell culture. In assays performed in water spiked with JCPyV Mad4 strain, a decay of 2 $\log_{10}$ was achieved for infectious JCPyV exposed to a UVC fluence of 1,400 J/m^2 revealing high UVC resistance of this dsDNA virus (Calgua et al., 2014). Polyomaviruses seem to be more resistant to UV radiation than are other small non-enveloped viruses such as the parvoviruses and caliciviruses (Nims et al., 2013). Chorine based disinfectants have displayed some level of efficacy against polyomaviruses. Data on disinfection of JCPyV present in seawater with chlorine available from de Abrêu Correa and coworkers evaluated by qPCR addressed the kinetics of inactivation of JCPyV Mad4, a culturable strain, in natural and artificial seawater to be of 1.5 and 1.1 $\log_{10}$ genome copy reductions after 30 min of contact time respectively at initial chlorine concentrations of 2,5mg/l (de Abrêu Correa et al., 2012). No infectivity assays were conducted for this virus. The results obtained provide data that might be applicable to seawater used in shellfish depuration. Data reported on tertiary treatment of secondary wastewater effluents containing JCPyV are diverse: JCPyV reductions up to 1 $\log_{10}$ after application of chlorination, filtration and coagulation and UV disinfection was reported by Rusiñol and coworkers (Rusiñol et al., 2015), while no JCPyV reduction was seen when equivalent treatment was applied in another plant (Fernandez-Cassi et al., 2016). The inactivation of MCPyV has been evaluated in a wastewater treatment plant where 28/37 raw sewage samples tested positive for the virus while 8/32 and 9/22 secondary and tertiary treated had the virus respectively. A 2 $\log_{10}$

reduction was observed after secondary treatment while no further reduction was observed when tertiary treatment (chlorination, filtration, coagulation and UV) was applied (Rusiñol et al., 2015). Rodriguez-Manzano and coworkers reported absence of detectable JCPyV DNA in tertiary treated wastewater samples after sand filtration and UV (Rodriguez-Manzano et al., 2012). No data on the infectious capability of detected virus in tertiary treated water were reported since these viruses cannot grow in cell culture as it has been previously explained. Although infectivity should be highly reduced after tertiary treatment, previous studies detected infectious human adenoviruses after UV disinfection at wastewater treatment plants (Rodríguez-Manzano et al., 2012).

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