GLOBAL WATER PATHOGEN PROJECT

**PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND
EPIDEMIOLOGY ASPECTS**

PAPILLOMAVIRUS

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Summary

Human Papillomaviruses (HPVs) are small, non-enveloped double-stranded DNA viruses of the Papillomaviridae family. These viruses infect squamous epithelia including the skin and mucosae and are associated with a variety of clinical conditions ranging from common warts to intraepithelial dysplastic lesions, which may progress to invasive carcinoma in several anatomical sites. More than 40 HPV genotypes infect the anogenital mucosa causing sexually transmitted diseases; 15 of these can cause cancer of the cervix, the second most common cancer among women worldwide. In addition to cervical cancer, HPV is causally associated with less common cancers at other sites, including cancer of the vulva, vagina, penis and anus, as well as some cancers of the head and neck. HPV viruses are classified as either 'high-risk' or 'low-risk' types depending on their association with the development of cancer.

HPV infection is most commonly transmitted through sexual activity. Other modes of transmission cannot entirely be ruled out, however. Indeed, there is evidence that HPV can survive well outside of its host to potentially be transmitted by non-sexual means. Recently, HPVs have been detected in urban wastewaters, and in surface waters as well, demonstrating that epitheliotropic viruses can find their way into sewage, and subsequently other water environments. The presumed source of contamination is the washing of skin and mucous membranes, but recent detection of HPVs in stool specimens suggests that they may also reach wastewaters as a result of shedding in the feces.

HPVs are not classic waterborne pathogens and their presence in water environments is a relatively recent discovery.

The present chapter describes the state of the art on human papillomaviruses and their presence in water environments. Unfortunately, since HPVs have only recently been identified as potentially waterborne pathogens, little data are available on the fate of these viruses in water environments and their potential for waterborne transmission.

The first section of the chapter covers the epidemiology of HPV infections, clinical manifestations and associated diseases, diagnosis, treatment, and prevention. The second section focuses on the occurrence of these viruses in different water environments. Finally, data on inactivation of HPVs is addressed.

Papillomavirus

Papillomaviruses are known to cause skin infections as well as important sexually transmitted disease but are also found in urine, feces and sewage, and may thus be considered potential waterborne contaminants.

1. Epidemiology of the Disease and Pathogen

1.1 Global Burden of Disease

1.1.1 Global distribution

Human papillomavirus infections are common skin infections and are commonly found in the genital tract of men and women with or without clinical lesions. Data on cervical HPV prevalence in women with normal cytology are provided by a meta-analysis that included 194 studies comprising over 1 million women in 59 countries with normal (non-cancerous) cytologic results. The estimated global prevalence of HPV was 11.7%, with the highest prevalence observed in Sub-Saharan Africa (24%), Eastern Europe (21.4%), and Latin America (16.1%) (Bruni et al., 2010; Bruni et al., 2016). The five most prevalent types worldwide are HPV16 (3.2%), HPV18 (1.4%), HPV52 (0.9%), HPV31 (0.8%) and HPV58 (0.7%), all characterized as involved with a high-risk for cervical cancer. HPV type 16 is the cause of approximately 50% of cervical cancers globally. Types 16 and 18 together account for about 70% of cases (Bruni et al., 2016). Cervical cancer ranks as the 4th cause of female cancers in the world and the 2nd most common female cancer in women aged 15 to 44 years (Bruni et al., 2016). Data on HPV DNA prevalence in men are scant, and there is great variation in the prevalence depending on anatomical sites sampled, sampling methods, and detection assays (Giuliano et al., 2008). A comprehensive literature review performed on the incidence and prevalence of anogenital warts between 2001 and 2012, showed that anogenital warts are common in both males and females throughout the world. The overall (females and males combined) reported annual incidence of anogenital warts (including both new and recurrent) ranged from 160 to 289 per 100,000. Incidence peaked before 24 years of age among females and between 25 and 29 years of age in males (Patel et al., 2013).

1.1.2 Symptomatology

Human papillomavirus (HPV) is a large group of epitheliotropic viruses, members of the family Papillomaviridae, associated with various genital, oral, and cutaneous conditions, both benign and malignant. They present a tropism towards either the cutaneous or mucosal epithelium. Of the types that infect mucosal keratinocytes, some cause benign neoplasms such as warts (low risk-LR types) and others cause malignant neoplasms such as cervical cancer (high risk-HR types). The majority of HPV infections do not cause symptoms or disease. They resolve spontaneously and are cleared within 6-24 months. HPV infections that do not clear involve a risk of progression to epithelial abnormalities with a related risk of subsequent development of HPV-associated diseases. Only about 10% of HPV infections persist. HPV is one of the most common causes of sexually transmitted diseases in both men and women worldwide. It is a well-established cause of cervical cancer and is involved in other anogenital cancers (anus, vulva, vagina and penis). The International Agency for Research on Cancer (IARC) currently defines 12 HR HPV types that are associated with cancers in humans (Group 1,

types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59), although the list may expand with further studies (IARC, 2012a). Cervical cancer is the fourth most common cancer in women, and the seventh overall, with an estimated 528,000 new cases and 266,000 deaths in 2012 (IARC, 2012b). Higher incidence and mortality rates are seen in non-industrialized versus industrialized countries (Bosch et al., 2013). This may be attributable to prevention strategies such as screening and vaccination programs, which are more likely to be available in industrialized countries. Nearly 100% of cervical cancers, 90-93% of anal canal cancers, 36-40% of penile cancers, 40-64% of vaginal cancers and 40-51% of vulvar cancers are attributable to

HPV infection (Nyitray & Iannacone, 2014). Human papillomavirus infection is also a major risk factor for a distinct subset of head and neck squamous cell carcinomas, involving five anatomic sites: oral cavity, oropharynx, nasopharynx, hypopharynx, and larynx. Clinical manifestations of low-risk non-oncogenic HPVs are anogenital warts, also known as condylomata acuminata, which are common, benign but highly infectious tumors that occur in both sexes. HPVs can also cause non-genital cutaneous disease, including common cutaneous warts, flat warts and plantar warts. Table 1 lists the diseases caused by human papillomavirus infection.

Table 1. Main diseases caused by human papillomavirus (adapted from Cobo 2012 and Cubie 2013)

Disease	Site	Types
Palmar and plantar warts	skin	1,2,4
Common warts		1, 2, 4, 7, 26, 27, 29, 41, 57
Flat warts		3, 10, 26-29 41, 49
Carcinoma and keratosis in epidermodysplasia verruciformis		5, 8; less commonly 14,17,20 and 47
Anogenital warts	Anogenital tract	6, 11, 30, 40-45, 51, 54, 61, 72, 81, 89
Anogenital cancers		16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58
Recurrent respiratory papillomatosis	Respiratory tract	6, 11
Oropharyngeal carcinoma		16,18

1.2 Taxonomic Classification of the Agent

1.2.1 Description of the Agent

The human papillomavirus is a member of the family Papillomaviridae. Viruses are non-enveloped, 45 to 60 nm in diameter, and contain a double-stranded DNA genome of ~7.000-8.000 base-pairs, associated with cellular histones. The icosahedral capsid is composed of 72 pentameric

capsomers, containing major (L1) and minor (L2) structural proteins. Figures 1 and 2 show the virion and genome structures of an Alphapapillomavirus (permission obtained from Philippe Le Mercier, ViralZone, SIB Swiss Institute of Bioinformatics).

Figure 1. Virion structure of an Alphapapillomavirus (permission obtained from Philippe Le Mercier, ViralZone, SIB Swiss Institute of Bioinformatics)

Figure 2. Genome structure of an Alphapapillomavirus (permission obtained from Philippe Le Mercier, ViralZone, SIB Swiss Institute of Bioinformatics)

1.2.2 Human types

Human papillomavirus are divided into 5 genera: Alphapapillomavirus (species alpha 1-14), Betapapillomavirus (beta 1-6), Gammapapillomavirus (gamma 1-20), Mupapillomavirus (species 1 and 2) and Nupapillomavirus (species 1) (<http://ictvonline.org/index.asp>). The alpha genus can generally be said to include the viruses associated with the development of mucosal tumors in humans, while the beta genus comprises those that are associated with the development of cutaneous tumors. Betapapillomavirus types are prevalent as asymptomatic infections in normal

skin and mucosa in the general population. The genera gamma, mu, and nu include further cutaneous HPVs. Below the species level, Papillomaviruses can be differentiated into types. HPV types belonging to different genera share less than 60% similarity of the L1 portion of the genome. Different viral species within a genus show between 60 and 70% similarity (Bzhalava et al., 2014). Each virus type is species-specific and tropic for a specific anatomical region of the cutaneous or mucosal epithelium. To date, the highest HPV type number awarded is HPV210 (www.hpvcenter.se, accessed on 21 August 2016). The list of Human Papillomavirus types recognized by the International Human Papillomavirus Reference Center (<http://www.hpvcenter.se/>) is shown in Table 2.

Table 2. Human Papillomavirus types (International Human Papillomavirus Reference Center, <http://www.hpvcenter.se/>)

Genus	Clinical manifestations	Species (Types)
Alpha	Alpha HPVs preferentially infect the anogenital and oral mucosa, causing both malignant and benign neoplasms. Cutaneous lesions have also been observed.	Alpha 1 (HPV32, HPV42); Alpha 2 (HPV3, HPV10, HPV28, HPV29, HPV77, HPV78, HPV94, HPV117, HPV125, HPV160); Alpha 3 (HPV61, HPV62, HPV72, HPV81, HPV83, HPV84, HPV86, HPV87, HPV89, HPV102, HPV114); Alpha 4 (HPV2, HPV27, HPV57); Alpha 5 (HPV26, HPV51, HPV69, HPV82); Alpha 6 (HPV30, HPV53, HPV56, HPV66); Alpha 7 (HPV18, HPV39, HPV45, HPV59, HPV68, HPV70, HPV85, HPV97); Alpha 8 (HPV7, HPV40, HPV43, HPV91); Alpha 9 (HPV16, HPV31, HPV33, HPV35, HPV52, HPV58, HPV67); Alpha 10 (HPV6, HPV11, HPV13, HPV44, HPV55 (reclassified as HPV44 subtype), HPV74); Alpha 11 (HPV34, HPV73, HPV64 (reclassified as HPV 34 subtype), HPV177); Alpha 13 (HPV54); Alpha 14 (HPV71, HPV90, HPV106)
Beta	Beta HPVs cause cutaneous lesions in humans. Infections exist in latent form in the general population and are activated under conditions of immunosuppression.	Beta 1 (HPV5, HPV8, HPV12, HPV14, HPV19, HPV20, HPV21, HPV24, HPV25, HPV36, HPV46 (reclassified as HPV20 subtype), HPV47, HPV93, HPV98, HPV99, HPV105, HPV118, HPV124, HPV143, HPV152, HPV195, HPV196); Beta 2 (HPV9, HPV15, HPV17, HPV22, HPV23, HPV37, HPV38, HPV80, HPV100, HPV104, HPV107, HPV110, HPV111, HPV113, HPV120, HPV122, HPV145, HPV151, HPV159, HPV174, HPV182, HPV198, HPV209); Beta 3 (HPV49, HPV75, HPV76, HPV115); Beta 4 (HPV92); Beta 5 (HPV96, HPV150, HPV185)
Gamma	Gamma HPVs cause cutaneous lesions in humans	Gamma 1 (HPV4, HPV65, HPV95, HPV158, HPV173, HPV205); Gamma 2 (HPV48, HPV200); Gamma 3 (HPV50, HPV188); Gamma 4 (HPV60); Gamma 5 (HPV88); Gamma 6 (HPV101, HPV103, HPV108); Gamma 7 (HPV109, HPV123, HPV134, HPV138, HPV139, HPV149, HPV155, HPV170, HPV186, HPV189, HPV193); Gamma 8 (HPV147, HPV112, HPV119, HPV164, HPV168, HPV176); Gamma 9 (HPV116, HPV129); Gamma 10 (HPV121, HPV130, HPV133, HPV142, HPV180, HPV191); Gamma 11 (HPV126, HPV136, HPV140, HPV141, HPV154, HPV169, HPV171, HPV181, HPV202); Gamma 12 (HPV127, HPV132, HPV148, HPV157, HPV165, HPV199, HPV210) Gamma 13 (HPV128, HPV153); Gamma 14 (HPV131); Gamma 15 (HPV135, HPV146, HPV179, HPV192); Gamma 16 (HPV137); Gamma 17 (HPV144); Gamma 18 (HPV156); Gamma 19 (HPV161, HPV162, HPV166); Gamma 20 (HPV163, HPV183, HPV194); Gamma 21 (HPV167) Gamma 22 (HPV172 ^a); Gamma 23 (HPV175 ^a); Gamma 24 (HPV178 ^a , HPV190 ^a , HPV197 ^a); Gamma 25 (HPV184 ^a); Gamma 26 (HPV187 ^a); Gamma 27 (HPV201); Gamma (HPV203)
Mu	Mu HPVs cause cutaneous lesions in humans	Mu 1 (HPV1); Mu 2 (HPV63); Mu (HPV204)
Nu	Nu HPV causes cutaneous lesions in humans	Nu 1 (HPV41)

^a Assignments are tentative and not official.

1.2.3 Novel types

A distinct type is established when the nucleotide sequence of the L1 gene differs from that of any other characterized type by at least 10% (Bernard et al., 2010; de Villiers et al., 2004). Novel HPV types are given a unique number only after the whole genome has been cloned, deposited at the International HPV Reference Center established at the German Cancer Research Center (Bernard et al., 2010; de Villiers et al., 2004), and resequenced for the purposes of verification (www.hpvcenter.se). The international HPV Reference Center also maintains the reference clones and distributes samples of the reference material for research use. Molecular methods most often used for identification and characterization of novel PV types include PCR, rolling circle amplification and next-generation sequencing (Kocjan et al., 2015). As for animal PV, the most recent published review reported 122 animal PV types, identified from 54

different animal species (Rector & Van, 2013). According to the Papillomavirus Episteme database (PaVE) website (<https://pave.niaid.nih.gov/>), 152 animal PV types exist, identified from 72 different animal species (accessed on 21 August 2016).

1.3 Transmission

1.3.1 Routes of transmission

HPV infections are primarily transmitted through direct skin-to-skin or skin-to-mucosa contact. Initial infection by HPVs is thought to occur through microabrasions of the epithelium, thus allowing entry of HPV particles into cells of the basal layer. Infection of the basal layer is the first step to the potential development of HPV-related disease. Human papillomavirus is the most common sexually transmitted infection. More than 40 HPV types can be easily spread through direct sexual contact by vaginal, anal,

and oral sex, from the skin and mucous membranes of infected people to the skin and mucous membranes of their partners. The Centers for Disease Control (CDC, <http://www.cdc.gov/>) estimates that more than 90 percent and 80 percent of sexually active men and women, respectively, will be infected with at least one type of HPV at some point in their lives (Chesson et al., 2014). The acquisition of HPV infection is associated with many risk factors such as a high number of sexual partners, early age at start of sexual activity, cigarette smoking, and previous sexually transmitted diseases. Since HPV is usually asymptomatic, most individuals are not aware that transmission of HPV has occurred and can unknowingly transmit the virus to others. Even though sexual transmission is well documented as the primary transmission route of HPV, the virus may also be transmitted by nonsexual routes. Indeed, HPV DNA has been detected in blood as well as reproductive and placental cells, and has been found among infants, children, and individuals who have never had sexual intercourse (Freitas et al., 2013). The potential routes of nonsexual transmission of HPV have been reviewed by Ryndock and Meyers (Ryndock & Meyers, 2014). Possible routes of horizontal transmission are the fingers and mouth, fomites, and skin contact other than sexual contact. A potential nonsexual route of HPV transmission – self-inoculation – is described in studies on HPV in female virgins (Tay et al., 1990). HPV infection by self-inoculation has also been documented in cases of genital warts among children that had not been sexually abused. Vertical transmission from mother to child is another route of transmission. The identification of HPV as an extremely stable virus as well as its ability to be resistant to common disinfectants suggests that this virus may be transmitted via contaminated fomites and surfaces, and through nosocomial transmission (Meyers et al., 2014; Ryndock & Meyers, 2014). Recently, the possibility of waterborne transmission has been raised (Fratini et al., 2013; Reynolds, 2012), due to the fact that HPVs are excreted both in the urine and in the feces. And indeed, HPV has recently been detected both in sewage and in sewage sludge (Bibby & Peccia, 2013; Cantalupo et al., 2011; La Rosa et al., 2013; Symonds, 2008). Consequently, these viruses may reach waters receiving sewage discharge. Although waterborne transmission has never been demonstrated, HPV DNA has recently been detected in different water environments, as discussed in the following sections.

1.3.2 Reservoirs

Papillomaviruses occur in many species within the animal kingdom. Papillomavirus genomes have been isolated and characterized from reptiles, birds, marsupials and many other mammalian species (Bernard et al., 2010; Rector & Van, 2013). The members of this family are species-specific, however, and humans are the only reservoir for HPV.

1.3.3 Incubation period

The incubation period of HPV is quite long, and rather variable depending on host immune status. The average

incubation period, which begins immediately after initial contact with an infected person, is usually two to three months, but can range from one to 20 months. However, when HPV is transmitted from one person to another, the virus infects the top layers of the skin and can remain inactive or latent for months or possibly years before warts or other signs of HPV infection appear. Prospective studies reported that the median time between infection with HPV types 6 or 11 and the development of anogenital warts was 11 to 12 months among males, and 5 to 6 months among young females (Patel et al., 2013).

1.4 Population and Individual Control Measures

1.4.1 Vaccines

The WHO recognizes the importance of HPV-related diseases as global public health problems, and recommends adopting and implementing national immunization programs. Vaccinating against HPV is the best way to prevent HPV-related cancers and disease. Two vaccines are currently available for the prevention of HPV-related disease: a quadrivalent vaccine, licensed in 2006, and a bivalent vaccine, licensed in 2007. These vaccines are based on virus-like particles assembled from recombinant main capsid protein L1 produced in eukaryotic systems. These vaccines have no therapeutic effect in infected individuals. An additional vaccine has been developed, in which the number of HPV types is increased to 9 by adding types 31, 33, 45, 52 and 58 to the quadrivalent vaccine (Pils & Joura, 2015). This recently licensed 9-valent-6/11/16/18/31/33/45/52/58 vaccine provides good, long-term protection against infection with those HPV types, and their related diseases, potentially preventing ~90% of cervical and other HPV-related cancers and precancers. Current HPV vaccines may drastically reduce the incidence of cervical cancer and other HPV-related cancers and diseases, if widely implemented.

1.4.2 Hygiene measures

It is not known whether hand washing and disinfection of surfaces reduces contamination and may aid in reducing transmission. One study, aimed at assessing the presence of HPV on frequently used equipment in gynecological practice (glove box, lamp of a gynecological chair, gel tubes for ultrasound, colposcope and speculum), found that 18% of samples were HPV positive, supporting the hypothesis of possible nosocomial transmission (Gallay et al., 2015). HPV transmission by endocavity vaginal ultrasound has been suggested, after high-risk HPV DNA was shown to persist on ultrasound probes despite disinfection with quaternary ammonium wipes and probe cover (Casalegno et al., 2012).

2. Environmental Occurrence and Persistence

2.1 Detection Methods

Human papillomaviruses cannot be propagated in tissue culture as they require differentiated epithelial cells in order to complete their life cycle (Doorbar, et al., 2012). This precludes the use of traditional virus culturing

techniques for HPV. Further, due to their strict species specificity, HPVs cannot be propagated in laboratory animals. The production of viral like particles has recently allowed the development of serologic assays; however, since the development of antibodies occurs 12-15 months after infection with HPV, these assays are not useful for the detection of acute infection. In addition, natural infection does not consistently lead to the production of a detectable antibody response. Thus, the traditional tools commonly employed in diagnostic virology are not suitable for HPV detection, a task which is therefore mainly based on molecular techniques. Several consensus or general PCR primers are used to amplify a broad spectrum of HPV genotypes by targeting the most conserved regions of the HPV genome - the L1 and E1 regions. The MY09/11 system identifies high-risk HPV genotypes by amplifying a 450-bp sequence in the conserved L1 region (Manos et al., 1989). The GP5+/GP6+ PCR system uses a pair of consensus primers to amplify a 140-bp DNA fragment in the L1 gene (de Roda Husman et al., 1995). This region is internal to the sequence flanked by the MY primer, therefore the GP5+/GP6+ primers may be used either as a single

oligonucleotide primer, or in "nested" PCR, following MY oligonucleotide primer amplification. This nested PCR system, validated on clinical samples, has proved to be a powerful tool for HPV detection even in environmental samples (La Rosa et al., 2013; Iaconelli et al., 2015; La Rosa et al., 2015). Recently, next generation sequencing has been shown to be effective in the detection of a wide range of HPVs, including novel types, in both clinical and environmental samples (Bibby & Peccia, 2013; Bzhalava et al., 2014; Cantalupo et al., 2011; Johansson et al., 2013; Kocjan et al., 2015). Since deep sequencing technologies are continuously evolving with increasing throughput and decreasing costs, it is likely that an expanding diversity of HPVs will continue to be revealed.

2.2 Data on Occurrence in the Environment

The surveillance of sewer systems, successfully employed for monitoring of enteric viruses, can also be applied to epitheliotropic viruses such as oncogenic HPVs, which can find their way into sewage and into waters impacted by feces, urine and sewage. Table 3 summarizes the studies on HPVs in environmental samples.

Table 3. Detection of HPVs in environmental raw sewage and other samples

Area	Viruses	Matrices analyzed	Percent Positive (# of samples)	Concentration Average (range), GC/L ^a	Reference
Italy	HPV6, 9, 11, 16, 20, 25, 76, 80, 104, 110, 111, 120, 145, and putative new types of the beta genus.	Raw Sewage	81.0% (34/42)	NR	La Rosa et al., 2013
Italy	HPV8, 17, 21, 25, 32, 80, 99, 105, and putative new types of the beta genus	River	56.0% (14/25)	1.08E+03 to 3.70E+04	Iaconelli et al., 2015
Italy	HPV8, 12, 23, 25, 120 and unclassified HPVs	Swimming pools	50.0% (7/14)	1.27E+03 to 1.13E+04	La Rosa et al., 2015
USA	HPV	Raw Sewage	17.0% (2/12)	NR	Symonds, 2008

NR - Not Reported

^aGC/L= *Genome copy* number per liter; assuming 1GC =1 viron Cantalupo et al., 2011; Bibby and Peccia, 2013 described observations of HPV 112 and 7, 10, 16, 18, 34, 49, 53, 63, 90, 92, 129 in sewage and sewage sludge in Spain and USA but no quantitative data were available.

2.2.1 Excreta

A recent study showed that a wide range of both alpha (including high-risk types) and beta, mucosal and cutaneous papillomaviruses are detected in human stool samples (Di Bonito et al., 2015). In this study, 13 samples (12.6%) were positive for nine genotypes: HPV32 (LR, alpha1), HPV39 (HR, alpha7), HPV44 (LR, alpha10), HPV8 (beta1), HPV9, HPV23, HPV37, HPV38 and HPV120 (beta2). Two putative novel genotypes of the beta genus, species 1 and 2, were also detected. The tissues of origin are unknown, since feces can collect HPVs throughout the entire digestive system, from the oral cavity, pharynx and esophagus to the stomach, and small and large intestine. Moreover, numerous studies have detected HPVs in anal-rectal cytology specimens, as well as from human colorectal mucosal biopsies (Bean & David, 2010). HPV is detected at low percentages (4 to 10%) in urine samples from low risk populations, such as sexually unexposed girls and healthy women with a normal cytology, and in higher proportions (>69%) among patients with cervical intraepithelial neoplasia grade 2+, with increasing prevalence rates the higher the grade of the lesion (Nicolau et al, 2014). During urination, urine gets contaminated by impurities lining the urethral opening, including mucus and debris of exfoliated cells from the vagina, cervix and uterus (Kitchener & Owens, 2014; Pathak et al., 2015). Very few studies have investigated HPV DNA levels in urine. Recently, Vorsters and coworkers used type-specific quantitative PCR to compare HPV DNA in cervical and urine samples in HPV16- and HPV18-positive women and detected 2.34×10^5 mean copies/mL of urine for HPV16 and 1.34×10^3 mean copies for HPV18 (Vorsters et al., 2016).

2.2.2 Sewage

The first evidence of the presence of HPVs in water environments comes from a study addressing the diversity of viruses in samples of raw sewage, final effluent, and the marine environment collected in 2006 and 2007 (Symonds, 2008). The study, conducted in the United States, found HPVs in 2/12 raw sewage samples but not in treated sewage or in seawaters. Broad range primers FAP59/FAP64 targeting the L1 gene were used to detect HPV, after whole genome amplification. Cantalupo and coworkers later studied the viral diversity in raw sewage collected from the United States, Spain, and Ethiopia by deep sequencing (Cantalupo et al., 2011). They identified 234 previously known viruses, belonging to many different viral families, including HPV112 and the newly discovered human polyomavirus 6, both tropic for skin, suggesting that viruses from human skin, as well as from stools, can find their way into sewage. Another study on viral pathogen diversity in sewage sludge – the semi-solid waste resulting from wastewater treatment – identified 43 different human viruses, including HPV (Bibby & Peccia, 2013). Shotgun metagenomics was applied to sewage sludge samples from five wastewater treatment plants throughout continental

United States, each serving between 100,000 and 1,000,000 people. Herpesvirus and Papillomavirus, both highly prevalent in the general population, were the most ubiquitous and abundant viruses identified, with 100% occurrence in the samples analyzed. The authors suggested that this high prevalence, coupled with the viruses' elevated relative abundance demonstrate the potential for these viruses to be used as indicator or source tracking organisms for human waste. Specifically, 11 different HPV types (both mucosal and cutaneous) were detected, belonging to the alpha, beta, gamma, and mu genera: Alpha-2 (HPV10), Alpha-6 (HPV53), Alpha-7 (HPV18), Alpha-8 (HPV7), Alpha-9 (HPV16), Alpha-11 (HPV34), Alpha-14 (HPV90), Beta-3 (HPV49), Beta-4 (HPV92), Gamma-9 (HPV129), and Mu-2 (HPV63). Of these, as we have seen, HPV16 and 18 are causal agents of cervical cancer (IARC group I), and are also linked to vaginal, vulval, anal and penile cancer. HPV34 and HPV53 are also "possibly carcinogenic" (IARC, 2012a; IARC, 2012b). More recently, the occurrence and genetic diversity of HPVs in urban wastewaters was investigated in Italy (La Rosa et al., 2013). A wide range of cutaneous (HPV9, HPV20, HPV25, HPV76, HPV80, HPV104, HPV110, HPV111, HPV120, HPV145) and mucosal (HPV16, HPV6, HPV11) types were detected in 81% of raw sewage samples collected. Multiple virus genotypes and multiple virus strains belonging to the same genotype (up to three different sequences) were present in a single sewage sample. Low-risk HPV6 and 11 were the most prevalent genotypes detected in sewage samples, but the high risk HPV16 genotype was also present, as well as subgenomic fragments from putative novel genotypes. Clearly, the presence of HPV-DNA in sewage samples does not necessarily imply that the viruses in question are viable and infectious. Unfortunately however, the study of infectivity presents considerable difficulties, both for the lack of in vitro systems able to ascertain HPV replication, and in light of the impossibility of using animal models due to the virus' species-specificity.

2.2.3 Surface waters

Viral pathogens can end up in fresh or marine waters when treated sewage effluent is discharged. Iaconelli and co-workers recently examined the occurrence of HPV in surface waters by monitoring two rivers in Northwestern Italy (Iaconelli et al., 2015). The study in question, using nested PCR assays, identified HPVs in 14/25 (56%) river samples. Eight known papillomavirus genotypes were detected, belonging to the alpha1 (HPV32), beta1 (HPV8, HPV21, HPV25, HPV99, and HPV105), and beta2 (HPV17 and HPV80) species. A number of putative new genotypes, showing less than 90% nucleotide identity with known prototypes were detected. Quantitative data, using a SYBR Green q-PCR assay targeting the FAP region within the L1 capsid gene, yielded an HPV viral load in river water samples ranging between 1.08×10^3 and 3.7×10^4 GC/L.

2.2.4 Recreational waters

A variety of viruses may be discharged in pool waters by infected individuals or asymptomatic carriers. The occurrence of HPV in swimming pool waters has recently been studied in Italy (La Rosa et al., 2015). Polyomavirus, enteric viruses (adenovirus, norovirus and enterovirus), and bacteriological parameters (fecal indicator bacteria, heterotrophic plate count, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) were also examined. Human papillomaviruses were identified in 7/14 (50%) samples. These belonged to five known types of the beta1 (HPV8, 12, 25) and beta2 (HPV23 and 120) genera. The study also found two putative new HPV genotypes. None of the enteric viruses tested were detected in the analyzed samples, and pool waters met the microbiological requirements defined by the national code. The implications on human health arising from the presence of HPV DNA in pool waters remain difficult to interpret.

2.3 Persistence

HPVs are very stable viruses, able to survive on fomites and surfaces for days (Ryndock & Meyers, 2014). HPV is resistant to heat and drying, and is able to survive on inanimate objects, such as clothing and laboratory equipment, that have come into contact with infected patients. The precise survival time is unknown, however. HPV has been shown to retain infectivity even after dehydration for 7 days (Roden et al., 1997). In vitro infectivity after desiccation was compared for pseudotype HPV-16 virions - a model for high-risk type genital HPV - and bovine papillomavirus type 1 (BPV-1), a papillomavirus known to be transmitted via fomites. The two viruses exhibited similar resistance to desiccation, with no inactivation, 0.33 log₁₀ and 0.15 log₁₀ reductions when dehydrated for 1, 3, and 7 days, respectively, at room temperature. More recently, Ding and co-workers demonstrated persistent infectivity of HPV16 pseudovirus in various scenarios of environmental contamination and durability of native HPV16 virions in both wet and dry

environments (Ding et al., 2011). They reported that HPV16 remains infectious for at least 7 days on a wet surface. This information provides the basis for the hypothesis regarding nonsexual HPV transmission. Although HPV have been identified abundantly in water environments, its resistance to inactivation and its stability in such environments are not known.

3. Reduction by Sanitation Management

3.1 Wastewater Treatment

No data are available on removal by wastewater treatment. However, there is some information on disinfection.

3.2 Disinfection

Little is known about human papillomavirus susceptibility to disinfection. This is due to the difficulty in producing sufficiently high titers of infectious HPV particles, and the lack of a suitable assay to test for infectivity. What little is known about HPV resistance to virucides derives from studies using recombinant particles, or from a hospital study on potentially infected equipment (Ryndock & Meyers, 2014). Meyers et al., (2014) tested the susceptibility of HPV16 virions to 11 common clinical disinfectants: 70% and 95% ethanol and isopropanol, 2.4% and 3.4% GTA (glutaraldehyde), 0.55% OPA (ortho-phthalaldehyde), a triple phenolic, a 0.25% and 1.2% PAA-silver-based disinfectant and 0.525% hypochlorite). Results showed that commonly used clinical disinfectants, including those used in medical and dental healthcare facilities, have no effect on HPV16 infectivity. HPV was shown to be resistant to inactivation by both GTA (shown to be effective against adenoviruses, parvoviruses, calciviruses and many enteroviruses), and OPA, a new alternative to GTA. Like most non-enveloped viruses, HPV was resistant to alcohol-based disinfection, including ethanol and isopropanol. HPV was, on the other hand, susceptible to hypochlorite and to high concentrations of PAA-silver-based disinfectant.

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