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Emerging and potentially emerging viruses in water environments

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Abstract. Among microorganisms, viruses are best fit to become emerging pathogens since they are able to adapt not only by mutation but also through recombination and reassortment and can thus become able to infect new hosts and to adjust to new environments. Enteric viruses are among the commonest and most hazardous waterborne pathogens, causing both sporadic and outbreak-related illness. The main health effect associated with enteric viruses is gastrointestinal illness, but they can also cause respiratory symptoms, conjunctivitis, hepatitis, central nervous system infections, and chronic diseases. Non-enteric viruses, such as respiratory and epitheliotrophic viruses are not considered waterborne, as they are not readily transmitted to water sources from infected individuals. The present review will focus on viral pathogens shown to be transmitted through water. It will also provide an overview of viruses that had not been a concern for waterborne transmission in the past, but that may represent potentially emerging waterborne pathogens due to their occurrence and persistence in water environments.

Key words: viruses, waterborne, emerging, drinking water, recreational water.

Riassunto *(Infezioni emergenti e potenzialmente emergenti in ambienti acquatici)*. I virus sono tra i principali agenti di infezioni emergenti, in quanto sono in grado di acquisire nuove caratteristiche biologiche attraverso fenomeni di mutazione, ricombinazione e riassortimento genico, adattandosi così a nuovi ospiti e nuove nicchie ecologiche. I virus enterici sono tra le principali cause di malattie infettive di origine idrica, che possono manifestarsi come casi sporadici o cluster epidemici. Le manifestazioni cliniche più comuni sono le gastroenteriti; tuttavia i virus enterici sono in grado di causare anche sintomi respiratori, congiuntiviti, epatiti, infezioni del sistema nervoso centrale e malattie croniche. La presente rassegna fornisce una panoramica dei virus enterici tradizionalmente associati alle malattie idrotrasmesse; mostra inoltre dati epidemiologici su alcuni gruppi di virus che non sono stati associati a malattie idrodiffuse in passato (inclusi virus respiratori ed epiteliotropici), ma che possono rappresentare nuovi patogeni emergenti a causa della loro presenza e persistenza in ambienti acquatici.

Parole chiave: virus, malattie idrotrasmesse, patogeni emergenti, acque potabili, acque ricreative.

INTRODUCTION

The human illnesses associated with enteric viruses are diverse and their severity varies. The symptoms most commonly associated with enteric viral infections are those of gastroenteritis (GE). Some enteric viruses, however, are responsible for respiratory symptoms, conjunctivitis, hepatitis, central nervous system infections (aseptic meningitis, poliomyelitis) and muscular syndromes (fibromyalgia, myocarditis). Enteric viruses have also been implicated in some chronic diseases, including diabetes and chronic fatigue syndrome. Patients suffering from viral infections may excrete 10^5 to 10^{11} virus particles per gram of stool. Consequently virus concentrations in wastewaters receiving fecal matter are high. The degree of wastewater inflow contamination depends on the season, the prevalence of viral infections and the characteristics of circulating viruses. Wastewater treatment systems, even when properly functioning, remove only about 20-80% of enteric viruses [1-6], allowing a significant viral load to be released in effluent discharge and spread in the environment, transported through groundwater, estuarine water, seawater and rivers. The concentration of enteric viruses in water can vary significantly in time and space, depending also on whether the source of pollution is continuous or the result of a sudden influx of fecal contamination.

Viruses cannot replicate outside their host's tissues and therefore cannot multiply in the environment. They can, however, survive in the environment for extended periods of time, longer than most intestinal bacteria, making it unsafe to rely solely on bacteriological water quality standards. Viruses have been reported to survive and remain infective for up to 130 days in seawater, for up to 120 days in freshwater, and for up to 100 days in soil at 20-30 °C [7]. Comparisons between enteric viruses also show variability, with adenoviruses potential-

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ly surviving longer in water than other enteric viruses, such as hepatitis A virus and poliovirus [8]. Despite the relatively low concentration of viruses in water, these micro-organisms carry health risks, since they have very low infectious doses (10-100 virions) so that even a few viral particles in water can pose health risks.

The global impact of waterborne disease is difficult to assess. This is due to the wide range of clinical manifestations associated with waterborne viruses, to potentially long latency periods, and to the fact that in some cases, diseases related to water can also be transmitted in other ways. Moreover, evidence of water contamination is not always available by the time an outbreak of disease is identified, mainly due to the difficulties associated with the detection of viruses in water. Indeed, viral detection in water environments presents particular challenges due to low pathogen concentrations, and the multitude of enteric viruses requiring different analytical methods. Data on viral waterborne diseases is fragmented, often focusing on specific countries and/or pathogens. In US, the Centre for Disease Control (CDC), the Environmental Protection Agency, and the Council of State and Territorial Epidemiologists established the Waterborne Disease and Outbreak Surveillance System (WBDOSS) to collect and report data related to occurrences and causes of waterborne disease outbreaks associated with drinking and recreational waters. Data from the WBDOSS drinking water surveillance from 1971 (a 36-year period) were recently reviewed by Craun and collaborators [9]: an etiology was identified only in 56.1% of the outbreaks reported (7.9% of viral origin). Since viral outbreaks linked to recreational or drinking waters are often underestimated [10, 11] enteric viruses could be responsible for a significant portion of outbreaks of "undetermined" etiology. According to the last WBDOSS report, during the 2007-2008 surveillance period, 13.9% of drinking and 4.8% of recreational water-associated outbreaks were caused by viruses [12, 13]. The number of outbreaks of unknown etiology was 11.1% for drinking and 21.6% for recreational waters, thus indicating a decrease of "unidentified" etiologies in recent years, probably due to the availability of improved methods for the detection of waterborne pathogens.

Emerging waterborne diseases can be defined as those that have newly appeared, or are rapidly increasing in incidence and/or geographic range, or those for which water transmission routes have only recently been recognized. Complex relations between the pathogen, the host, and the natural environment determine the emergence of pathogens. In the globalized world, the circulation of microbes are facilitated so that infectious agents are capable of spreading rapidly anywhere in the world. Among microorganisms, viruses are best fit to become emerging pathogens since they are able to adapt not only by mutation but also through recombination and reassortment and can thus become able to infect new hosts and to adapt to new environments. Emerging waterborne enteric viruses belong to the families *Caliciviridae* (norovirus), *Picornaviridae* (enterovirus and hepatitis A virus) and *Adenoviridae* (adenovirus). These pathogens are included in the Environmental Protection Agency Contaminant Candidate List 4 - CCL, a list of contaminants that are currently not subject to any proposed or promulgated primary drinking water regulations in the USA, but are known or anticipated to occur in public water systems. All these viral pathogens have been detected in sewage, surface water, groundwater and drinking water sources around the world. Other virus groups are considered to be potentially emerging waterborne pathogens and include hepatitis E virus, the viral agent of avian influenza, coronavirus, polyomavirus, picobirnavirus, and papillomavirus.

The present review will focus on viral pathogens shown to be transmitted through water. It will also provide an overview of viruses that had not been a concern for waterborne transmission in the past, but that may represent potentially emerging waterborne pathogens due to their occurrence and persistence in water environments. *Table 1* shows human viruses described in this review, that are potentially transmitted by the waterborne route.

Table 1 | *Human viruses potentially transmitted by the waterborne route.*

Virus group	Genus	Family	Disease caused
Norovirus	Norovirus	*Caliciviridae*	Gastroenteritis
Human enterovirus A-D	Enterovirus	*Picornaviridae*	Paralysis, herpangina, meningitis, respiratory disease, hand-foot-and-mouth disease, myocarditis, heart anomalies, rush, pleurodynia, diabetes
Hepatitis A virus	Hepatovirus	*Picornaviridae*	Hepatitis
Human adenovirus A-G	Mastadenovirus	*Adenoviridae*	Gastroenteritis, respiratory disease, conjunctivitis
Hepatitis E virus	Hepevirus	*Hepeviridae*	Hepatitis
Influenza A virus	Influenza A virus	*Orthomyxoviridae*	Influenza
Human coronavirus	Coronavirus	*Coronaviridae*	Gastroenteritis, respiratory disease
Human polyomavirus	Polyomavirus	*Polyomaviridae*	Skin diseases, progressive multifocal leukoencephalopathy, nephropathy, hemorrhagic cystitis
Human picobirnavirus	Picobirnavirus	*Picobirnaviridae*	Diarrhea
Papillomavirus	Papillomavirus	*Papillomaviridae*	Genital warts, throat warts (rarely), skin warts, cervical cancer and other, less common but serious cancers

VIRAL PATHOGENS TRANSMITTED THROUGH WATER

Norovirus

Noroviruses (NoV) are non-enveloped, single-stranded RNA viruses, belonging to the family *Caliciviridae*, currently subdivided into five genogroups (GI, GII, GIII, GIV, GV), comprising at least 40 genetic clusters, subdivided into various types. Genotypes infecting humans are those belonging to GI, GII and GIV. Human NoV is emerging as the leading cause of epidemic GE and as an important cause of sporadic GE in both children and adults worldwide. It is responsible for nearly half of all GE cases and for more than 90% of non-bacterial GE epidemics worldwide [14, 15]. NoV infection induces vomiting, diarrhea, mild fever, abdominal cramping, and nausea. Although typically with a self-limiting disease of short duration, new evidence suggests that the illness can be severe and sometimes fatal, especially among vulnerable populations -young children, the elderly and the immunocompromised- and is a common cause of hospitalization. Numerous reports have associated NoV with clinical outcomes other than GE, such as encephalopathy, disseminated intravascular coagulation, convulsions, necrotizing enterocolitis, post-infectious irritable bowel syndrome, and infantile seizures. Outbreaks have often been reported from institutional settings (*e.g.*, nursing homes, hospitals, day care, cruise ships), where NoV can be especially difficult to control due to efficient person-to-person transmission, and virus resistance to common cleaning agents.

In Europe and the United States, NoV epidemics have been reported to increase in both incidence and severity, probably as a result of an increased pathogenicity and/or transmissibility of new strains [16, 17]. The availability of improved detection techniques, combined with an enhanced awareness to the potential risks associated with this pathogen, has led to the establishment of several surveillance systems, national (Calicinet in the US, Vironet in Canada, the Norovirus Surveillance Network in Australia and New Zealand) as well as global (Noronet). The Foodborne Viruses in Europe (FBVE) network has been collecting laboratory and epidemiological data on NoV outbreaks in Europe since 1999.

The primary mode of transmission is faecal-oral and occurs through the ingestion of contaminated water, consumption of contaminated food, or direct contact with environmental surfaces or infected persons. Numerous outbreaks have originated from sewage-polluted drinking and recreational water. Norovirus belonging to GI are significantly more likely to have been transmitted via water than by other routes of transmission, and are therefore thought to be more stable in water than GII strains. Noroviruses have been detected in a wide range of water environments such as sewages, municipal water, rivers, recreational waters, and groundwater throughout the world [18-30].

Noroviruses are highly resistant to adverse environmental conditions. They can survive chlorination in concentrations up to 10 ppm and temperatures ranging from below 0 °C to 60 °C and higher. The NoV genome may persist for 1-3 months in different types of water (mineral, tap water and river) [31]; GI NoV stored in groundwater was still infectious in human volunteers after 2 months, and GI NoV RNA stored in groundwater was still detectable by RT-PCR after 588 days [32, 33].

Several reviews on waterborne NoV outbreaks have been published [10, 20, 34]. The most recent paper reviewed all publications on the PubMed database reporting waterborne NoV outbreaks from the Norwalk epidemic that led to the discovery of the virus in 1969 up to the year 2007. In the 38 years covered, the review found 43 waterborne NoV outbreaks in various parts of the world (involving NoV alone or together with other enteric viruses) [20]. In the four years between 2008 and the present, on the other hand, a similar review found 24 reported outbreaks (La Rosa, unpublished data) associated with drinking water from private wells, community water systems, fountains, and surface water such as lakes and swimming pools. NoV are believed to be the major cause of recreational waterborne outbreaks (45%, followed by adenovirus 24%), documented in the published literature [11].

The explosion of data on NoV outbreaks in recent years is reason for concern. It is, however, unclear whether this is the result of improved reporting, surveillance and detection, or an actual increase in incidence.

Enterovirus

Human enteroviruses, members of the family *Picornaviridae*, consist of non-enveloped virus particles containing a 7,500-nucleotide single-stranded RNA genome protected by an icosahedral capsid. They comprise more than 100 serotypes, which are currently grouped into four species, Human enterovirus A-D, based on molecular and biological characteristics [35]. The health effects associated with enterovirus infections are varied, ranging in severity from mild to life threatening. Mild symptoms include fever, malaise, sore throat, vomiting, rash and upper respiratory tract illnesses, acute gastroenteritis (less common). Viraemia can occur, transporting enteroviruses to various target organs and resulting in potentially serious complications such as meningitis, encephalitis, poliomyelitis and myocarditis. Other complications include myalgia, Guillain-Barré syndrome, hepatitis and conjunctivitis. Enteroviruses have also been implicated in the aetiology of chronic diseases, such as inflammatory myositis, dilated cardiomyopathy, amyotrophic lateral sclerosis, chronic fatigue syndrome, and diabetes mellitus. Since these viruses are common, are shed in extremely high numbers from infected individuals, and are stable in the environment for extended periods of time, they have been suggested as a parameter for evaluating viral pollution of environmental waters [36, 37]. Enteroviruses are resilient organisms, able to survive drastic changes in temperature and pH [6, 38]. Enteroviruses have been detected in both raw and treated sewages [5, 39-44]. Studies on removal efficiencies at wastewater treatment plants, based on real-time PCR, showed that enteroviruses are more resistant to treatment than other enteric viruses (adenoviruses and noroviruses) [5]. Moreover, live virions have been detected in treated waters through infectivity assays, pointing to potential public health risks [5, 40]. The occurrence of enteroviruses in coastal waters in both bathing and non-bathing sites is documented worldwide [45-50]. Enteroviruses have also been found in rivers [49, 51-55], lakes [56, 57], groundwater [29, 58-61] and in both untreated and finished drinking water supplies [30, 38, 55, 62, 63]. Despite the widespread presence of these viruses, however, few drinking water-related outbreaks have been reported [64-67], only two outbreaks of recreational waterborne coxsackievirus, nine echovirus outbreaks concentrated between 1990 and 2005 [11] and sporadic echovirus 30 outbreaks in swimming pool. The limited knowledge on the role of waterborne enterovirus transmission could be related to a number of factors, including the wide range of clinical symptoms, frequent asymptomatic infection, the diversity of serotypes and the dominance of person-to-person spread.

Hepatitis A virus

Among the types of hepatitis viruses identified so far, only two types, hepatitis A (HAV) and hepatitis E (HEV) are transmitted via the faecal-oral route and therefore associated with waterborne transmission. HAV is an icosahedral non-enveloped, single-stranded RNA virus belonging to the *Picornaviridae* family, genus Hepatovirus. HAV infections result in numerous symptoms, including fever, malaise, anorexia, nausea and abdominal discomfort, followed by jaundice; the infection can also cause liver damage. Fulminant hepatitis is a rare complication, posing an increased risk to elderly patients and to patients with chronic liver disease. The incidence of infection varies between regions of the world, with the highest rate in non-industrialized countries where sewage treatment and hygiene practices can be poor. Conversely, the number of reported cases of HAV infection has declined substantially in countries with effective immunization programs. The virus is excreted in the feces (and urine) of infected persons and can contaminate soil, water (fresh or seawater) and food, including shellfish (mussels and oysters) harvested from contaminated water. The virus can survive for up to 60 days in tap water, over 6 weeks in river water, over 8 weeks in groundwater and even up to 30 weeks in sea water [68]. HAVs have been detected in different water environments: wastewaters [69-74], treated effluents [75], surface waters [51, 76-80], and drinking waters [81-83]. Outbreaks of hepatitis A among persons who use small private or community wells have been reported [84-87]. According to a review based on WBDOSS drinking water surveillance data spanning a 36-year period, 45.3% of the 64 viral drinking water outbreaks were attributed to hepatitis A virus [9]. As for recreational waterborne hepatitis A, four outbreaks were identified, linked to lakes, pool, thermal pool/spa [11]. The morbidity contributed by these four outbreaks is low when compared to outbreaks of other etiologies.

Adenovirus

Human adenoviruses (HAdVs) are non-enveloped, icosahedral viruses of the genus Mastadenovirus, family *Adenoviridae*. These viruses have a linear, double stranded DNA genome (26-45 kb) encapsidated in an icosahedral protein shell. There are 51 serotypes classified into six species, A–F, defined using biological characteristics. Additional types continue to be identified and characterized using genomics and bioinformatics. Clinical manifestations are highly heterogeneous, ranging from upper and lower respiratory tract infections to gastroenteritis, pneumonia, urinary tract infection, conjunctivitis, hepatitis, myocarditis and encephalitis [88, 89]. Adenoviruses can cause severe or life-threatening illness, particularly in immunocompromised patients, children and the elderly. Adenoviruses have been widely detected in wastewaters (both influent and effluent sewages) worldwide [88]. Different studies reported AdV concentrations to be the highest of all enteric viruses tested in these matrices [5, 90, 91]. The viral load in the feces of infected individuals is high (~10^6 particles/g of fecal matter). In addition, AdVs are very resistant to UV light and may survive longer than fecal indicator bacteria both in sewage and in the environment. Adenoviruses have also been frequently detected in surface waters. In comparative studies, AdVs usually outnumbered enteroviruses in surface waters [88]. A Europe-wide surveillance study carried out to determine the frequency of AdV and NoV in recreational waters (marine and freshwater samples), detected AdVs in 36.4% of samples, of which approximately 25% were infectious, supporting the case for considering AdVs a good indicator of bathing water quality [27]. Quantitative data on AdV in these waters showed mean values of 3.2×10^2 genome copies per 100 ml of water [92]. Infectious AdVs have been detected in conventionally treated and disinfected drinking water in Africa and Asia, using genome detection with PCR in cell culture. Limited data suggest that AdVs survive longer in water than either enteroviruses or HAV. They are capable of surviving for months in water, especially at low temperatures. Their increased stability in the environment may be in part attributable to their double-stranded DNA, and/or to their ability to repair damaged DNA by activating cell repair enzymes. This may also enhance their resistance to inactivation by UV light. AdVs have been implicated in drinking water outbreaks, although they were not the only pathogens involved [93, 94]. As for recreational outbreaks, 14 conjunctivitis or pharyngoconjunctival fever outbreaks, linked to pool, lake and pond, have been described, spanning six decades, making AdV the second etiological agent, after NoV [11, 95] for such epidemics.

Hepatitis E virus

Hepatitis E virus (HEV) infections are caused by a positive-sense, non-enveloped RNA virus of the Hepevirus genus. The four major genotypes (GI to GIV), all belonging to a single serotype, are known to infect humans. While GI and GII are restricted to humans, GIII and GIV are zoonotic and may infect animals (swine, chickens, deer, mongooses, and rabbits), as well as humans, in both industrialized and non-industrialized countries [96]. Symptoms include malaise, anorexia, abdominal pain, arthralgia, fever and jaundice. The incubation period for HEV varies from 14 to 63 days. HEV infection usually resolves in 1-6 weeks after onset. The fatality rate is 0.5-3%, except in pregnant women, for whom the fatality rate can approach 20-25%. HEV has been included in the National Institute of Allergy and Infectious Diseases NIAID List of Emerging and Re-emerging Diseases (Group I-Pathogens newly recognized in the past two decades). Illnesses associated with HEV are rare in industrialized countries, with most infections being linked to international travel. HEV is transmitted via the fecal-oral route and is easily spread by water contaminated with human fecal matter. Large waterborne outbreaks with high attack rates among young adults have been described in regions characterized by poor sanitary conditions. Since the first retrospectively documented hepatitis E outbreak in India in 1955-1956, many large waterborne outbreaks have been reported in Asia and Africa [97], affecting up to 79 000 and even 119 000 persons in India and China in 1991, respectively. Most of the outbreaks occurred after monsoon rains, heavy flooding, contamination of well water, or massive uptake of untreated sewage into city water treatment plants. No reports of HEV linked to recreational water were found in the published literature. Two cases of HEV were reported in persons who swam in the River Ganges, but they also drank unboiled or unfiltered water while in India [11]. In industrialized countries, while there have been sporadic cases of locally acquired hepatitis E, no epidemics have been reported. HEV has been detected, however, in different water environments, including urban sewages, in Spain, Italy, France and the United States [98-102]. Moreover, infectious HEV particles have been reported to occur in sewage, indicating the existence of a potential public health risk from the contamination of surface water with HEV [103]. HEV has also been detected in rivers [104, 105] and in bivalves [106].

Influenza virus

Influenza is a highly contagious, acute respiratory illness caused by a virus, member of the *Orthomyxoviridae* family, containing a segmented negative-sense RNA genome. The segmented nature of the genome allows for the reassortment or exchange of segments between two virus strains co-infecting the same cell; thus, influenza viruses are constantly reemerging through changes in their genome. All subtypes of influenza A viruses (H1 to H16 and N1 to N9) have been isolated from wild waterfowl. Most infected birds are asymptomatic, even when they are excreting large quantities of infectious virus in their feces as well as in their saliva and nasal secretions, and act as "silent" reservoirs of the virus. Domestic waterfowl (*e.g.*, ducks) may also act as a two-way intermediary in the transmission pathway of avian influenza between wild waterfowl and domestic terrestrial poultry (*e.g.*, chickens) [107]. Viral transmission occurs mainly by direct contact between infected birds and essentially the respiratory tract of susceptible hosts, but the role of an indirect waterborne transmission linked to feces-contaminated water has also been confirmed [108, 109]. It had been thought that avian viruses, although highly pathogenic for domestic poultry, did not replicate efficiently or cause disease in humans. However, human cases of avian influenza have recently become increasingly frequent. The threat posed by highly pathogenic avian influenza A H5N1 viruses to humans remains significant, given the continued occurrence of sporadic human cases, the endemicity in poultry populations in several countries, and reassortment, which may produce novel viruses of potential threat to public health. Contaminated lakes and ponds play an essential role as environmental virus reservoirs. Avian influenza virus has been isolated from lakes and pond waters and other water environments, where they can persist for extended periods of time [107-111]. There is very little information on the role of water in the transmission of influenza viruses among waterfowl or to other animals, including humans. De Jong described two simultaneous H5N1 cases in a single family in Vietnam, suggesting that exposure to possibly contaminated canal water via swimming or washing may have resulted in infection. The role of water in transmission however, could not be confirmed [112]. More recently, two studies identified contaminated water as a potential risk factor for H5N1 infection in a Cambodian village [111, 113]. Nevertheless, observational and analytical studies have not yet identified exposure to a contaminated water environment as an established risk factor for influenza H5N1.

Coronavirus

Coronaviruses, of the *Coronaviridae* family, are enveloped, single-strand RNA viruses that range from 60 to 220 nm in size. They are primarily respiratory pathogens, a frequent cause of the common cold in both children and adults. Although its major source of transmission is person-to-person contact through respiratory secretions, the fecal-oral transmission may be possible as well. Coronaviruses were not considered of concern for waterborne transmission, until a new coronavirus, the causative agent of severe acute respiratory syndrome (SARS), was detected in the feces of infected patients. In addition, large clusters of cases suggest the possibility of environmental contamination via sewage or ventilation systems [114]. In 2003, during the investigation of an outbreak in Hong Kong, transmission by aerosolized wastewater was suspected [115]. A recent study investigated the survival of representative coronaviruses in tap water and wastewater [116]. Inactivation of coronaviruses in the test water was highly dependent on temperature, level of organic matter, and presence of bacteria. Coronaviruses were inactivated faster in water at 23 °C (10 days) than in water at 4 °C (> 100 days); they died off rapidly in wastewaters (2-4 days). Research is still needed to shed light on the persistence of this virus in the environment, its zoonotic reservoirs, and its potential transmission through waterborne routes.

Polyomaviruses

Human polyomaviruses (HPyV) are members of the *Polyomaviridae* family. Polyomaviruses are approximately 38 to 43 nm in size. Their double-stranded DNA is thought to contribute to their heat stability and increased resistance to UV light treatment. Polyomavirus infections appear to be lifelong, but are generally asymptomatic in healthy individuals. In the immunocompromised host, however, reactivation of the latent viruses appears to occur in the kidneys and brain tissue leading to potential tumor development. Five HPyV have been known for some time (*i.e.*, JCV, BKV, MCV, WUV, and KIV) and four additional types have been recently characterised [117]. Polyomaviruses of primary concern include the JC virus (JCV), BK virus (BKV) and the Merkel cell virus (MCV), responsible for diseases such as progressive and fatal multifocal leukoencephalopathy (brain cancer), nephropathy (kidney cancer), and Merkel cell carcinoma (MCC), a rare but aggressive skin cancer, respectively. BK virus and JC virus are ubiquitous in the human population with up to 90% and 60%, respectively, of the adults having antibodies against these viruses [117]. MCC is extremely unusual before the age of 50 and people at risk include those with fair skin, excessive UV light exposure, and immunosuppressed patients. The incidence rate is 0.44 cases per 100 000 individuals in the USA and 0.15 cases per 100 000 individuals in Japan; the number of MCC cases has, however, tripled the last 15 years [118]. Exposure to HPyV occurs early in childhood, generally by the age of five to 10. Polyomaviruses are excreted in the urine and feces of infected individuals, both healthy and symptomatic, and are thus subject to environmental transmission via the waterborne route. JC and BK PyVs have been detected in urban sewage from various geographical areas [119, 120]. Recently Bofill-Mass *et al.*, [121] analyzed the presence and characteristics of newly described human HPyVs (KI, WU and Merkel cell PyV) in urban sewages and river waters. This study indicates that the MCV, a virus strongly associated with human cancer, is prevalent in the population and may be disseminated through the fecal/urine contamination of water.

Picobirnavirus

Human picobirnavirus (HPBV) are fairly small (35 nm), non-enveloped, spherical viruses. They have been found in a wide range of hosts, including humans, with or without GE. These viruses have not been successfully cultured in the laboratory, and their pathogenesis is unknown. High levels of occurrence in wastewater samples have been reported. Symonds and coworkers detected HPBV in 100% of raw sewage samples and 33% of final effluent samples [122]. Whether the presence of HPBV in aquatic environments is specific to human fecal contamination or whether animals may act as a source of contamination is unclear. The authors suggested that these viruses could be used, along with AdVs as potential markers of fecal contamination. However, a recent study detected HPBVs in only 25% of wastewater samples, casting doubt on their suitability as indicators of fecal contamination in water [123]. Further research will be needed to determine if these candidate viruses have the necessary characteristics of a microbial water quality indicator.

Papillomaviruses

Papillomaviruses are small epitheliotropic viruses detected in all vertebrates. Human Papillomaviruses (HPVs) belong to the *Papillomaviridae* family and are distributed over five genera (alpha, beta, gamma, mu and nu). They are small (approx. 50-60 nm), with a circular double stranded DNA genome measuring 7-8 kb in length. Infections due to papillomaviruses are common and lead to a wide variety of clinical manifestations that involve the epidermal surfaces, including common warts, palmoplantar warts, oral warts and genital warts. Strong evidence indicates that certain papillomaviruses are involved in cervical and genital cancers. Some have also been implicated in laryngeal/oral cancer and some lung cancers [124]. Recent evidence suggests that epitheliotropic viruses can find their way into sewage as enteric viruses [125]. A recent study detected HPVs in the vast majority (81%) of sewage samples, with a wide range of genotypes belonging to both the alpha and the beta genus, including putative novel genotypes [126]. Moreover, not only cutaneous HPVs, but also three of the most important anogenital types, such as the oncogenic HPV-16 and the low-risk HPV-6 and HPV-11 genotypes, were detected [126]. Although large-scale studies will be required before the health significance of the presence of HPVs in the environment is fully understood, these data pave the way for investigations on HPV transmission through contaminated water. In fact, this could reveal a hitherto unexplored mode of HPV transmission which may account for anogential HPV infection among people who had never been sexually active (*e.g.*, children and virgins).

CONCLUSIONS

Globalization, new technologies, and the genetic evolution of pathogens, humans, and vectors stimulate the emergence of new microbial threats to water quality. Despite the progress in water and wastewater treatment technology, waterborne diseases continue to have far reaching public health consequences in both non-industrialized and industrialized countries. Efforts to improve the detection, investigation, and reporting of outbreaks at the local and national levels worldwide, modeled on the WBDOSS surveillance system, are crucial to identify the causes of outbreaks and to understand the environmental factors contributing to these outbreaks.

Future studies are needed to provide valid and reproducible methods for the detection of waterborne viral pathogens in order to determine the extent of contamination of water environments, the types of pathogens involved and the correlation between viral contamination and environmental factors. Also, quantitative microbial risk assessment (QMRA) analysis should be improved, using water quality data, pathogen-specific characteristics, prevalence data, and exposure data. Such information will lead to a better understanding of the health risks related to water systems, and to improved methods of control.

In summary, research efforts to mitigate the effects of infectious threats, focusing on improved surveillance and diagnostic capabilities, including reliable viral indicators of fecal contamination of water, are crucial.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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