WATERBORNE VIRAL PATHOGENS: DETECTION, CONTROL AND MONITORING OF WATER QUALITY FOR HUMAN CONSUMPTION

Lígia C. Faccin-Galhardi¹, Nayara Lopes¹, Samantha F. Espada¹, Rosa Elisa C. Linhares¹, Jacinta S. Pelayo², Carlos Nozawa¹*

Departamento de Microbiologia, CCB, Universidade Estadual de Londrina, Caixa Postal 6001, 86051-990, Londrina, PR, Brasil.
1. Laboratório de Virologia
2. Laboratório de Bacteriologia

ABSTRACT

The viral contamination of water for human consumption is a common cause of diseases outbreaks, therefore, a matter of great relevance for public health, especially in developing countries. In most countries, legislation recommends the study of coliforms to indicate the potability of water, however, this is not correlated with the presence of virus. The present review sought to characterize the epidemiological importance of waterborne viral diseases and the limitations of laboratorial detection and elimination of these pathogens. Besides, the state of art of virus as a tool to assess the quality of the water, as well as, the use of a single virus as the indicator of environmental contamination by virus itself are presented. Reports have demonstrated that some viruses are resistant to conventional water treatment process. Changes in water treatment policy should be achieved, and, should involve viral detection and resistance to physical and chemical agents, amongst other methods. However, we have to bear in mind that the decrease of waterborne infectious diseases is greatly dependent upon the improvement of sanitation, water treatment, together with preventive measures. All those measures together could ensure appropriate conditions for the promotion of human health and the reduction of environmental impacts.

Keywords: Virus detection, Waterborne infectious diseases, Control, Indicators.

INTRODUCTION

The water has a direct influence on health, quality of life and human development. According to World Health Organization (WHO), all people have the right to an adequate supply of potable water that ensures all the needs of the population, without causing risk to health (WHO 2008). This should be guaranteed by governmental health policies through of collective systems of distribution, however, this does not occur in most of developing countries. There are still 780 million people without access to an improved drinking water source. And even though 1.8 billion people have gained access to improved sanitation since 1990, the world remains off track for the sanitation target (UNICEF 2012). The lack of basic sanitation found in developing and developed countries can result in contamination of water by sewage discharge directly on aquatic ecosystems. Moreover, the progressive and uncontrolled urbanization of the cities, leads the occupation of areas unsuitable for housing, without infrastructure and sanitation (Sá et al. 2005). Another source of contamination is the occurrence of cracks in the pipeline water distribution system, which may be located within open ditches or near the sewage. Beyond this, the inappropriate destination of domestic waste and leachate also contribute to increase water sources contamination (Fong & Lipp 2005).

Brazil possesses 15% of the freshwater of the planet, however, one seventh of the cities has no access to good quality water. In 2008, about 99% of the Brazilian cities had some type of water supply service and, among them, 87% had water treatment systems, 6.2% had water partially treated and 6.8% without any treatment. However, only 55.2% of the municipalities possessed sewage collection system (IBGE 2008).

The absence or poor protection of water resources can introduce several pathogens, such as virus, bacteria, protozoa or helminthes, making them unsuitable for consumption and causing outbreaks of various diseases, representing a heavy burden for public health services. According to the Brazilian Ministry of Health, Decree nº 2914/2011, the microbiological standard of water...
potability is determined by the absence of pathogens, specifically, total coliforms and *Escherichia coli*. The decree also recommends the inclusion of assays for enterovirus, *Giardia* spp e *Cryptosporidium* spp (Brasil 2011). These pathogens are more resistant than bacteria to standard water treatment, and their presence, even in low density represents high risk, therefore, it is essential the use of methods for their detection (Ali et al. 2004).

**Waterborne viral pathogens**

The presence of viruses in water is evidence of fecal contamination because they are excreted by infected individuals and do not belong to natural microbial population. Haas et al. (1993) found that the risk of waterborne viral infection is 10-100 times greater than that by bacteria. Virus excretion in feces occurs in extremely high number, usually $10^5$ - $10^{11}$ virus particles/gram of feces (Farthing 1989). About 100 species of virus can be transmitted by water and can be the cause of human diseases (Bosch 1998). Among them, the most common belong to the families *Picornaviridae* (poliovirus, coxsakievirus, echovirus and hepatitis A virus [HAV]), *Adenoviridae* (adenovirus), *Caliciviridae* (norovirus and sapovirus), *Astroviridae* (astrovirus), *Reoviridae* (reovirus and rotavirus) and *Hepeviridae* (hepatitis E virus [HEV]).

In developed countries, 10% of hospitalizations of children under five years old occur due gastroenteritis, and the viral agents are responsible for 80% of these hospitalizations (Domínguez et al. 2009). It is estimated the occurrence of more than 100 million episodes of diarrhea each year, resulting in approximately 24 million medical visits, 2.4 million hospitalizations and more than 500,000 infant deaths (Greenberg & Estes 2009). In Brazil, it was shown that of 154 children hospitalized with acute diarrhea, 72.8% were positive for enteropathogens. The prevalent agents were bacteria (51.3%) and rotavirus (37.6%), with a high percentage of mixed infections. Bacteria were detected in 21.4% of individuals without diarrhea, however, rotavirus was mostly diagnosed in individuals with diarrhea, that would explain the higher prevalence of viral agent (Souza et al. 2002). Morillo et al. (2010) analyzed 134 fecal samples collected from 2004 to 2008, from day-care centers of São Paulo State (Brazil). Rotavirus was detected in 28.3% (38/134) of the samples, with 65.7% (23/35) in samples collected in 2004, 50% (9/18) in 2007 and no case in 2008. A drastic reduction in the number of outbreaks/rotavirus infection was demonstrated in the same State, 35 outbreaks/10,481 cases in 2004; 24/3,144 in 2005; 35/2,084 in 2006; eight/164 in 2007 and one outbreak/3 cases in 2008. That reduction could be explained by the vaccination campaign begun in 2006 and also by the improvement of the sanitary conditions, including water and sewage treatments. The incidence of rotavirus infection is similar in developed and developing countries regardless of socioeconomic condition (Gurgel et al. 2008). This finding has suggested an alternative respiratory route for the disease transmission, other than fecal-oral. Although rotavirus is considered the most common etiological agent of infantile diarrhea worldwide, other enteric viruses are associated with gastroenteritis. Frost et al. (2002) observed the occurrence of 625 outbreaks of gastroenteritis in USA, from 1971 to 1998, due to the ingestion of contaminated water and 161 outbreaks caused by the use of contaminated recreational water. Of the total of 786 outbreaks, 30 were caused by HAV, 29 by norovirus, 2 by adenovirus, 2 by enterovirus and 2 by rotavirus. In the same country, from 1999 and 2000 other outbreaks associated with norovirus were reported related to recreational water and the ingestion of untreated water in which 202 people were infected (Lee et al. 2002). From 1995 to 2000 outbreaks caused by norovirus or rotavirus were reported in Europe, 24% occurred in Finland, 17% in the Netherlands, 14% in Slovenia, 7% in Spain and 7% in England (Lopman et al. 2003). According to Jurzik et al. (2010) norovirus infected 344 and 1,699 people, respectively, in southern Italy and in Podgorica (Montenegro) due to contaminated water, and, additionally, 460 people were infected with enterovirus (EV) during an outbreak in the Republic of Belarus. In Brazil, Morillo et al. (2008) also showed the importance of norovirus in waterborne infection.

In the last two decades there has been a growing concern worldwide about waterborne viral infections either with drinking or recreational water. In Brazil, from 1987 to 1999, Mehnert & Stewien (1993) and Mehnert et al. (1999) conducted the first investigation of rotavirus and adenovirus in sewage and polluted streams in the city of Sao Paulo (Brazil). Other studies also carried out in drains, streams and wells, in the city of Sao Paulo, identified HAV (Sassaroli et al. 2000), rotavirus (Queiroz et al. 2001; Pauli et al. 2003) and adenovirus (Santos et al. 2001; Garrafa et al. 2003; Martins et al. 2003). Sdiri-Loulizi et al. (2010) analyzed 250 samples of sewage, in Tunisia, from 2003 and 2007 and found 43% (72/166) positive samples for rotavirus, 11 (4.4%) for norovirus and 1 (0.4%) for enteric adenovirus. Hassan et al. (2010) collected 93 samples of water in treatment station also in Tunisia and found that 67.5% were contaminated with calicivirus, 35.13% with enterovirus and 10.81% with HAV.

The contamination of marine environment is also of great concern and involves contamination of sea food, especially, shellfish once they get their nutrient by filtering water. Virus can remain infectious and at the high concentration in the tissues of mollusks enabling their transmission and causing diseases when consumed raw or undercooked. Studies carried out in Florianópolis (Brazil) demonstrated the presence of HAV, rotavirus, poliovirus and adenovirus in mollusks (Santos et al. 1999; Vinatea et al. 2002; Rigotto et al. 2003; Souza et al.
Dilution is a crucial element in the aquatic milieu in that concentration of great volumes of water is essential for the detection or isolation approaches. Early studies of enteric virus for monitoring the microbiological quality of water and possible sources of contamination occurred in the 40s (Fong & Lipp 2005). However, studies in the field of aquatic virology (now called environmental virology) started only after the epidemic of hepatitis A in New Delhi, in 1955. In that occasion, Jumna River was contaminated by domestic sewage treated with chlorine and aluminum which eliminated bacteria, but not virus, causing about 30,000 cases of hepatitis A. Since then, environmental virology has been viewed as an issue of great concern due to the industrial and medical impact (Bosch 1998).

For the detection of viruses in aquatic environments, several studies have been conducted to find out a virus that can be used as an indicator of (viral) contamination (Jurzik et al. 2010). It is known that bacteria have been used since the XIX century (Medema et al. 2003) as indicators of fecal pollution in water, especially total coliforms and entecocci. However, these groups of microorganisms do not reflect the presence of virus. The high amount of virus excreted in feces and the maintenance of its infectivity for sufficient time period to cause diseases, among other characteristics, make it impossible to use bacteriological profile in the evaluation of viral presence in water (Bosch 1998). Bosch et al. (1991) reported the occurrence of water samples contaminated with rotavirus, enterovirus and HAV, however, free of bacterial indicators. Moresco et al. (2012) detected the presence of E. coli in all water samples collected from Florianópolis city beaches. After water concentration, PCR could be used or the concentrate inoculated into appropriate and susceptible cell cultures depending on the virus studied. Molecular techniques have been used in the detection of enteric viruses, in environmental samples, from the beginning of the 90s. Assays such as, PCR and hybridization detect parts of the viral genome, highly conserved and with homology with virus specific groups (De Leon et al. 1990; Allard 1992). The advantages over the cell culture lay on their rapidity, specificity and sensitivity. The disadvantage is that they do not evaluate virus infectivity and the high sensitivity increases the risk of false positivity (Fong & Lipp 2005). Other methods, such as, radioimmunoaassay, enzyme immunoassay, immunofluorescence, and even, complement fixation are used for virus detection in clinical samples, however, are difficult or have low sensitivity for use in water samples (Griffin et al. 2003). Detection in cell culture is mainly based in the observation of the cytopathic effect (CPE). Among the disadvantages, virus isolation is time consuming, and, again, depends on the development of CPE, and many viruses are fastidious, such as, norovirus (Fong & Lipp 2005). The concentrates can contain substances that influence negatively the virus detection, particularly for molecular analysis, therefore, removal of inhibitors should be carefully assessed. An integrated cell culture/polymerase chain reactions (ICC/PCR) technique has been developed for environmental samples and has been considered as a reliable and practical method for viral pathogens, such as, enteroviruses, HAV, enteric adenovirus and astrovirus. An extension of the integrated techniques can be used, for example, for HAV and adenovirus, such as, ICC-strand-specific RT-PCR (detects the intermediate negative strands of positive-stranded viruses, in cell culture) and ICC-mRNA RT-PCR (detects viral mRNA [adenovirus] in cells) (Reynolds 2004, Rodriguez et al. 2009).

### Indicators of viral contamination

Due to the difficulties of isolation and identification of virus in water, several studies have been conducted to find out a virus that can be used as an indicator of viral contamination.
Adenovirus and HAV were detected in approximately 50% of the samples, however, polyomavirus and norovirus were detected only in 3% and 7.5% of the samples, respectively. Yet again, demonstrating no correlation between the presence of virus and bacteria.

According to Griffin et al. (2008), coliforms also can not be considered indicators of viral contamination because they are more sensitive to environmental variations, such as, pH, temperature and salinity. Furthermore, they are more easily removed/inactivated by physical (filtration, ultraviolet radiation) and chemical (chlorination) processes in water treatment. Havelaar (1993) and Jofre et al. (1995) proposed the use of Bacteroides fragilis RNA phage, F-specific, and somatic coliphage as potential indicators of viral contamination. Amaral et al. (1994) suggested coliphage as an indicator of viral presence due to its correlation with coliform bacteria in the environment. Moreover, Nasser & Oman (1999) found that the F+ bacteriophage persisted actively longer than E. coli in various sources of water. Considering the morphology, structure and composition of bacteriophages, they could exhibit similar behavior to enteric virus in water. In fact, the use of bacteriophages as indicators due to their greater resistance to environmental factors in comparison to bacteria has been suggested, with a close relation with water contamination by enteric virus. Coliphages continue to replicate even after excretion of the host, which does not occur with enteric virus, therefore, its determination could lead to a misleading conclusions (Grabow 2001). Wiggins & Alexander (1985) determined a minimum density of 10³ CFU/ml of bacteria for a significant bacteriophage replication. Similarly, Cornax et al. (1991) suggested that in water with 10⁵-10⁶ CFU/ml fecal coliforms the numerical relationship with coliphage is almost inexistente. These findings suggest that quantitative and/or qualitative assaying of phage may not represent the scenery of the enteric virus presence, inconsistent with the concept of phages as an indicator agent of fecal contamination (Amaral et al.1994; Jurzik et al. 2010). Adenovirus has been suggested as indicator of viral contamination, a part from enterovirus, because of its physicochemical stability and presents little seasonal variability. It is also more easily detected and has no quantitative correlation with the coliforms (Mehnert et al. 2001; Jiang 2002; Heerden et al. 2003). However, no relationship was observed with the presence of enterovirus and HAV (Jiang 2002). The European government officials also include the search of enterovirus as virological indicator (Mehnert et al. 2001). Poliovirus-1 vaccinal strain was also proposed, however, no correlation was shown with the presence of HAV and rotavirus (Sobsey et al. 1988; Abad et al. 1994). The Torque Teno virus (TTV) was mentioned as a potential indicator by supposedly being characterized as the first commensally enteric virus and resistant to environmental conditions (Griffin et al. 2008). Dalla Vecchia et al (2013) detected the TTV genome in 11.7 % of the water collected in schools of three municipalities located in south of Brazil. In addition, other investigations in Brazil, have also considered the use of enterovirus, rotavirus and adenovirus for this purpose (Dalla Vecchia et al. 2012, Fongaro et al. 2012, Fongaro et al. 2013). Therefore, microorganisms other than E. coli, coliforms and enterococci (Jurzik et al. 2010) have to be searched for to be elected as the most adequate indicator of animal virus in surface water.

**Water treatment and elimination of viral pathogens**

The enteric viruses can be inactivated by chemical, physical and biological agents. The chemical process includes treatment with strong acids, oxidizing agents, alcohols, formaldehyde, etc. The chemical oxidizing, such as, chlorine affects the structure of viral proteins turning the agents noninfectious. Physical agents include heat, dehydration, and electromagnetic radiation. High temperature, 95 °C or higher, degrades capsid protein, however, nucleic acids structures are preserved by their thermostability. Therefore, virus inactivation is based on the degradation of the capsid or nucleic acid by physicochemical or enzymatic methods (Nuanualsuwan & Cliver 2002; Nuanualsuwan & Cliver 2003; Cliver 2009).

Conventional treatment of drinking water is performed by the following sequence, lime application, coagulation, flocculation, sedimentation, filtration, chlorination, fluoridation and correction of pH. The filtration and chlorine disinfection are the main procedures to eliminate pathogens. The pre-chlorination, coagulation and sedimentation are also very effective in reducing the number of microorganisms in water (Payment et al.1998). Under filtration, particles and microorganisms are retained on filters or membranes and the remaining microorganisms are eliminated by the chlorination step. By traditional process in water treatment stations the viral clearance is ineffective due to the size of these agents. In addition, some viruses are resistant to chlorination, therefore, remain viable. A number of enteric viruses in sewage remain associated with the solid matter, suggesting greater virus concentration in biosolids than in wastewater (Simmons & Xagoraraki 2011). Lodder & de Roda Husman (2005) showed that treated sewage in the Netherlands dumped in the river Maas, contained a great concentration of enterovirus. Fourteen percent of the water samples from the Gulf of Mexico revealed the presence of enterovirus, however, the virus was present in, as much as, 72% of the marine sediment. In U.S.A. 30% of water samples from 35 States were positive for enterovirus (Abbaszadegan et al. 1999).

The enteric virus are resistant to acidic pH, for example, enterovirus in general resists to pH 3-5 by 1-3 hours. Poliovirus, also an enterovirus, is resistant to pH
Ozone is an alternative chemical agent extremely effective in eliminating pathogens in water. According to Payment (1998), ozone disinfection is the most efficient method for inactivating pathogens during the treatment of water. Wedemeyer et al. (1978) demonstrated that concentrations lower than 0.01 mg/l inactivated the virus of necrotizing pancreatitis. Disinfection with ozone showed a reduction of norovirus infectivity, accompanied by molecular testing (Shin & Sobsey 2003). Ali et al. (2004) used activated carbon (5 mg/l) during water clarification and filtration steps and found that enterovirus particles were completely eliminated, as confirmed by inoculation in cell culture and RT-PCR.

It is noteworthy that chemical disinfectants such as chlorine, chloramines and ozone commonly used in water treatment and have been effective in the elimination of microbiological contaminants, except for some enteric viruses. The main problem with the use of these products is the hundreds of carcinogenic components produced in the natural environment (Qilin Li et al. 2008). UV radiation itself beyond damaging the nucleic acid of the microorganisms is a possible cause of by-products generation that may be harmful for higher mammals (Liltved et al. 2006).

Better sanitation conditions are important preventive measures against infections not only bacterial but those caused by enteric virus or whatever. In Brazil, the conditions of water and domestic sewage treatments have improved in recent years, according to the Brazilian Institute of Geography and Statistics, overcoming a historic setback of this sector. New parameters to monitor the sanitary conditions are emerging, mainly due to increased degradation of aquatic ecosystems and environmental impacts and health. Public policies have encouraged surveillance in environment preservation, including monitoring systems and integrated database. These systems include indicators of environmental conditions and data on the sanitary services effectiveness and the establishment of goals to ensure health of the population, and prevention of environmental impacts.

Although there are recommendations for virological analysis of water for consumption by governmental organizations, the procedure is not mandatory, including in Brazil. There is not a single virus that fulfills all the requirements to be considered indicator of viral contamination. The identification of these pathogens would reduce the risk of outbreaks of viral diseases, however, technical difficulties are the limitations. Molecular techniques have been used, but again, they do not differentiate whether virus is infective or not. In conclusion, studies of enteric virus in water sources for human consumption should be greatly encouraged to provide high standard water quality to promote health and decrease medical and hospital costs.
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