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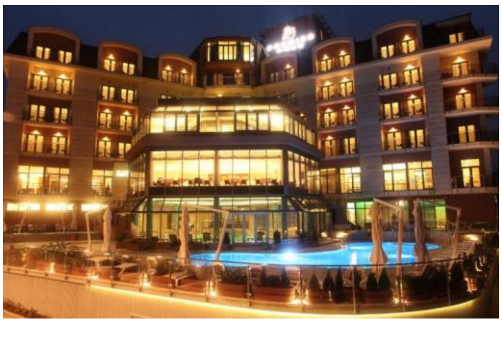

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VIRUSES IN ENVIRONMENT: SITUATION IN VOJVODINA PROVINCE OF SERBIA

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Abstract

The environment receives, maintains and transports etiological agents to susceptible hosts. The presence of animal and human, especially zoonotic viruses in environment is intensively studied and monitored in a lot of countries worldwide. Viruses are shed in extremely high numbers in the faeces of infected individuals. Surface water is subject to faecal contamination from a variety of sources. Water could be contaminated through direct inflow of untreated sewage. There is also more direct faecal contamination of the environment from humans and animals, for example by bathers or by defecation of farm animals and free-range or wild animals onto surface waters or soil. After the enteric viruses are discharged into water, they can survive for prolonged periods in the aquatic environment. Some of the human, animal and zoonotic viruses were chosen to estimate the possible existence of route of human and animal faecal contamination, as well as indicate whether the examined surface waters could present a possible hazard for animal and public health.

In Vojvodina Province in Serbia, in total 60 surface water, and 6 untreated sewage samples were collected at 33 locations during two sampling periods: summer (July-October), and autumn (November-December). The presence of seven ((human (HAdV, NoV GI, NoV GII and HAV), animal (PAdV and BPyV) and zoonotic (HEV)) viruses in surface water and sewage samples was tested by real-time PCR (qPCR) and reverse transcription real-time PCR (RT-qPCR) assays. The results show that the potential risk for public and animal health exists if the examined surface waters are used in agricultural and recreational purposes and present the need for assessing water sources for viral contamination to help protect public health.

Keywords: environment; human, animal and zoonotic viruses; Vojvodina; Serbia

Introduction

The environment receives, maintains and transports etiological agents to susceptible hosts. Viruses can be transmitted through various environments (water, sewage, soil, air, or surfaces) and persist enough in these vehicles to represent a health threat. Pathogenic viruses are routinely introduced into the environment through the discharge of treated and untreated wastes, as current treatment practices are unable to provide virus-free wastewater effluents (Bosh et al., 2006). The animal, human and especially zoonotic viruses in environment is intensively studied and monitored in a lot of countries worldwide. They are shed in extremely high numbers from infected individuals and they are stable in the environment for extended periods of time. Human exposure to even low levels of some pathogenic viruses in the environment, such as norovirus, adenovirus, hepatitis E and A viruses, can cause infection and disease. Some of these viruses have been suggested as a parameter for evaluating viral pollution of environmental waters (La Rosa et al., 2012). The viruses transmitted by the respiratory and faecal-oral routes, transport and persistence in environment is directly related to the potential for and risk of transmission, host exposure, infection and disease (Sobsey and Meschke, 2003). Enteritic viruses can survive the water disinfection processes that eliminate bacteria (Carratala et al., 2013). Several waterborne outbreaks of viral gastrointestinal illness have been documented. Besides gastrointestinal illnesses, enteric viruses have been linked to more acute conditions, including meningitis and paralysis (Spinner and di Giovani, 2001). Many of the viruses are important pathogens of their human and animal hosts, although some of them do not always cause severe illness or high mortality rates (Sobsey and Meschke, 2003).

Faecal Contamination of the Environment

The presence of viruses in water is evidence of faecal contamination because they are excreted by infected individuals and do not belong to natural microbial population (Faccin-Galhardi et al., 2013). Especially important are a variety of non-enveloped enteric and respiratory viruses. These include adenoviruses, astroviruses, caliciviruses, papovaviruses, parvoviruses, picornaviruses (enteroviruses and hepatitis A virus), and other nonenveloped viruses that can be shed in faeces (and in some cases urine) from infected individuals and can be present in faecal wastes and faecally contaminated environmental samples (Sobsey and Meschke, 2003). Sewage, especially from slaughterhouses, may contain animal adenoviruses, sapoviruses, and HEV, which may also be zoonotic (Wyn-Jones et al., 2011; Petrović, 2013). The prevalence and distribution of animal-specific viruses in environmental waters must be determined in order to validate the use of these viruses for source tracking purposes (Fong and Lipp, 2005).

The treatment of waste water and sewage, especially from small towns and villages, as well as from some large cities in Serbia, is seldom implemented, or works only with partial function. Rivers, lakes, streams, and coastal waters are regularly contaminated by septic tanks, storm water runoff, agricultural run-off or run-off of the animal manure used in agriculture and effluents from inefficiently operated sewage treatment plants. Additionally, water could be also contaminated from overflows of treatment plants impacted by flooding events, or through direct inflow of untreated sewage (Lazić et al., 2015).

Viral pathogens transmitted through water

Water is an important vehicle for the transmission of enteric viruses. These agents are adapted to the hostile environment of the gut and in most cases, can persist for a very long time in water.

Adenovirus

Adenoviruses are members of the family *Adenoviridae* and the genus *Mastadenovirus* (Sinclair et al., 2009). Human adenoviruses (HAdV) have been proposed as indicators of the presence of human viral pathogens in the environment, because they are prevalent in environmental waters and successfully detected in different environmental samples, such as waters in lakes and rivers, sewage systems, treated waters and swimming pools (Bofill-Mas et al., 2010; Silva, 2011). HAdV has also shown to be very stable in the environment and resistant to water treatments (Rodrıguez-Lazaro et al., 2011). In spite of the existence of reported cases of pneumoenteritis or encephalitis, porcine adenoviruses (PAdV) do not normally produce clinically severe pathologies (Maluquer de Motes et al., 2004). PAdV have been often isolated from apparently healthy pigs (Fong and Lipp 2005). PAdV have been proposed as porcine faecal contamination indicators (Maluquer de Motes et al., 2004; Girones and Bofill-Mas, 2013).

Norovirus

Noroviruses belongs to the family *Caliciviridae* and has its own genus *Norovirus*. Since less than 10 virus particles can lead to infection and disease, noroviruses (NoV) are very common cause of both endemic and epidemic gastroenteritis (Teunis et al., 2008; Atmar, 2010). These viruses are extremely contagious. The burden of calicivirus (including NoV) has been clearly documented in numerous geographical areas worldwide (Rodrı´guez-La´zaro et al., 2012) In US, noroviruses causes an estimated 23 million cases per year (Sinclair et al., 2009). In Europe 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 (La Rosa et al., 2012).

Hepatitis E virus

Hepatitis E viruses (HEV) excreted in faeces and urine constitutes a significant proportion of pathogens present in sewage (Sinclair et al., 2009). The four major genotypes (GI to GIV) belonging to a single serotype. While GI and GII are restricted to humans, GIII and GIV are zoonotic and may infect animals (La Rosa et al., 2012). HEV has been detected in different water environments, including urban sewages, in Spain, Italy, France and the United States (La Rosa et al., 2012). 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 (La Rosa et al., 2012).

Hepatitis A virus

Hepatitis A virus belongs to the family *Picornaviridae* and has its own genus *Hepatovirus* (Sinclair et al., 2009). HAV infections result in numerous symptoms, including fever, malaise, anorexia, nausea and abdominal discomfort, followed by jaundice; the infection can also cause liver damage. HAVs have been detected in different water environments: wastewaters, treated effluents, surface waters and drinking waters (La Rosa et al., 2012). Water is considered to be the most important source of infectious virus because it can survive for long periods in this environment. For example, the virus can survive over 6 weeks in river water (Rodrı´guez-La´zaro et al., 2012).

Polyomaviruse

Bovine polyomaviruses (BPyVs) are members of the family *Poliomaviridae* and the genus *Poliomavirus*. BPyV do not lead to clinically severe diseases in cattle (Girones and Bofill-Mas, 2013). These viruses have been suggested as potential bovine markers (Hundesa et al., 2006; Girones et al., 2010; Girones and Bofill-Mas, 2013).

Situation in Vojvodina Province comparing to the situation in Europe

A number of studies have been carried out with the aim of estimating the risks from viral contamination related to the release of wastewater into surface waters. In Serbia, untreated urban sewage and wastewater inflow directly into the different surface waters. The sewage treatment plants usually do not exist, or there are only mechanical and partial- treatment sewage systems on place. The surface water samples were collected at 30 locations during two sampling periods: summer (July-October), and autumn (November-December). The sampling locations for surface waters were chosen near all larger towns and as close as possible to a few intensive animal production farms. Among these water samples, 10 samples were collected from 5 urban beaches and 5 locations in protected natural areas. In total, 60 samples of untreated surface water and 6 samples of untreated urban sewage samples were tested by qPCR and RT-qPCR. The most prevalent virus found was HAdV which was detected in 43.33% (26/60) samples in Danube, Sava, Begej and Krivaja Rivers, and DTD, Great Backa, KCIII canals and Palic Lake. HAdV has been proposed as an indicator of the presence of human viral pathogens in the environment (Bofill Mas et al., 2006; Silva et al., 2011). Results of our study confirm this observation, and are in line with the results of many other studies conducted around the world (Rusiñol et al., 2014; Kern et al., 2013; Silva et al., 2011; Pina et al., 1998). In Poland, 60 water samples were collected in 2007 from four sampling sites situated along the river Wieprz. Human pathogenic viruses were detected in 35% of samples. HAdVs were detected in 28.3% of samples, and were present throughout the whole year (Kozyra et al., 2011). In Spain, 23 river water samples from two sites with different levels of faecal pollution were tested, and the human virus most often detected was HAdV, being present in 15 of the 23 samples (65%) (Pina et al., 1998).

NoV GII was found in 40% (24/60) samples in Danube, Sava, Begej and Krivaja Rivers, and DTD, Great Backa, KCIII canals, Palic Lake, and Rakovac stream. NoV GI was found in 10% (6/60) samples in Danube and Sava Rivers, and Great Backa Canal. Our results are in accordance with the results of the studies conducted in Hungary, where noroviruses were detected in 30% examined surface water samples (Kern et al. 2013) and in the region of North-Eastern Greece where NoV GII strains were found in 34% samples (Parasidis et al., 2013b).

PAdV, BPyV and HEV were detected in 5 (8.33%), 4 (6.67%) and 2 (3.33%) samples in Krivaja, Sava, Danube and Begej Rivers, DTD and KCIII canals. In our study we found PAdV and BPyV presence in tested surface waters in Serbia in much lower extent than it is published in some other studies in Europe. One of the possible reasons for these results is because the animal production in Serbia has been quite low in recent years due to the economic crisis (Lazić et al., 2015). As HEV is highly prevalent in pig population in Serbia (Lupulović et al., 2010; Petrović et al., 2014); the source of the HEV detected in the samples could be infected humans and / or pigs.

HAV was not found in any of analyzed surface waters or urban sewage samples. Viruses were not detected in 25% (3/12) of the examined surface waters (Tisa and Jegricka Rivers, and the Special Nature Reserve Obedska bara - wetland).

Among 6 analyzed sewage samples, 5 (83.33%) were found positive for the target viruses. On both sampling occasions in two urban sewage systems HAdV and NoV GII and in one occasion NoV GI were detected, and in the third urban sewage system only NoV GII was found in one of two sampling occasions. Viruses such as norovirus and adenovirus may be highly prevalent in sewage-polluted recreational waters (Wyn-Jones et al., 2011). Autochthonous strains of HEV have been reported in urban sewage in several highly developed countries, as well as related cases of sporadic acute hepatitis caused by these non-imported strains (Pina et al., 2000).

Conclusion and recommendations

The surface water samples tested positive for human, animal and zoonotic viruses indicating that the contamination must have originated from a variety of sources. The results show that the potential risk for public and animal health exist if the examined surface waters are used in agricultural and recreational purposes, and suggest the necessity for further and more extensive studies. Methods related to virus purification and detection of viral particles should be improved such that survival of human pathogenic viruses in the environment can be followed reliably. Legislation on handling and treatment of water and sewage should be adapted as needed to reduce the risk of environmental virus contamination.

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