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Presence of human and animal pathogenic enteric viruses in water environments reflects fecal contamination and indicates a risk to public health. Those enteric viruses are introduced in surface waters through direct inflow of untreated sewage or leaking sewage and septic systems, urban run-off, agricultural run-off or run-off of animal manure used in agriculture. In aquatic environment viruses can survive for prolonged period of time. Human exposure to even low levels of these pathogenic viruses in the environment, such as norovirus (NoV) where just a few virus particles are required for the infection, can cause infection and disease. The treatment of waste water and sewage, especially of small towns and villages, as well as of some large cities in Serbia, doesn’t really exist or work only with partial function. Furthermore, there are no publically available data on presence of viruses in Serbian surface waters.

For the first time in Serbia, a small surveillance study was conducted in order to estimate the presence and frequency of occurrence of selected human (adenoviruses (HAdV), noroviruses, (NoV GI, NoV GII) and hepatitis A virus (HAV)), animal (porcine adenovirus (PAdV) and bovine polyomavirus (BPyV)) and zoonotic (hepatitis E virus (HEV)) viruses in selected surface waters in the country. Adenoviruses were selected as faecal indicators based on their almost universal shedding and stability in the environment; and noroviruses (NoV) and hepatitis A virus (HAV) were selected as being the most prevalent gastroenteritis agent worldwide and/or because many viral waterborne outbreaks are restricted to those viruses. Animal and zoonotic viruses PAdV, BPyV and HEV were chosen to estimate if a route of animal faecal contamination exists, as well as possible hazard that the examined surface waters could present for animal and public health.
Twelve surface waters (6 rivers, 1 stream, 1 lake, 1 national park wetland and 3 canals) and 3 urban sewage systems (from towns Subotica, Sombor and Odzaci) were sampled on the territory of Vojvodina Province in Serbia between July and December 2013. In total, 60 surface water samples were collected twice at 30 locations, with a different time frame between two samplings (1 - 5 months). In addition, 6 sewage effluent samples were collected twice at one sampling site per each of the three town sewage system (in two months intervals) before their discharge into the surface waters. Ten liters of untreated surface water or 5 liters of untreated urban sewage samples were collected and concentrated by the adsorption/elution technique with glass wool filtration followed by glycine beef extract buffer elution and centrifugation after low pH flocculation. Nucleic acids were extracted from concentrated surface water samples using NucliSENS miniMAG technology (Biomerieux). Tested viruses were detected by specifically targeted real-time (RT)-PCR methods including internal amplification controls. The whole process of samples concentration, nucleic acids extraction and molecular detection were controlled using murine norovirus as a sample process control.

The most prevalent virus found were HAdV which was detected in 43.33% (26/60) samples from 60% (18/30) tested locations and in 66.67% (8/12) examined surface waters (Danube, Sava, Begej and Krivaja rivers, and DTD, Great Bačka, KCIII canals and Palić Lake). NoV GII was found in 40% (24/60) samples from 56.57% (16/30) locations and in 75% (9/12) surface waters (Danube, Sava, Begej and Krivaja rivers, and DTD, Great Bačka, KCIIII canals, Palić Lake, and Rakovac stream). NoV GI was found in 10% (6/60) samples from 16.17% (5/30) locations and in 25% (3/12) surface waters (Danube and Sava rivers, and Great Bačka Canal), and PAdV, BPyV and HEV were detected in 5 (8.33%), 4 (6.67%) and 2 (3.33%) samples from 5 (16.67%), 4 (13.33%) and 2 (6.67%) tested locations and in 4 (33.33%; Krivaja, Sava and Begej rivers and DTD canal), 3 (25%; Danube, Sava and Krivaja rivers) and 2 (16.67%; Sava river and KCIII canal) examined surface waters. HAV was not found in any of analyzed surface waters or urban sewage samples. Viruses were not detected in 25% (3/12) examined surface waters (Tisa and Jegricka rivers, and the National park Obedska bara wetland). In three rivers (Danube, Sava and Krivaja) simultaneous presence of four viruses was detected (NoV GII, NoV GI, HAdV and BPyV or PAdV). Simultaneous presence of three viruses (NoV GII or NoV GI, HAdV and PAdV or HEV) was found in four surface waters (Begej river and all 3 examined canals). Two viruses (HAdV and NoV GII) were simultaneously detected in Palić Lake and the
presence of just one virus (NoV GII) was found in Rakovac stream. Among 6 analyzed sewage samples 5 (83.33%) were found positive on presence of viruses. On both sampling occasions in two urban sewage systems HAdV and NoV GII and in one occasion NoV GI were detected, and in third urban sewage system only NoV GII was found in one of two sampling occasions.

To our knowledge this was the first study on human and animal enteric virus presence in surface water environments in Serbia. The obtained results confirm the presence of pathogenic enteric viruses of both human and animal origin in surface waters in Serbia indicating the existence of diverse contamination sources. Furthermore, the results show the potential risk for public and animal health that will exist if the examined surface waters are used in agricultural and recreational purposes, and suggest the necessity for further and more extensive studies.

Keywords: surface waters; environmental surveillance; human and porcine adenovirus; norovirus; hepatitis A virus; hepatitis E virus; bovine polyomavirus; real time (RT)-PCR; Serbia