



Full length article

Large scale survey of enteric viruses in river and waste water underlines the health status of the local population

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ABSTRACT

Although enteric viruses constitute a major cause of acute waterborne diseases worldwide, environmental data about occurrence and viral load of enteric viruses in water are not often available. In this study, enteric viruses (i.e., adenovirus, aichivirus, astrovirus, cosavirus, enterovirus, hepatitis A and E viruses, norovirus of genogroups I and II, rotavirus A and salivirus) were monitored in the Seine River and the origin of contamination was untangled. A total of 275 water samples were collected, twice a month for one year, from the river Seine, its tributaries and the major WWTP effluents in the Paris agglomeration. All water samples were negative for hepatitis A and E viruses. AdV, NVGI, NVGII and RV-A were the most prevalent and abundant populations in all water samples. The viral load and the detection frequency increased significantly between the samples collected the most upstream and the most downstream of the Paris urban area. The calculated viral fluxes demonstrated clearly the measurable impact of WWTP effluents on the viral contamination of the Seine River. The viral load was seasonal for almost all enteric viruses, in accordance with the gastroenteritis recordings provided by the French medical authorities. These results implied the existence of a close relationship between the health status of inhabitants and the viral contamination of WWTP effluents and consequently surface water contamination. Subsequently, the regular analysis of wastewater could serve as a proxy for the monitoring of the human viruses circulating in both a population and surface water.

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1. Introduction

Human enteric viruses are a major cause of acute waterborne diseases in both developed and developing countries (Enserink et al., 2015; Patil et al., 2015). In addition to long term persistence in environmental water and strong resistance to disinfection treatment, they are able to cause illness after ingestion at low infectious dose (Yezli and Otter, 2011). Human infections by enteric viruses are often asymptomatic or pauci-symptomatic, but may also induce various symptoms such as intestinal and respiratory illness, hepatitis or conjunctivitis. They can even present a high risk of morbidity and mortality in high-risk populations such as young children, immunocompromised patients and elderly people (Gerba et al., 1996).

Human enteric viruses have the ability to multiply within gastrointestinal tract of their hosts and are then excreted in feces in large quantities (up to 10^{11} viruses/g stool) for a period ranging from several days to several months (Blacklow and Greenberg, 1991). Consequently, wastewaters are likely to contain a large amount of enteric viruses (Cantalupo et al., 2011; Lodder et al., 2013). These effluents are then treated by wastewater treatment plants (WWTPs) which are not

designed to specifically eliminate enteric viruses (Kitajima et al., 2012). WWTP effluents flow in rivers that are potentially used for different purposes such as shellfish farming (Rajko-Nenow et al., 2013), recreational activities (Dorevitch et al., 2012) and market gardening (Cheong et al., 2009) but also as catchment sources to produce drinking water (Maunula et al., 2005).

In order to appreciate and model the risk assessment of viral contamination associated with surface water, it is necessary to acquire more data on viral contamination of surface water and WWTP effluents. However, there were a few studies reporting the spatial and temporal dynamics of the different enteric viruses in surface water and treated wastewater. If the water microbiological quality is generally based on the monitoring of fecal indicators (*Escherichia coli* (*E. coli*) and intestinal enterococcus), these bacteria have generally a capacity of persistence in water and a resistance to disinfection treatments lower than the human enteric viruses and can rarely serve as a valuable proxy to survey viral contamination (Contreras-Coll et al., 2002; Tree et al., 2003).

This study monitored the evolution of various circulating enteric viruses (adenovirus, aichivirus, astrovirus, cosavirus, enterovirus, hepatitis A and E viruses, norovirus of genogroups I and II, rotavirus A and salivirus) over one year in the river Seine through the Paris urban area. To our knowledge, for the first time, the estimation of viral fluxes identified clearly the main viral contamination source, WWTP effluents,

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in an urban river and permitted the monitoring of their evolution over time. Finally, this study implied a close relationship between the health status of the population connected to a sewage system and the viral contamination of surface water.

2. Materials and methods

2.1. Water sample campaign

Starting from May 2013 until May 2014, 11 water samples of 10 L were collected twice a month at different points of the river Seine, upstream to downstream the Paris urban area (Fig. 1), for a total of 275 analyzed water samples. Each water sample was stored at 4 °C until 24 h maximum before the concentration of the viral particles. The treated wastewater samples were collected from the four major Paris area WWTPs which are designed to eliminate classical pollutants (carbon, nitrogen, and phosphorus). Sewage of the four plants is mainly treated by activated sludge (\blacktriangle^2 , 600 000 m³/day, 1.5 million equivalent inhabitants), by biological filtration (\blacktriangle^5 and \blacktriangle^{10} , 240 000 m³/day, 100 000 m³/day corresponding to 1 million and 400 000 equivalent inhabitants, respectively) and by an association of these two previous processes (\blacktriangle^7 , 1.5 million m³/day, 6 million equivalent inhabitants) (Fig. 1). The large urban Ile de France area is connected to the WWTP using both combined and separated sewer network (usually combined in the downtown area and separated in the suburban area as described in (Lucas et al., 2014)).

2.2. Primers and probes design

For aichivirus, cosavirus, enterovirus, hepatitis E virus and salivirus, RNA was detected using previously published primers and probes (Table 1). For adenovirus, astrovirus, hepatitis A virus, bacteriophage MS2, norovirus of genogroups I and II and rotavirus A, new primers and probes were developed (Table 1) using recently available genome sequences.

All developed primers and probes were designed using AlleleID® version 7.01 software (Premier Biosoft, Palo Alto, CA) by multiple sequence alignment of complete genomes, collected in NCBI GenBank database. Their specificity to various serotypes or genotypes of viruses and their strict specificity to human virus were evaluated *in silico* (Table 1). The developed primers and probes were evaluated using stool samples kindly provided by the French reference national center for enteric viruses. In addition, the performance of amplification reaction for norovirus, adenovirus and enterovirus was evaluated using external quality assessment panels from Quality Control for Molecular organization (QCMD, Glasgow, Scotland).

2.3. Viral concentration from water samples

Water samples were concentrated by three successive filtration/concentration steps previously described (Wurtzer et al., 2014). Briefly, 10 L of water sample was filtered using electropositive filters (NanoCeram® Virus Samplers, Argonide, Sanford, FL). Filters were then sonicated at 4 °C for 1 h in an elution buffer composed of 1%

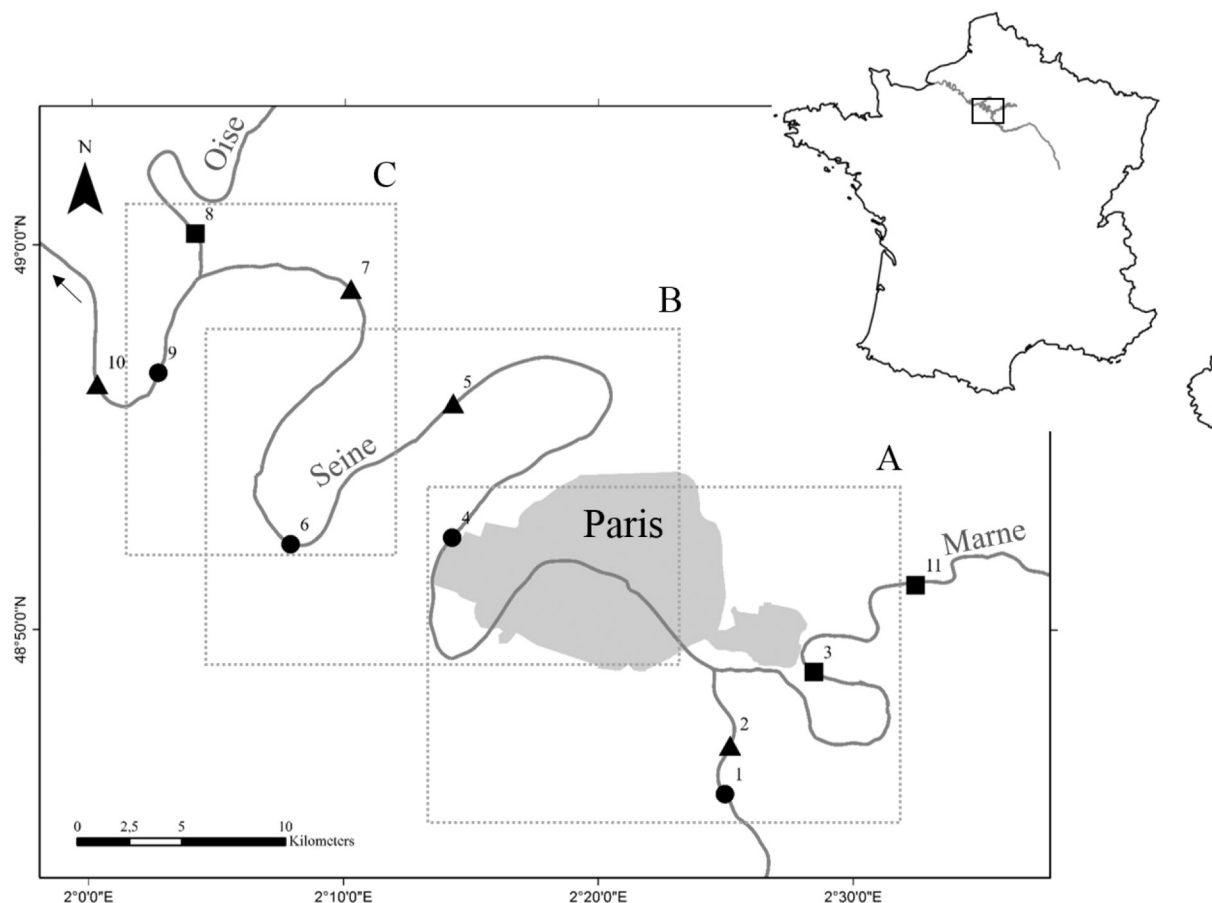


Fig. 1. Map representing a section of the Seine River and all sampling points from the Seine River ●, the tributaries ■ and WWTP effluents ▲. This section of the Seine River was divided into three sub-sections: A, B and C. The arrow indicates the direction of the Seine River flow.

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