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

B. Prevost [a], F.S. Lucas [a], A. Goncalves [b], F. Richard [b], L. Moulin [c,*], S. Wurtzer [c]

[a] LEESU (UMR MA 102, Université Paris-Est, Agro ParisTech), Université Paris-Est Créteil, 61, Avenue du Général-de-Gaulle, 94010 Créteil cedex, France
[b] SIAAP, Direction du développement et de la prospective, 82, Avenue Kléber, 92700 Colombes, France
[c] Eau de Paris, DRDQE, R&D biologie, 33, Avenue Jean Jaurès, 94200 Ivry sur Seinze, France

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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.

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,

* Corresponding author.
E-mail address: Laurent.moulin@eaudeparis.fr (L. Moulin).

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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, nitrogenous, and phosphorus). Sewage of the four plants is mainly treated by activated sludge (▲[2], 600 000 m^3/day, 1.5 million equivalent inhabitants), by biological filtration (▲[5] and ▲[10], 240 000 m^3/day, 100 000 m^3/day corresponding to 1 million and 400 000 equivalent inhabitants, respectively) and by an association of these two previous processes (▲[7], 1.5 million m^3/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%

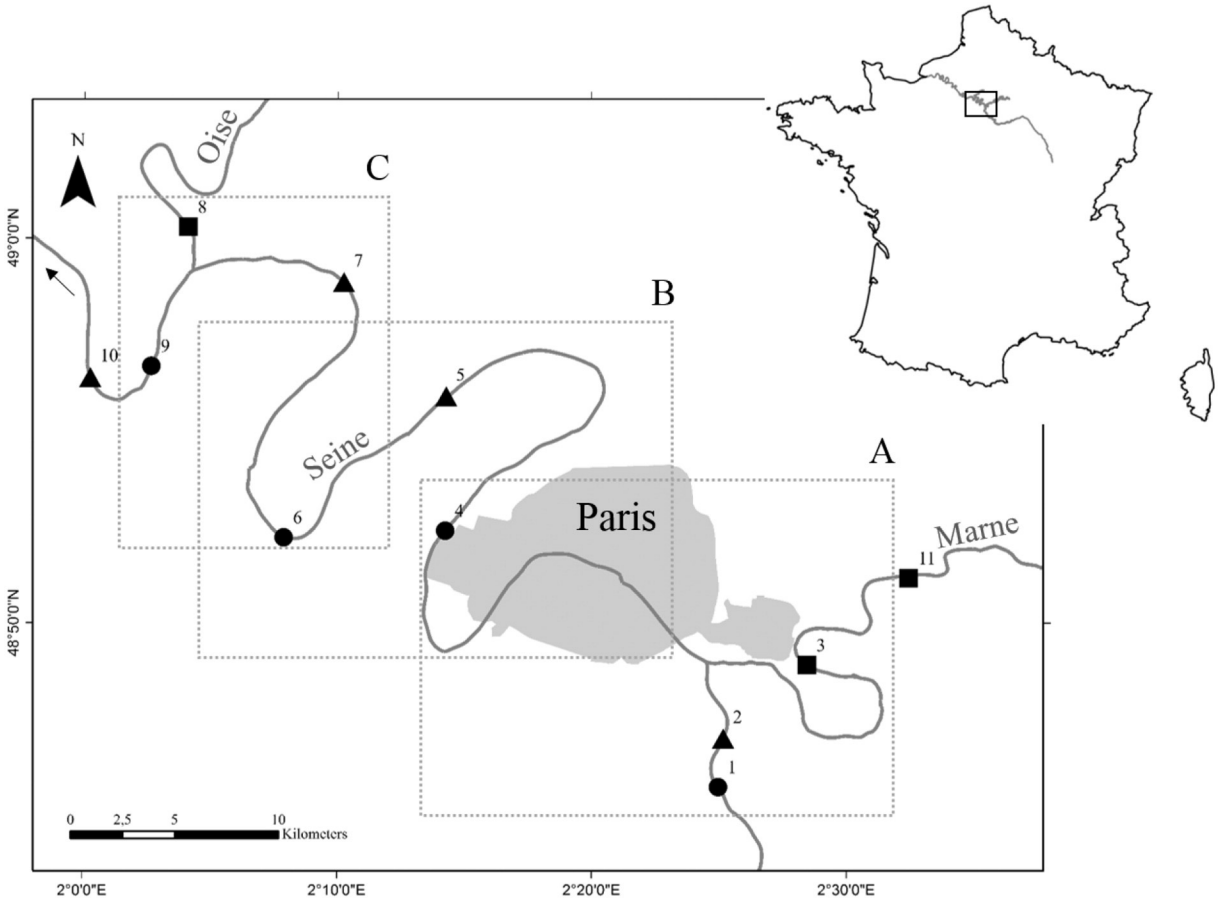

Fig. 1. Map representing a section of the Seine River and all sampling points from the Seine River ●, the tributaries ■ and WWTP effluents ▲. This section of the Seine River was divided into three sub-sections: A, B and C. The arrow indicates the direction of the Seine River flow.

Table 1
Sequences of primers and probes used for detection and quantification of enteric viruses. {G} = LNA G base; {T} = LNA T base; Y = T or C base; R = A or G base; S = C or G base; D = A, G or T base; M = A or C base; K = G or T base; V = A, C or G base; W = A or T base.

Viruses	Primers and probes	Quantification limits (genome copies/reaction)	nM	Sequences 5′–3′	Targets	References
Adenovirus ABCDEFG (AdV)	AdV_ABDEFG_F17676	10	300	TACATGCAYATCGCCG	*Hexon*	This study
	AdV_ABDEFG_R17727		900	CGGGCRAAYTGCACC		
	AdV_ABDEFG_P17694		400	FAM-CAGGAYGCYTCGGARTAYCT-BHQ1		
	AdV_C_F1767		300	TACATGCACATCTCGG		
Adenovirus 5 LacZ ΔE1 ΔE2	Vector_F	10	300	CGCATAGTTAAGCCAGTATC	Linking sequence	This study
	Vector_R		300	CTTGCCTTGTTGTAGCTTAA		
	Vector_P		100	DFO-CTACTCAGCGACCTCCAACAC-BHQ2		
Aichivirus (AichiV)	AichiV_F	10	1000	CCCAGTGTGCGTAACCTTCT	*5′-UTR*	Nielsen et al. (2013)
	AichiV_R		1000	GTACCTGCCTGGCATYCCTA		
	AichiV_P		200	HEX-ACGCCCTGTGCGGGATGAAA-BHQ1		
Astrovirus (AstV)	Astrovirus_F_4126	100	400	ATCACTCCATGGGAAGCTCCT	*ORF1–ORF2 junction*	This study
	Astrovirus_R_4264		400	GCGATGGAGTTGCTCTTCTGTG		
	Astrovirus_P_4221		200	FAM-TCCAGAVTCACGAAGCTGCTTWGCAGTCC-BHQ1		
Cosavirus (CosaV)	CosaV_F	100	1000	CTCCCGTTCCTTCTTGGAC	*5′-UTR*	Nielsen et al. (2013)
	CosaV_R		1000	CACTGTGTGGGTCCTTTCG		
	CosaV_P		200	FAM-AGCGATGCTGTGCGTGTGTG-BHQ1		
Enterovirus (EV)	EV_F453	10	900	GCCCCTGAATGCG	*5′-UTR*	Wurtzer et al., (2014)
	EV_R583		900	TGTCACCATAAGCAGY		
	EV_P536		100	FAM-CCAAAGTAGTCGGTTCC-NFQ MGB		
Hepatitis A virus (HAV)	HAV_F276	100	400	GGTCAACTCCATGATTAG	*5′-UTR*	This study
	HAV_R442		900	GCATCTCTTCATAGAAGTA		
	HAV_P301		100	FAM-CTGTAGGAGTCTAA-NFQ MGB		
Hepatitis E virus (HEV)	HEV_F	10	250	GGTGGTTTCTGGGGTGAC	*Capsid*	Jothikumar et al. (2006)
	HEV_R		250	AGGGGTTGGTTGGATGAA		
	HEV_P		100	FAM-TGATTCTCAGCCCTTCGC-BHQ1		
Bacteriophage MS2	MS2_F632	10	100	GTCGCGGTAATTGGCGC	*Maturation protein*	this study
	MS2_R708		300	GGCCACGTGTTTTGATCGA		
	MS2_P650		300	FAM-AGGCGCTCCGCTACCTTGCCCT-BHQ1		
Norovirus (NVGI and NVGII)	NVGI_F5290	10	600	CGYTGGATGCGSTTCCAT	*ORF1–ORF2 junction*	This study
	NVGI_R5374		300	CTTAGACGCCATCATCATTTAC		
	NVGI_P5318		300	FAM-CGACYCCGTCACA-BHQ1		
	NVGII_F5012		900	ATGTTYAGRTGGATGAGRTTCTC		
	NVGII_R5080		300	CGACGCCATCTTCATTCACA		
	NVGII_P5042		200	HEX-AGCACGTGGGAGGGCGATCG-BHQ1		
Rotavirus A (RV-A)	RAV_F9	10	300	ATGSTTTTCAGTGGTTGMTGC	*nsp3*	This study
	RAV_R83		600	AGCDACAACTGCRGCTTC		
	RAV_P35		100	FAM-ATGA{G}TC{T}ACDCARCA{G}A{T}GG-BHQ1		
Salivirus (SaliV)	SaliV_F1	100	1000	TAGTCGTCTTCCGGCTTGTC	*5′-UTR*	Nielsen et al. (2013)
	SaliV_R1		1000	CCTGGGTGGTCTTGAGKTGT		
	SaliV_P1		200	FAM-TGCCCAACGCCCGTACTTTGG-BHQ1		
	SaliV_F2		1000	CCTCTCATGTGTGTGCTTGG		
	SaliV_R2		1000	GTCCATTRCTGGACTGGTCT		
	SaliV_P2		200	HEX-CTGAGACGATGTTCCGCTGTCCC-BHQ1		

beef extract (Bacto® Beef Extract Dessicated, BD Bioscience, Franklin Lakes, NJ), 0.05 M glycine (Merck®, Darmstadt, Germany), 0.1% Tween 80 (Sigma Aldrich, St Louis, MO) and 0.1% sodium polyphosphate (Sigma Aldrich, St Louis, MO), adjusted at pH 9.5. Then the viruses were eluted in an inverted flow. Second, the pH was adjusted at pH 3.5, allowing virus flocculation under slow magnetic agitation for 1 h and followed by a centrifugation at 4000 ×*g* at 4 °C for 2 h. Third, the pellet was resuspended in PBS 1× pH 9 and was finally ultracentrifugated on 1 mL of 40% sucrose at 150,000 ×*g* at 4 °C for 2 h. The pellet was resuspended in 1 mL of 40% sucrose. The mean recovery rate of the complete detection method in spiked experiments (3 experiments with 10 L of surface water and 3 experiments with 10 L of WWTP effluents) ranged from 18 to 42% for AdV type 41 and AdV type 5 *LacZ ΔE1 ΔE3*, 31 to 57% for AstV type 1, 57 to 83% for coxsackievirus B3, 40 to 68% for NVGI.4, 42 to 61% for NVG.4 and 39 to 65% for RV-A. The endogenous viral contamination, measured before the spiking, was negligible compared to the concentration of spiking solutions and did not affect the estimation of recovery rates.

2.4. Extraction of viral nucleic acid

All viral nucleic acids from concentrated water samples were extracted with a MagNA Pure Compact extractor and MagNA Pure Compact Nucleic Acid Isolation Kit I — Large Volume (Roche Applied Science, Bâle, Switzerland), allowing the processing of samples up to 1 mL according to the manufacturer's instructions. Extracted nucleic acids were immediately analyzed and the leftover was stored at −80 °C.

2.5. Real-time PCR assay conditions

Each independent reaction was carried out with 5 μL of total nucleic acids extracted, using specific primers and probes for each virus (Table 1) and TaqMan® Fast Virus 1-Step Master Mix (Life Technologies, Carlsbad, CA) according to the manufacturer's recommendations. All reactions were performed with a ViiA™ 7 real-time PCR system (Life Technologies, Carlsbad, CA). The thermal cycling profile was one step of reverse transcription at 50 °C for 5 min, one step of initial denaturation at 95 °C for 20 s, 45 cycles of 5 s denaturation at 95 °C and 40 s annealing/extension at 60 °C. Fluorescence was measured at the end of annealing/extension step on FAM, HEX and DFO channels. Each amplification run included a no template control and a positive amplification control based on plasmids used to perform standard curves. Results reported for each sample were means of duplicate. The raw amplification data were collected with ViiA™ 7 software version 1.2.1 (Life Technologies, Carlsbad, CA) and then processed with Excel software (Microsoft, Redmond, WA).

2.6. Viral quantification

For each targeted virus, standard curves were performed using pGEM-T-easy vector (Promega, Madison, WI) containing the amplified sequence. The plasmid concentrations were determined by spectrophotometry using a BioSpec-nano Micro-volume UV–Vis Spectrophotometer (Shimadzu, Kyoto, Japan) and then used to establish standard curves by 10-fold serial dilutions, ranging from 10^8 to 1 genome units/reaction. These standard curves permitted quantifying the virus number in each water sample. The results were expressed as number of genome copies/L.

2.7. Viral concentration controls and analysis validation criteria

Three controls were included in order to confirm the good recovery rate of this method. A global control, an adenovirus 5 *LacZ ΔE1 ΔE3* produced by transfection of pAD/CMV/V5-GW/LacZ vector (Life Technologies, Carlsbad, CA) in 293A cells, was beforehand seeded at 10,000 genome units in each water sample before the concentration steps. A bacteriophage MS2, used as an extraction control and as a control of reverse transcription step, was seeded at 10,000 genome units in each concentrated water samples just before the extraction step. A competitive inhibition control of the amplification step, made of a partial sequence of human β-actin gene cloned into pCR2.1 TOPO vector (Life Technologies, Carlsbad, CA) and flanked by EV_F453 and EV_R583 sequences (Table 1) and 1000 genome units was added after extraction of total nucleic acids. If the recovery rate of the global control was at least equal to the lower limit determined in spiked experiments (i.e., 18%) and if no inhibition of extraction and amplification controls was observed, then the results would be validated. If an inhibition of extraction and/or amplification controls was observed (i.e., a Cycle threshold (Ct) shift greater than 2 cycles between the water sample and blank sample), then a dilution of extracted nucleic acids could be tested to overcome the inhibition. If the recovery rate was not validated and if any inhibition of the two other controls was observed, the sample would be excluded of the analysis.

2.8. Calculation of viral fluxes

For each water sample, the viral flux was calculated by multiplying the estimated viral load and the average daily flow measured at each sampling point (Table S1, supplementary data). All flow-rate data were measured and provided by the Parisian public sanitation service using both ultrasound and venturi devices.

$$\text{Viral Flux/day} = \text{Viral load} \times \text{Daily flow}$$

2.9. Statistical analysis

All statistical analyses were performed using GraphPad Prism version 6.01 software (GraphPad, La Jolla, CA). Wilcoxon matched-pairs signed rank tests were performed to compare the viral loads of the Seine River upstream and downstream of the Paris urban area. Kruskal–Wallis tests with Dunn's multiple comparisons post-test were performed to highlight the seasonality of enteric viruses. Spearman correlation tests were performed between observed viral loads and the number of bacterial fecal indicators in the surface water samples.

3. Results

3.1. Detection frequency and viral load in water samples

A total of 275 water samples collected from 11 different sample points (see sampling location in Fig. 1) were analyzed: 100 samples from WWTP effluents, 100 surface water samples from the Seine River and 75 samples from its tributaries. For each water sample, recovery rate of the global control allowed to validate the analysis. Moreover, the extraction control and the competitive amplification control did not reveal any significant inhibition in accordance with the validation criteria defined in section 2.7 (supplementary data — Table S2). All water samples were negative for HAV and HEV. During the whole year, in all water samples from the Seine River (n = 100), AdV, AstV, NVGI, NVGII and RV-A were the most abundant and frequent populations with a median viral load ranging from 10^2 to 10^3 copies/L and detection frequency of 93%, 36%, 88%, 92% and 57%, respectively (Fig. 2a). AichiV, CosaV, EV and SaliV had a median viral load inferior to 10^2 copies/L, with a detection frequency of 21%, 14%, 6% and 13% respectively (Fig. 2a). In water samples from the tributaries (n = 75), AdV, AstV, CosaV, AichiV and NVGII had a median viral load ranging from 10^2 to 10^3 copies/L with a detection frequency of 65%, 16%, 12%, 9% and 73%, respectively (Fig. 2b). EV, NVGI, RV-A and SaliV presented a median viral load inferior to 10^2 copies/L with a detection frequency of 13%, 72%, 21% and 1% respectively (Fig. 2b). In the WWTP effluents (Fig. 2c), the viral loads were higher compared to surface water (Fig. 2a and b). In all WWTP effluent samples (n = 100), AdV and RV-A were the most abundant and frequent viruses, with a median viral load comprised between 10^4 and 10^5 copies/L and a detection frequency of 100% and 86% respectively (Fig. 2c). In treated sewage, AstV, NVGI and NVGII had a median viral load between 10^3 and 10^4 copies/L with a detection frequency of 84%, 98% and 98% respectively (Fig. 2c). AichiV, CosaV and SaliV were less abundant and frequent in treated sewage, with a median viral load ranging from 10^2 to 10^3 copies/L, and a detection frequency of 61%, 65% and 44%, respectively. Meanwhile, EV presented the lowest viral load in the effluents, with a median viral load below 10^2 copies/L, and a detection frequency of 64% (Fig. 2c). At the end of this one year sampling campaign, four major enteric viral populations could be distinguished from others in their frequencies and their viral loads: AdV, NVGI, NVGII and RV-A. The following results focused on these four viral populations. During the whole sampling campaign, bacterial fecal indicators (*E. coli* and intestinal enterococcus) were measured in parallel at the same day (or in most cases, with a time difference inferior to 24 h). No correlation was found between these four viral populations and the usual fecal indicators (supplementary data — Fig. S1).

3.2. Influence of the Paris urban area on the river Seine

The influence of the Paris urban area on the Seine River was estimated by comparing the viral loads between the sample collected the most upstream (n = 25) and the most downstream (n = 25) of the Paris urban area (Fig. 1). The downstream viral loads were significantly higher than the upstream loads (Wilcoxon matched-pairs signed rank test with all p values <0.0001) (Fig. 3). The average difference of viral loads between the most upstream and the most downstream samples was 4.90×10^3 copies/L ($\pm 5.61 \times 10^3$) for AdV, 9.25×10^2 copies/L ($\pm 1.13 \times 10^3$) for NVGI, 1.41×10^2 copies/L ($\pm 1.62 \times 10^3$) for NVGII and 2.84×10^3 copies/L ($\pm 3.84 \times 10^3$) for RV-A. The detection frequency in the upstream samples (80% (20/25) for AdV, 64% (16/25) for NVGI and 84% (21/25) for NVGII) was lower than the detection frequency in the downstream samples in which these viruses were always detected. To a lesser extent, the detection frequency of RV-A also increased from 28% (7/25) in the upstream samples to 84% (21/25) in the downstream samples.

3.3. Identification of viral contamination sources

The estimation of the daily viral flux for each sampling point took into account the flow of the WWTP effluents and the tributaries, and thus allowed to assess their real impact in terms of viral contamination of the Seine River. Therefore, in order to model the contribution of tributaries and effluents of each WWTP, the Seine River was divided into

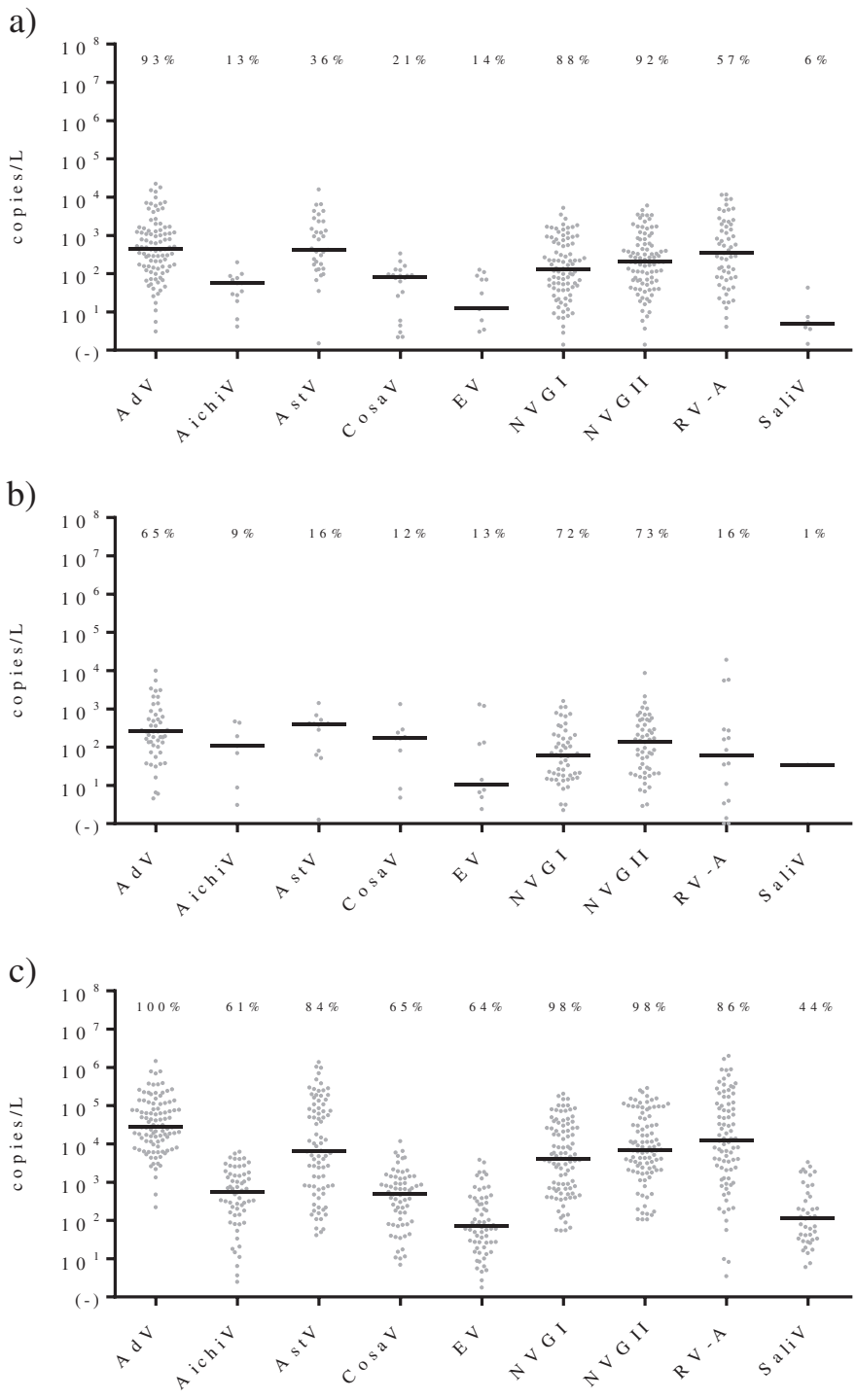

Fig. 2. Viral load of each detected enteric virus determined by real-time RT-PCR assays in water samples from (a) the Seine River (n = 100), (b) the tributaries of the Seine River (n = 75) and (c) the WWTP effluents (n = 100). Percentage represents the detection frequency of each enteric virus. The black lines represent median viral loads for each detected enteric virus. (–) means not detected.

three sections A, B and C (Fig. 1). For sections A and C, a WWTP effluent and a tributary were considered while for section B, there was only a WWTP effluent. In Fig. 4, for each sampling time, the left bar represents the viral flux addition from the upstream sampling point of the Seine River section and the viral flux from the potential sources (WWTP effluent and/or tributary), while the right bar represents the viral flux from the downstream sampling point of the Seine River section. The comparison between these bars allowed validating the consistency of the viral flux approach. Fig. 4 shows that a major part of the viral contamination resulted from WWTP effluents while the tributaries of the Seine River played a minor role. In section A, the Marne River contribution to the viral contamination of the Seine River could be considerable at times. In section C, the Oise River did not seem to have a real impact on the Seine River contamination. Overall, the most important viral fluxes in the river Seine were recorded between December and March, and were mainly due to an increase in viral fluxes from the WWTP. Indeed, the incidence rates of acute diarrhea in the Ile-de-France population available from the Sentinelles network exceeded 100 cases per 100,000 inhabitants between October and April which was consistent with the viral flux increase from WWTP effluents (Fig. 4).

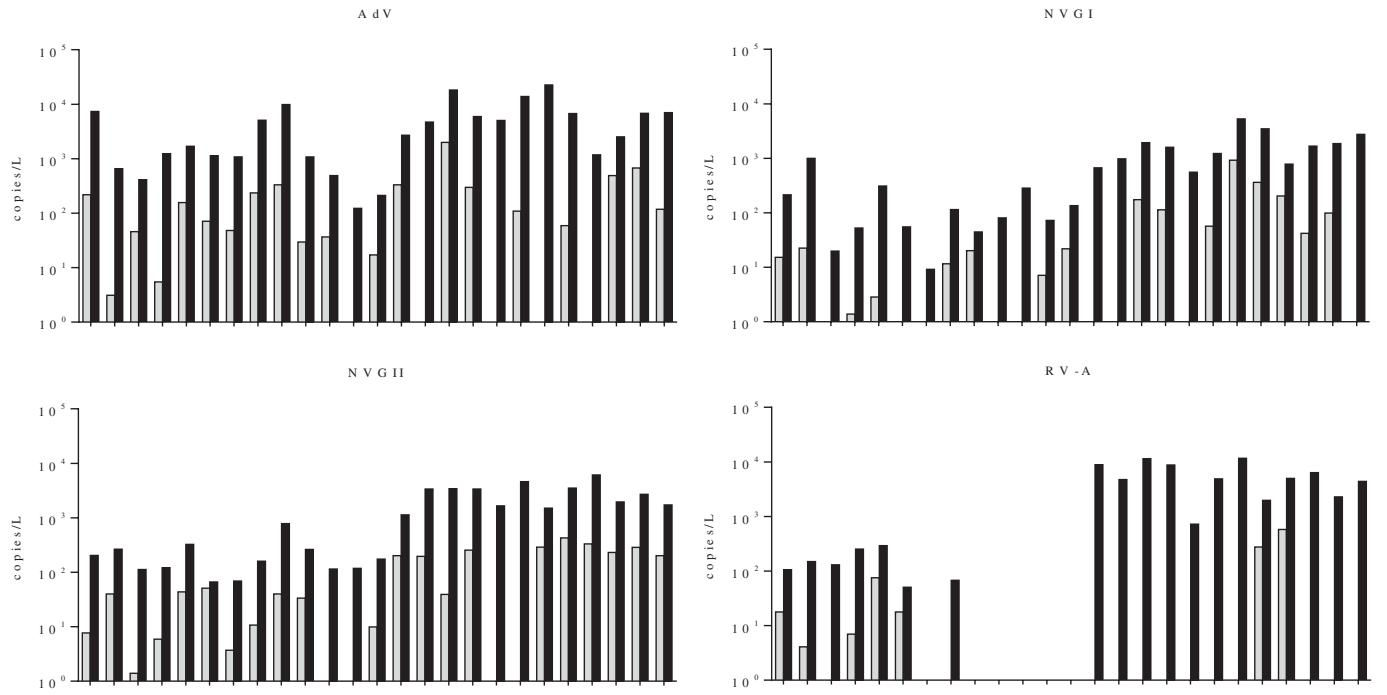

Fig. 3. Comparison of viral loads between the most upstream sampling point (n = 25, ▭) and the most downstream sampling point (n = 25, ■) of the Paris urban area during the whole sampling campaign. Each bar represents a sampling time. (–) means not detected.

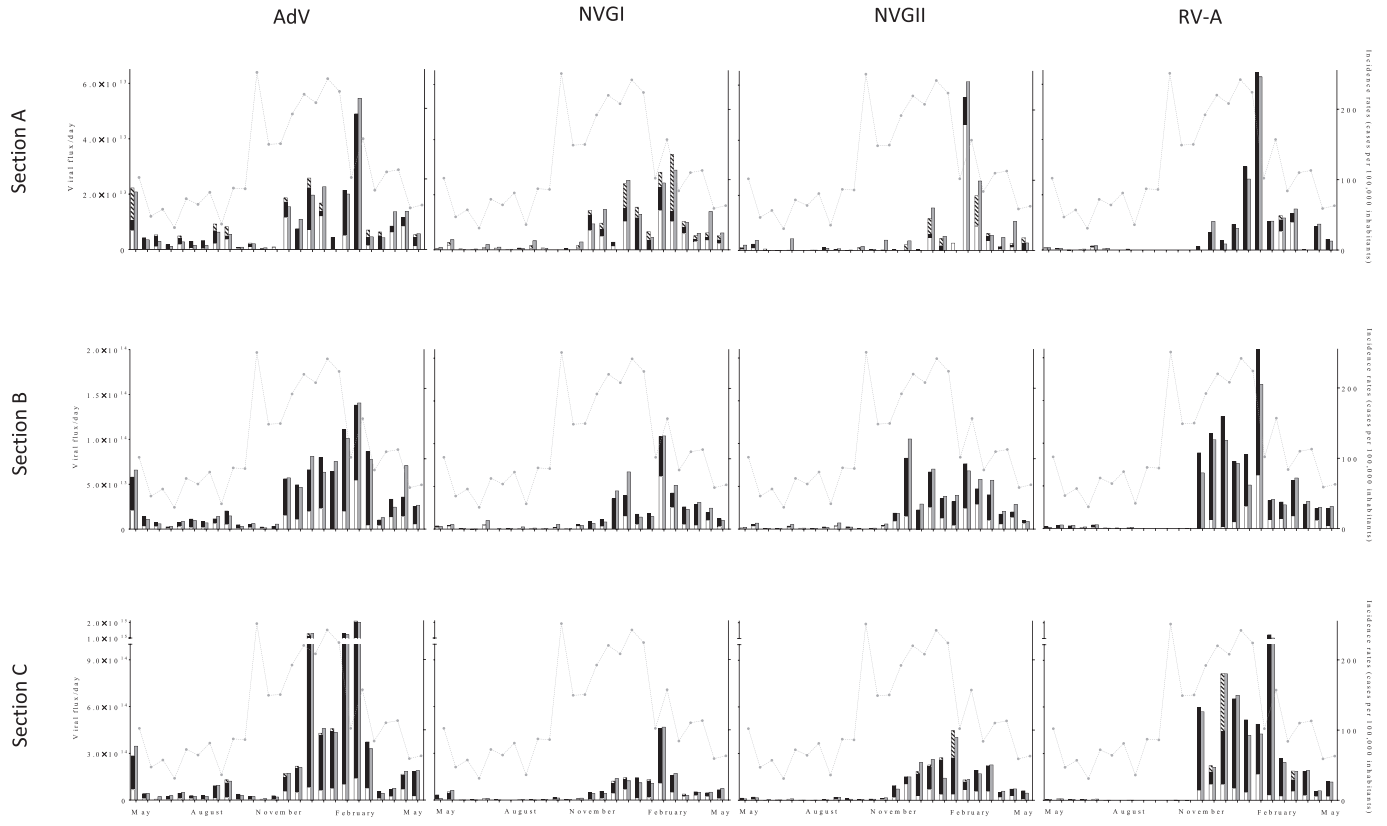

Fig. 4. Observed viral fluxes of AdV, NVGI, NVGII and RV-A over time for three different sections of the Seine River. The top, middle and down rows of viral flux graphs represent respectively sections A, B and C. Each sampling time (n = 25) is composed of 2 bars. The left one is the addition of the viral flux from the sampling point the most upstream ▭ of the Seine River section and the viral flux from WWTP effluents ■ and/or tributaries ▨ of the Seine River. The right one represents the viral flux from the sampling point the most downstream ▭ of the Seine River section. -●- Incidence rates (cases per 100,000 inhabitants) of acute diarrhea in Ile-de-France population.
Data from Sentinelles network, INSERM/UPMC, http://www.sentiweb.fr.

3.4. Seasonality of enteric viruses

In order to investigate the seasonal concentration of human enteric viruses in the WWTP effluents, a statistical test was performed for each viral population (Kruskal–Wallis test with Dunn's multiple comparisons post-test, n = 100 p values <0.05 were considered as significant). During winter, the viral loads of AichiV, AstV, NVGII, and RV-A were significantly higher than all other seasons with a median viral load of 1.55×10^3 copies/L, 1.08×10^5 copies/L, 9.57×10^4 copies/L and 2.11×10^5 copies/L respectively. However the viral loads of AdV, NVGI and CosaV in winter were significantly higher than those measured only in summer and autumn. No significant seasonal difference of viral load was shown for EV and SaliV (Fig. 5). Similar results were observed in the Seine River (data not shown).

4. Discussion

Given that human enteric viruses are excreted in feces of infected people and are mainly transmitted by ingestion of contaminated water and/or food, the monitoring of virus circulating in the environment is necessary in order to better understand the virus epidemiology and to identify the viral contamination sources. The aim of this study was to investigate the real impact of WWTP effluents in a 90 kilometer section of the Seine River for one year. In this study, the prevalence and concentration of a very large panel of human enteric viruses were evaluated using real time RT-PCR assays in different water samples from the Seine River, its tributaries and the major wastewater effluents in the Paris urban area. To our knowledge this is the first study that clearly demonstrates the quantitative contribution of WWTP effluents. We also developed several primer sets and probes that could be valuable for the monitoring of a large panel of enteric viruses. In this study, all viral loads of human enteric viruses were calculated without considering any loss of viral particles during the steps of concentration and detection. However, the global control, the extraction control and the inhibition amplification control permitted to compare all water samples analyzed even if concentrations of these viruses were probably under-estimated. Real-time PCR assays detect viral nucleic acids of both infectious and non-infectious viruses but this approach allowed to look for various enteric viruses.

4.1. Viral population circulating in the Seine River and WWTP effluents

A huge diversity of human enteric viruses could be detected in this study with especially AdV, NVGI, NVGII and RV-A that were remarkable in their high detection frequency and viral load in analyzed water samples. In accordance with previous studies, no correlation was observed between fecal indicators and these four viruses, confirming that specific analysis is needed to describe any viral contamination (Contreras-Coll et al., 2002; Tree et al., 2003). These four viruses are frequently detected in different types of water, wastewater effluents (Prado et al., 2011), river water (Hamza et al., 2009) and groundwater (Jung et al., 2011). Few studies with quantitative data for these four viruses are available in the literature to compare their occurrence in different waters and countries. In river water, the average viral load varied for NVGI and II and rotavirus between 2×10^2 and 2×10^3 copies/L in Netherlands (Lodder and de Roda Husman, 2005). For AdV the concentration was estimated between 10^1 to 10^4 copies/L in Spain, 6.10×10^2 to 8.51×10^2 copies/L in Taiwan (Huang et al., 2014) and 4.20×10^2 to 2.70×10^3 copies/L in Japan (Kishida et al., 2014). In treated sewage, the average viral load varied for NVGI and II between 8.96×10^2 to 7.49×10^3 copies/L in Netherlands (Lodder and de Roda Husman, 2005) and up to 2.15×10^7 copies/L for AdV in Norway (Grøndahl-Rosado et al., 2014). For RV-A the estimated concentration was 5.98×10^2 to 2.9×10^4 copies/L in Netherlands (Lodder and de Roda Husman, 2005). Despite the low number of studies concerning the viral load and the discrepancy between detection methods, it was noteworthy that these concentrations were in the same range. Additionally, if the size of the river was quite different between the studies, the results were similar. Further studies should focus on the population density connected to a river. In addition, 88% (241/275) of the collected samples were positive for AdV which was consistent with many studies that proposed AdV as a potential viral indicator of water contamination in several countries, for example in Spain (Albinana-Gimenez et al., 2009), in New Zealand (Hewitt et al., 2013) and in the United States of America (Kundu et al., 2013). Moreover, AichiV, CosaV and SaliV were detected for the first time in the Seine River. Their presence in surface water was not surprising in view of precedent studies realized around the world (Alcala et al., 2010; Blinkova et al., 2009; Haramoto et al., 2013). In this study, the lack of HAV and HEV detection in all water samples was quite consistent with the low seroprevalence for HAV and HEV in Paris population (Cadilhac and Roudot-Thoraval, 1996; Maylin et al., 2012). The identification of WWTP as major contributor to the contamination of the Seine River by human enteric viruses was possible by estimating the viral fluxes. For instance, the relative contribution of WWTP effluents to viral contamination measured downstream of each section (A, B and C) was 56%, 72% and 73% for AdV averaged over the year, respectively. This relative contribution of WWTP effluents was 42%, 57% and 56% for NVGI, 28%, 56% and 58% for NVGII and 60%, 72% and 63% for RV-A, respectively. Even if the tributaries contribution was minor, the relative contribution of the Marne River was higher compared to the Oise River. This observation could be explained by the upstream contamination of the Seine River in each section. Indeed,

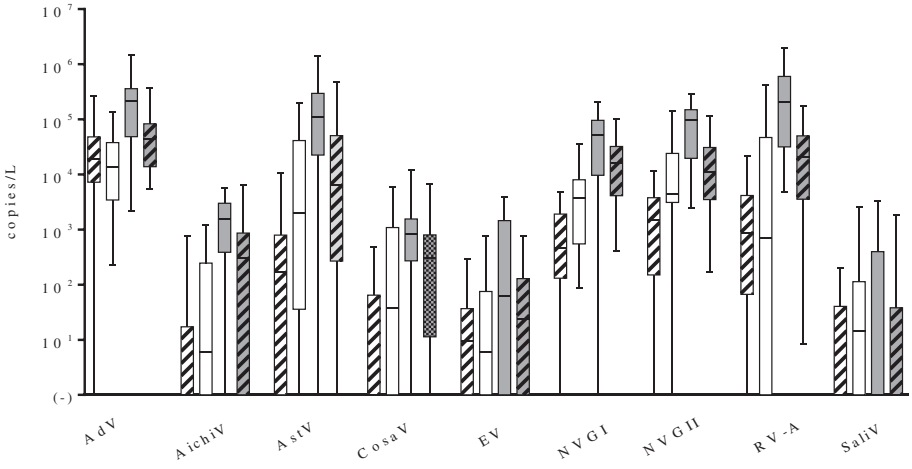

Fig. 5. Viral load of each enteric virus in WWTP effluents according to season (▨ in summer (n = 28), ▭ in autumn (n = 24), ▬ in winter (n = 24), ▨ in spring (n = 24)) during the sampling campaign (n = 100). The median value is represented by a line inside the box and the minimum and maximum values by bars. (–) means not detected.

despite the fact that viral fluxes of the Marne and Oise Rivers were comparable, the Seine River was less contaminated in section A (upstream to Paris area) than section C (downstream to Paris area).

4.2. Temporal variation and relation with the health status of the population

All calculated viral fluxes from WWTP effluents were higher between December and March. This would mean the existence of a seasonal prevalence of human enteric viruses in the population connected to the Parisian wastewater network. This result was consistent with the observed seasonality in the epidemiology of gastroenteritis (Lorrot et al., 2011). We confronted our results with the database from the French Sentinelles network, which reports weekly the French case number of gastroenteritis requiring specialized consultation. The data comparison (Fig. 4) suggested the existence of a close relationship between the health status of a population and viral contamination of WWTP effluents and surface water. However, a potential discrepancy between viruses circulating in population and isolated viruses after hospitalization could be observed. Such a difference could result by the fact that many infections are asymptomatic or pauci-symptomatic and do not need any hospitalization (Bucardo et al., 2010). Individual host factors (age, immune system, genetics …) can probably explain the severity of infection, but differences in pathogenicity between various genotypes could also impact the care of patient. The regular survey of wastewater viral quality could serve as surrogate for the evaluation of the circulating human viruses both in the human population and in the surface water. The use of high throughput sequencing approach on surface water and wastewater could permit a better prediction of epidemic outbreaks in order to optimize vaccination strategies, furthermore it could also allow an identification of potential human virus genotypes resistant to the WWTP treatments. Moreover, these viral loads present in river water are likely to have a real impact on the viral shellfish contamination (Iritani et al., 2014), on the virological quality of irrigation water and consequently on the viral quality of raw vegetables and fruits (Cheong et al., 2009). As a resource for drinking-water, the river contamination could influence the tap water quality. For example in this study, the median viral load of RV-A was about 3.50×10^2 copies/L. According to the guidelines for drinking-water quality suggested by the World Health Organization, drinking-water treatment plants related to the Seine River should provide a reduction of the viral load at least 6 $\log_{10}$ for such concentrations of RV-A (WHO, 2011). In the Paris area plants, the multistep disinfection treatments (i.e., ozonation, UV and chloration) performed after the usual physic chemical clarification were necessary. However in few cases where the viral loads were beyond the limit of 10^4 copies/L (maximal value of 1.16×10^4 copies/L in the Seine River for RV-A), the reduction should be confirmed.

5. Conclusions

The surveillance of the viral contamination of surface water in an urban area showed the huge diversity of isolated virus in water. This survey provided an overview of the viral dynamic in the Seine River around Paris area for one year. With this data, identification of the viral contamination sources could be realized by a viral flux approach, showing WWTP as the main source. The huge diversity of human enteric viruses was identified in WWTP effluents meaning that all of these viruses circulated across the local population. Moreover, in the winter period, the number of gastroenteritis cases increased, impacting directly the viral quality of the river water. Consequently, the viral quality of the river water was closely linked to the health status of the local population.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2015.03.004.

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