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Effectiveness of passive sampling for the detection and genetic characterization of human viruses in wastewater†

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Wastewater-based epidemiology is a promising tool to anticipate potential viral outbreaks not only at city-scale but also at precincts-scale or even buildings. Passive samplers (PS) and, particularly, torpedo-shaped devices containing electronegative membranes, are cost-effective alternatives to traditional sampling methods to trace the circulation of pathogens amongst a community through wastewater. Here we evaluated the effectiveness of torpedo devices to detect eight different viral targets in wastewater from an urban WWTP and from a nursing home (NH), in comparison to 24 h composite active sampling (AS). The use of one or two membranes within the torpedo devices, their potential use for semi-quantification analysis and the performance of torpedoes to conduct studies of the wastewater virome was examined. Two membranes analysis provided more certain results than one single membrane. Considering PS as a semi-quantitative approach, it produced equivalent sensitivity to AS at the NH, but lower sensitivities at the WWTP. Less viral sequences were detected with torpedoes as compared to AS when analyzing the WWTP samples while no differences at the level of viral families detected were obtained at NH when applying both approaches. Our results suggest that PS is a powerful tool for viral detection and characterization at a building scale.

Water impact

Passive sampling approaches are becoming promising tools in wastewater-based epidemiology. Torpedo devices fitted with electronegative membranes are useful, affordable and practical tools to monitor viral pathogens in small scale scenarios (*e.g.* nursing homes). They can be used to study the presence of a diversity of viruses as well as to characterize the wastewater virome.

1. Introduction

Viruses that infect humans are shed through multiple body secretions. Wastewater surveillance is advantageous because it can be used to estimate disease burden without having to collect individual clinical specimens. This approach is simple, cost-efficient, non-invasive and allows us to obtain rapid results. Wastewater-based epidemiology (WBE) for virus surveillance has been used since the 1980s to determine the spread of poliovirus, the etiological agent for poliomyelitis.[1–3] It has recently reemerged as an important tool for public health monitoring to trace the communal circulation of SARS-CoV-2 and to anticipate potential COVID-19 outbreaks caused by pre-symptomatic or asymptomatic infected individuals.[4]

Most WBE studies focus on wastewater collected from wastewater treatment plants (WWTPs) and provide useful city-scale or suburban-scale information.[5] Most studies conducted at WWTPs rely on automatic samplers that are deployed at the inlet of the WWTP. Autosamplers collect a composite sample proportional to the daily flow and/or proportional to a given time interval[6] and give a representative view of the spread of a virus in the population served. Studies focusing on small sewerage systems such as nursing homes (NH), schools, hospitals or university campuses are important for early warning to target early actions in vulnerable communities. The use of autosamplers

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at smaller settings are limited by their cost, the need of a power supply and, in some cases, the difficulty to deploy and operate such complex devices in small drainage systems.[7] Passive samplers have thus been proposed as affordable and easy-to-use alternative for virus surveillance in these smaller scale scenarios.[8] Their deployment and further collection are easy and fast, they operate without electricity, so they can be used in any point of the sewer system.

The first passive sampler described was a folded gauze with a string, known as the "Moore swab" that was immersed for 48 h in a sewer for the detection of *Salmonella typhi*.[9] Moore swabs have been used to detect several bacterial and viral pathogens such as coxsackieviruses, *Vibrio cholera*, *E. coli* O157:H7, norovirus, and poliovirus.[10–18] During the COVID-19 pandemic, Moore swabs and other passive samplers were used for wastewater surveillance in both large and small catchments.[19] Schang and coworkers developed a 3D-printed sampler unit that resembles a torpedo.[5] Among gauzes, electronegative membranes, and cottons swabs, electronegative membranes were reported to be the best collecting material to be placed within the torpedoes.[4,5] According to the available literature at the time of writing, a deployment time of 24 hours was recommended to allow viruses to concentrate into the membranes.[20] Since the beginning of the COVID-19 pandemics, passive samplers have been proposed as affordable and easy-to-use alternative for virus surveillance in small scale scenarios.[8]

Next generation sequencing (NGS) techniques are excellent tools to monitor and identify viral pathogens circulating among the population. Sewage contains a high number of viruses and viral metagenomics (*i.e.*, viromics) is a very promising tool that could be paired to WBE for a more complete description of viruses circulating within a population as well as for the study of viral (re)emergences and discovery.[21,22]

The hypothesis of this study was that passive sampling (PS) could complement and even replace data obtained from the analysis of composite samples actively collected (AS) for the presence of human viruses in wastewater. To do that, a comparison between 24 h wastewater samples from a WWTP and a NH were collected using AS and PS. The variability and semi quantification of replicate samples was evaluated. The second aim of this study was to evaluate the potential of the torpedo devices to study the sewage virome.

2. Materials and methods

2.1. Study site, passive and active wastewater sampling

An urban WWTP serving 1.5 million inhabitants and the sewer from a NH, hosting 300 residents and 200 workers were selected to validate the use of passive samplers for the recovery of a diversity of viruses (see next section). From January 10th till March 22nd, 2022, a total of 30 samples from the inlet of the WWTP were collected using the two sampling methodologies. Fifteen AS using an ISCO 6712FR autosampler (Teledyne, Lincoln, NE, USA), that collects 24 h influent time-proportional samples (100 ml per hour over a 24 h period), and 15 PS devices, also known as torpedo sampling units (kindly donated by David McCarthy), containing 3 electronegative membranes. Torpedoes were deployed and retrieved 24 h later. Over the same time period, 30 samples from the residence building were also collected using both methods (a HACH-Bühler 2000 autosampler (HACH-LANGE GmbH, Germany) was used for AS (at the rate of 100 ml per hour over a 24 h period)). Passive and active samples (in a final volume of 250 ml of the total 2.4 litres of wastewater collected by the autosamplers) were handled using protective equipment (lab coats, glasses, gloves) in sterile containers (sealable plastic bags or 250 ml bottles respectively) and transported into a cooler to the laboratory for processing in less than 2 h.

2.2. Concentration of viruses, extraction of nucleic acids and analysis using quantitative PCR (qPCR)

Concentration of viral particles from composite wastewater samples (hereafter active sampling, AS), was performed by first removing debris by centrifuging 100 mL of the total 250 ml of sample at 4750 × *g* for 30 min. The resulting supernatant (80 mL) was then ultrafiltered using the automatic Concentration Pipette (CP-Select™) with 150 KDa tips (Innovaprep) into a final volume of 300 μL as previously described.[23] All water samples were spiked with the bacteriophage MS2 as process control, at a final concentration of 1×10^5 GC ml^{-1}, before any processing was carried out. Viral nucleic acids (NA) were extracted with the QIAamp Viral RNA mini kit using the QIAcube automatic system (Qiagen). The sample concentrates (140 μL) were used for the extraction in an elution volume of 70 μL. A negative control of the viral nucleic acid extraction, using PBS was added per batch of samples. PS units were dismantled on the day of retrieval. Two electronegative membranes fitted into the PS were collected and placed in a Petri dish with 0.5 ml of RNA preservative (RNA shield, Zymo research) and maintained at 4 °C until extraction on the next day. The third membrane was stored at −80 °C as a counter sample. Viral RNA extract was carried out using the RNeasy Power Microbiome Kit (Qiagen) into a final volume of 50 μL following manufacturer instructions and adding a step of bead-beating for 30 s at 4 m s^{-1} using FastPrep-24™ (MP Bio, USA).

Specific qPCR and RT-qPCR assays were used to quantify MS2 bacteriophage,[24] human adenovirus (HAdV)[25,26] JC polyomavirus (JCPyV),[27] human enterovirus (EV),[28] rotavirus (RoV),[29] norovirus genogroup I (NoV GI),[30–32] and norovirus genogroup II (NoV GII)[33,34] as previously described. For SARS-CoV-2, the N1 and N2 assays[35] targeting the gene encoding for the viral nucleocapsid protein were selected. The TaqMan® Environmental Master Mix 2.0 (Thermofisher Scientific) and the RNA Ultrasense™ One-Step RT-qPCR System (Invitrogen) were used for DNA and RNA viruses, respectively. In exception of the synthetic SARS-CoV-2 control (control 51 from Twist Biosciences), all the qPCR standards were prepared (as described by Rusiñol *et al.*, 2020 (ref. 36))

using synthetic gBlocks Gene fragments (IDT), quantified with a Qubit fluorometer (Thermofisher Scientific), and diluted serially from 10^0 to 10^7 copies per reaction. Quantification was performed in a QuantStudio™ Real-Time PCR System from ThermoFisher Scientific. Undiluted and 10-fold dilutions of the NA extracts were analysed, and MS2 was also assessed in wastewater samples collected by AS, to test for inhibition. Non-template controls were included in each qPCR plate. All qPCR preparations were performed in a clean laboratory and template addition was done inside a PCR cabinet. Standard qPCR curves were accepted under the following parameters: mean slope, between −3.1 and −3.5; r^2 = 0.999; and mean efficiency between 85 and 110%. The limit of detection (LoD) of the qPCR method was calculated by running six replicate tenfold dilutions of target SARS-CoV-2, JCPyV, HAdV suspensions around the detection end point (2.5, 5, 25 and 50 GC per well). The concentration that produced at least 95% positive replicates was assumed to be the LoD of the assay. The limit of quantification (LoQ) was estimated using the procedure described previously.[37]

2.3. Target enrichment sequencing (TES)

2.3.1. Sequence-independent, single-primer amplification (SISPA). A total of 24 samples (6 AS and 6 PS from the NS and 6 AS and 6 PS from the WWTP) were prepared prior to the library construction following the protocol previously described.[38] To analyse both RNA and DNA viruses, NA were retrotranscribed using in this study the SuperScript IV enzyme (Invitrogen) and random nonamer primers. The second cDNA strand was obtained using Sequenase 2.0 (Applied Biosystems) and then amplified following 25 PCR cycles using AmpliTaq Gold DNA polymerase (Applied Biosystems) to obtain enough dsDNA for downstream analyses. The PCR products were further purified with Zymo DNA Clean & Concentration kit (Zymo Research) and the resulting cDNA quantified using Qubit 2.0 (Life technologies) and the Qubit dsDNA HS Assay Kit (Invitrogen).

2.3.2. Library construction. Libraries were constructed using KAPA HyperPlus Library Preparation Kit (KAPA Biosystems, Roche). Following the manufacturer's instructions, enzymatic fragmentation was conducted from a starting quantity of 100 ng. After fragmentation, an end-repair, an A-tailing reaction and an adapter's ligation was performed following manufacturer instructions. Each sample was paired with the desired index using the KAPA UDI Primer mixes (KAPA Biosystems, Roche). A post-ligation clean-up was followed with the magnetic selection using KAPA HyperPure Beads (KAPA Biosystems, Roche). Libraries were then amplified using a 7 cycles LM-PCR and purified. The concentration of the resulting libraries was measured using Qubit 2.0 (Life technologies).

2.3.3. Capture of viral sequences by VirCapSeq-VERT capture panel. The VirCapSeqVERT Capture Panel (Roche) consisting in sequences from vertebrate viral pathogens was used to enrich the sample with vertebrate virus sequences. The libraries were carefully mixed to obtain a pool of 1 μg. Using the HyperCap Target Enrichment Kit (Roche) and the HyperCap Bead Kit (Roche), the pool was hybridised for 20 h at 47 °C and recovered with the Capture Beads (HyperCap Bead Kit, Roche). The captured DNA was amplified with 14 cycles LM-PCR and purified using HyperPure Beads (Roche). The concentration was measured using Qubit 2.0 (Life technologies) and sequenced on an Illumina NextSeq platform (400 M reads).

2.3.4. TES bioinformatic processing. Pair-end FASTQ files generated from the sequencing were analysed using IDseq portal, a cloud-based, open source bioinformatic platform.[39] First, short-read sequencing data was validated performing a subtraction of host sequences *via* STAR (spliced transcripts alignment to a reference) of a raw read to a host-specific database (STAR, RRID: SGR_015899).[40] Illumina adapters were trimmed *via* Trimmomatic[41] and low-quality reads, duplicates, and low complexity reads were removed using the Paired-Read Interactive Contig Extension (PRICE) computational package (PRICE, RRID:SCR 013063),[42] the CD-HITDUP tool v4.6.8 (CD-HIT, RRID:SCR 007105),[43] and a filter based on the Lempel–Ziv–Welch (LZW) compression score, respectively. Viral reads were taxonomically classified using an assembly-based alignment to the NCBI nucleotide (nt) and non-redundant protein (nr) databases[44] using GSNAPL[45] and RAPsearch2.[46] Only viral reads with a 70% identity and >100 nt length were selected.

3. Results and discussion

3.1. Determination of the number of membranes needed for a representative passive sampling

To our knowledge and since the first use of the torpedo-shaped passive samplers for SARS-CoV-2 surveillance in wastewater, only Habtewold and coworkers used more than one electronegative membrane from each passive sampler to test for the presence of viral pathogens and indicators.[4] To our knowledge our study is the first one testing the rationality of using two membranes from the same sampling device to give reliable results.[5,7,47,48] In the current work, two electronegative membranes were tested per torpedo device to evaluate the presence of 3 different viral targets using qPCR: two viral indicators of human faecal contamination (HAdV and JCPyV) and SARS-CoV-2 (N1). A total of 15 PS, each containing 2 membranes, were collected in parallel to two 24 h-AS replicates. Fig. 1 shows the concentration (in genome copies per litre) of the target viral indicators and SARS-CoV-2 (N1 assay) obtained from AS replicates while Fig. 2 shows the values obtained when analysing the torpedo membranes.

As shown in Fig. 1, whereas no noticeable differences were observed comparing viral GC values obtained from different AS replicates, (Pearson's correlation coefficient test 0.76, 0.61 and 0.96 for JCPyV, HAdV and SARS-CoV-2 (N1 assay) respectively) a lower correlation was obtained between the PS membranes (Pearson's correlation coefficient test below

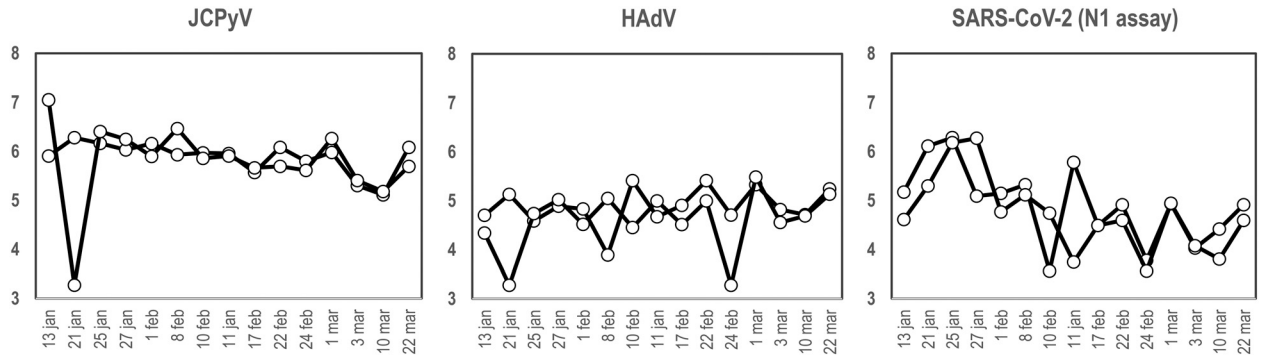

Fig. 1 Quantification (in $\log_{10}$ GC L^{-1}), of JCPyV, HAdV and SARS-CoV-2 (N1 assay), from two different wastewater replicates collected at different dates (*X*-axis) using AS in a WWTP.

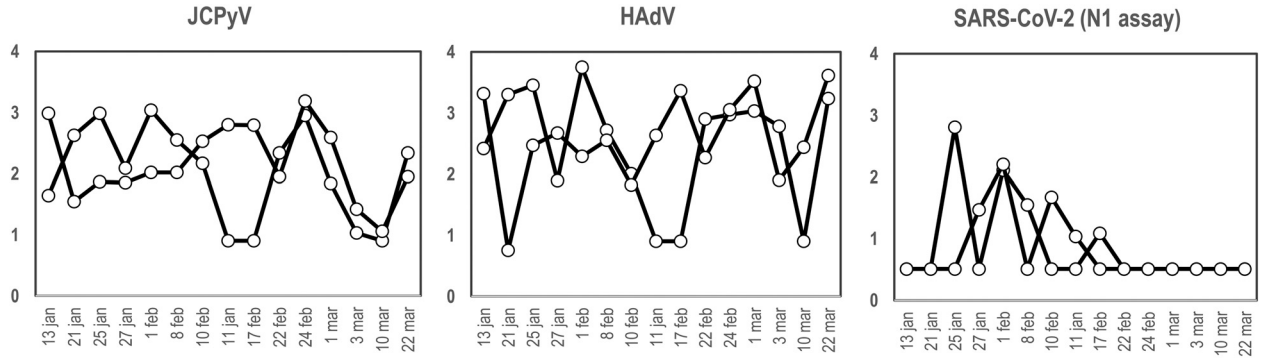

Fig. 2 Quantification (in $\log_{10}$ GC/reaction), of JCPyV, HAdV and SARS-CoV-2 (N1 assay), from two different membranes from a PS at different dates (*X*-axis) at the inlet of a WWTP.

0.33). Note that for PS membranes results are expressed in copies per reaction since the exact volume of wastewater passing through the torpedo is unknown and the calculation of viral concentrations is, thus, unattainable. Exceptional differences were detected on January 21st in JCPyV and HAdV quantifications, which may arise from errors in the analytical procedure (*e.g.* ultrafiltration tips, pipetting errors).

Membrane replicates showed greater variability (Fig. 2) probably due to irregular distribution of the wastewater flowing inside the torpedo. In that case a positive detection was considered when qPCR result was above the limit of detection (LoD) (10, 8 and 3 genome copies (GC) for JCPyV, HAdV and SARS-CoV-2 respectively). Considering positive and negative results, membrane replicates matched 12 out of 15 days for JCPyV and HAdV and 11 out of 15 days for SARS-CoV-2. For the further analysis in this study, it was decided to include in the analysis at least 2 membranes to obtain a representative result from each sample. A result was considered positive when at least one of the membranes resulted positive in virus detection.

3.2. Detection of human viruses at a WWTP and a NH by applying two different sampling/detection strategies

SARS-CoV-2 (N1 and N2 assays), JCPyV, HAdV, EV, RoV, NoV GI and GII, were analysed in a total of 60 samples collected using AS and PS at both WWTP and the NH from January 10th to March 22nd, 2022.

At the WWTP, all composite samples collected tested positive for all viruses analysed while the percentages of detection using PS ranged from 20% to 100% as summarized in Fig. 3. The full dataset of quantification/semi quantification values is shown in the ESI† Tables S1–S3. Inhibition was observed by quantifying JCPyV, as internal process control in 5 out of 120 total samples (Table S4†). The calculated MS2 recovery in the WW samples resulted in 40% of recovery of the seeded material (data not shown).

While human faecal viral indicators, at the WWTP, showed 100% positivity for both AS and PS, the other viral pathogens analysed resulted in 100% positivity by AS between 20% and 73% when using PS. Regarding SARS-CoV-2, between 40–47% positives were reported in PS samples, in contrast to 100% positive samples from AS. This comparison is based on the different performances between PS and AS over a 24 h period while some studies have reported higher PS sensitivities compared PS samples with grab samples.[5,7,19,47] High levels of suspended solids in wastewater may affect the adsorption of viruses to the electronegative membranes. Hayes and coworkers[49] demonstrated that adsorption of the virus to the sampling material is highly dependent of the characteristics of the water. Retention of solids can improve RNA recovery but and excessive retention can inhibit de RNA extraction process.

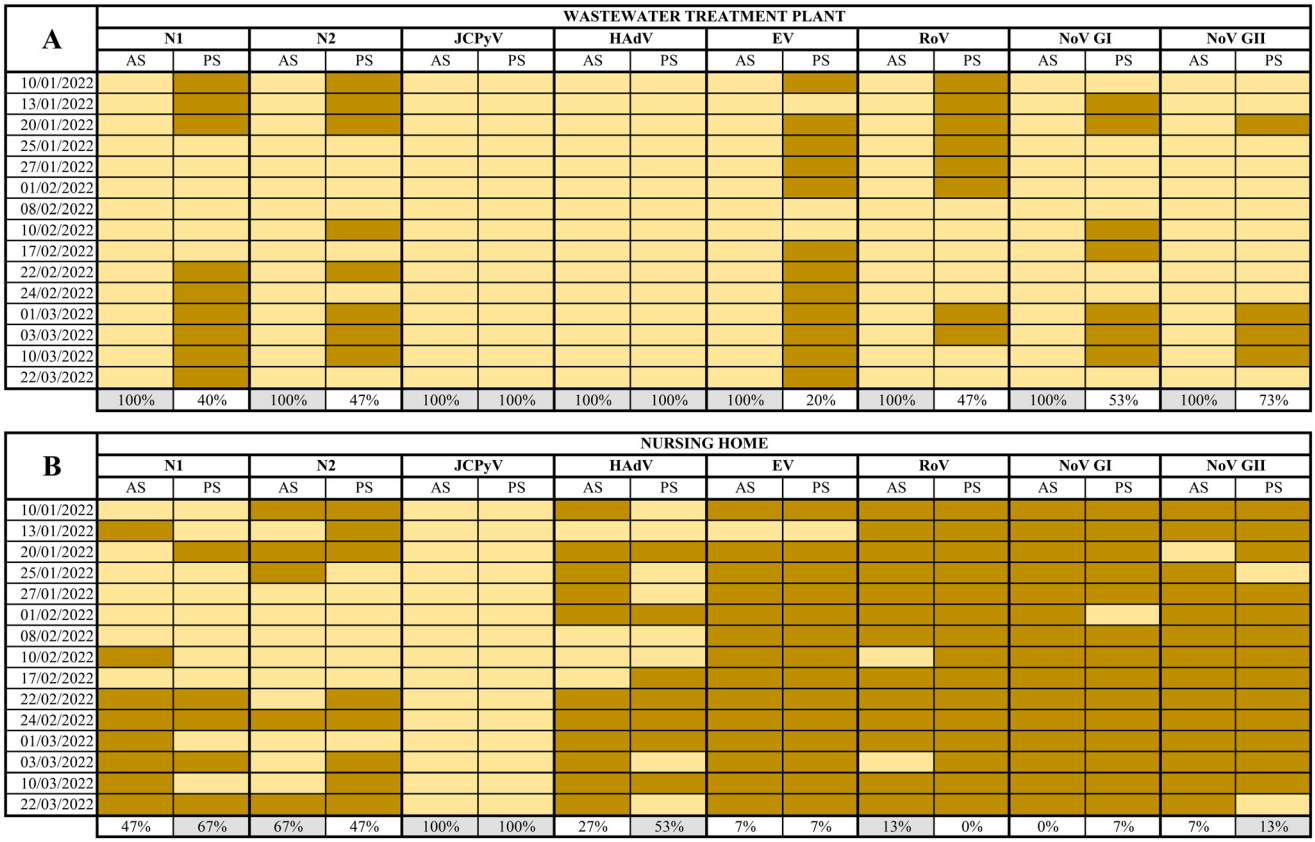

Fig. 3 Detection of SARS-CoV-2 (N1 and N2 assay), JCPyV, HAdV, EV, RoV, NoV GI and GII in wastewater samples collected using PS and AS from a WWTP (A) and wastewater samples collected using PS and AS from a NH (B). Yellow cells indicate detection and brown cells no detection.

It should be noticed that nucleic acids analysed in the current study were extracted using kits designed to provide highly pure RNA isolation by using inhibitor removal. PS performance might also have been altered by temperature, pH, or dissolved solids concentration.[50] It is also expected that different viruses show different presence patterns despite being adsorbed and concentrated with the same method, since they present different physical properties. Li and coworkers[51] suggested that the different structure between non-enveloped viruses (EV, HAdV, RoV, NoV) and enveloped ones (SARS-CoV-2) could vary their behavior in the affinity for membranes or in the recovery process. All in all, our experimental design did not allow us to differentiate whether the virus adsorption or sample processing were the critical steps in the process. According to recent studies, a low viral recovery could also have been caused by a short deployment time.[50,52] Li and coworkers recently demonstrated that the maximum viral accumulation using electronegative membranes was obtained after 48 h of deployment.[50] Wilson and coworkers[7] also concluded that sensitivities of PS could overcome grab sampling when more than 24 h of wastewater flow were considered.[7] Since the concentration of viruses from AS and PS was performed using different methods for viral recovery, the direct comparison of results is risky if not impossible.

On the other hand, at the NH, the percentage of virus detection using AS ranged from 0% for RoV to 100% for JCPyV and from 0 to 100% for the same viruses when using PS (Fig. 3, the complete data set is available in ESI† Table S3). In general, nearly equivalent results were observed when applying both methods: the more frequently detected virus was JCPyV followed by SARS-CoV-2 (N1 and N2) and HAdV while EV, NoVs and RoV were rarely detected. Negative detection for SARS-CoV-2 by PS corresponded to samples collected during a period of low prevalence of infections in the assisted community[53] suggesting lower sensitivity (in terms of the capacity to produce a positive detection of viral nucleic acid) than traditional detection using AS.

It is remarkable to notice that JCPyV and HAdV showed 100% positivity in both AS and PS in the WWTP and JCPyV also showed 100% positivity in the NH samples. These viruses are used as process controls and fecal indicators because of their abundance in wastewater and their correlation with fecal pollution.[54] It is clear that all NH samples analyzed presented fecal contamination and that JCPyV is more appropriate as fecal indicator at small-scale sewage systems as previously proposed.[55] This is a relevant output of our work since it may be used as a tool to rule out false negative samples. Unlike the WWTP, in this specific site, the presence and spread of a symptomatic viral infection within a small community is more infrequent. Consequently, the likelihood of detection of viral pathogens in wastewater is very low despite the sampling protocol. This is sustained by

the high number of negative samples obtained by both methods for RoV, EV, NoV GI, and NoV GII. The obtained results confirm that PS is a very good wastewater sampling method for viral studies in small-scale scenarios where AS is not feasible.

3.3. Evaluation of the PS as semiquantitative approach

The PS method here evaluated is based on the free flow of viral particles through the membranes and since we could not assume that all the flow contacts the PS membrane, results cannot be expressed per unit volume (*e.g.*, CG mL^{-1}). Although most studies using this methodology report qualitative results, some authors have shown a significant correlation between the measured SARS-CoV-2 RNA concentrations in samples collected using AS and PS methods.[5,20] These results suggest that PS can express semi-quantitative results for virus detection in wastewater, meaning that a higher quantity of virus in wastewater correlates with a higher accumulation of viruses in membranes and, therefore, a higher GC quantification. To perform a semiquantitative analysis, and by using all the GC values obtained in this study for the different viral species determined, we considered half of the LoD when a result was below the LoD was considered. The semiquantitative detection of JCPyV in both sampling sites presented the highest Pearson's correlation observed, being $R^2 = 0.42$ and $R^2 = 0.38$ at the WWTP and the NH respectively. The results indicate low correlation for SARS-CoV-2 (N1 assay) in both sites (0.13 at the WWTP and 0.18 at the NH). In other words, fluctuations in the genome copy detection at the qPCR would indicate increases/decreases in the genome copies per ml of wastewater.

For SARS-CoV-2, correlations improve when considering results above the LoD, being 0.78 at the WWTP and 0.8 at the NH. Other authors reported correlations between 0.27 and 0.76, in PS collected from WWTP inlets or manholes.[5,20] All in all, when viral concentrations are above the LOD, PS results correlates positively with qualitative results obtained after AS.

3.4. Virome characterization from samples obtained using PS

A secondary aim of this study was to evaluate the performance of torpedoes to study the sewage virome. With this aim, and to increase the sensitivity of the mass sequencing on-wards viruses that infect vertebrates, a target enrichment sequencing was conducted using Vir-CapSeq-VERT panel.

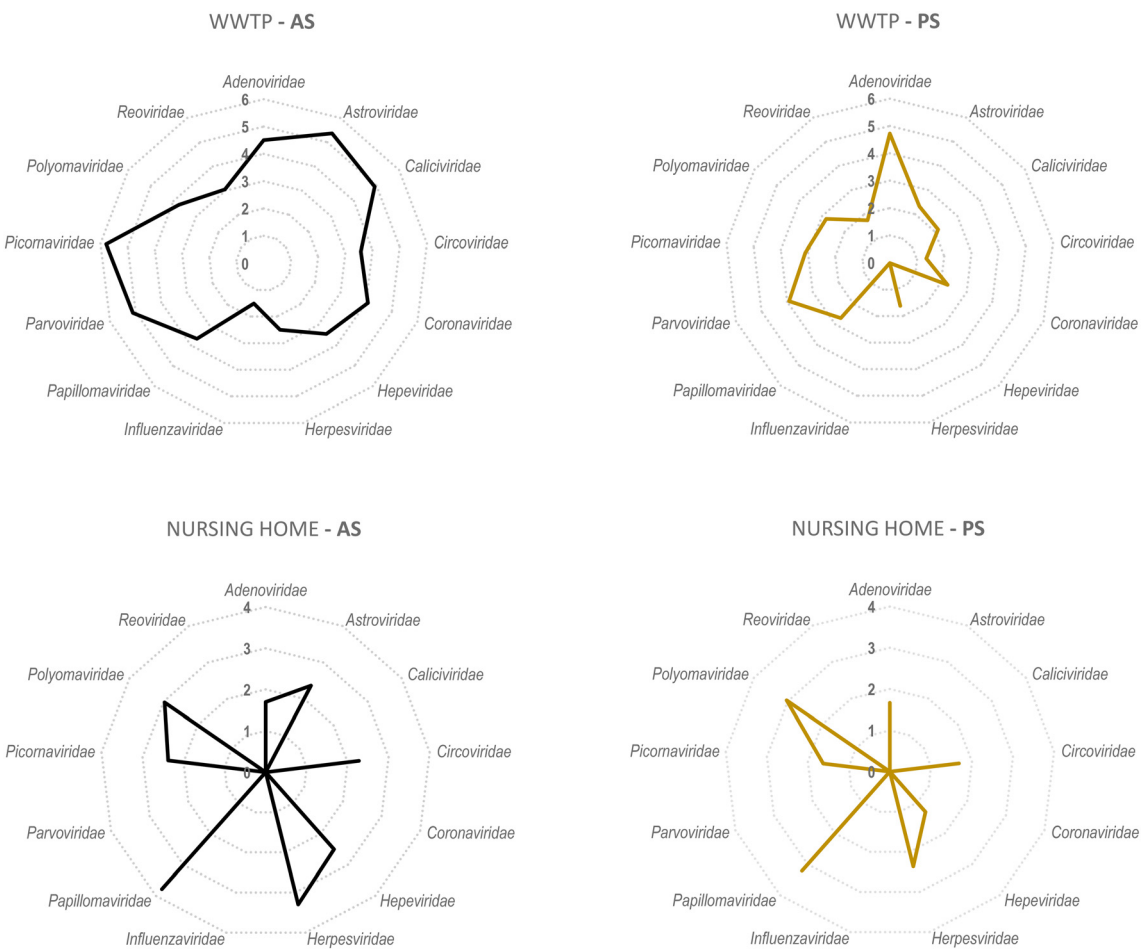

Fig. 4 Comparison of the number of log_{10} viral reads for each viral family obtained from 6 different wastewater samples when using a AS *versus* PS in both a WWTP and a NH. Numbers 1 to 6 indicate log_{10} of number of reads.

At the WWTP, viral families observed were those expected and reported in other studies where the same viral enrichment method was applied.[21] Results showed a higher diversity of viral families and genus present at wastewater samples when collected by AS (Fig. 4 and 5). The number of reads and the number of samples presenting viruses of specific viral groups were higher in samples collected using AS rather than PS. However equivalent viral groups were detected.

The most importantly excreted human pathogens are members of nine families: *Adenoviridae*, *Astroviridae*, *Caliciviridae*, *Hepeviridae*, *Parvoviridae*, *Papillomaviridae*, *Picornaviridae*, *Polyomaviridae*, and *Reoviridae*.[56] All these families have been also described as members of the sewage virome[21,57] and more recently also *Coronaviridae* and *Influenzaviridae* have been included in this list.[58] Aligned with the bibliography,[21] the *Picornaviridae* family, including important pathogens like enterovirus, poliovirus or hepatitis A virus, were the most abundant in raw wastewater. *Reoviridae* and *Hepeviridae* were found to be the least abundant when using AS. Although lower number of reads were obtained using PS at the WWTP (2 log fold), most viral families representing the sewage virome were also found using this passive approach. Viruses belonging to the *Reoviridae* family were not represented when using this sampling methodology probably because of a lower sensitivity of the methodology.

Pathogenic viruses belonging to *Adenoviridae*, *Astroviridae*, *Hepeviridae*, *Picornaviridae* and *Polyomaviridae* were present at the NH. Both methodologies provided similar abundances except for *Astroviridae* for which the AS provided higher number of viral sequences. Interestingly, a high diversity of *Herpesviridae* and *Papillomaviridae* reads were detected in the building WW. Among those viruses herpesviruses-2 and -3 as well as human papillomaviruses β and γ, highly prevalent in older people compared to young adults,[59] were found at the NH facility. The absence of *Coronaviridae* or *Picornaviridae* at NH over the sampling period could be explained because the virus was not circulating over the period of sampling in the tested community.

Passive sampling seems to be less sensitive in terms of detection and number of viral families detected by massive sequencing. Despite providing a lower number of sequences, equal diversity of viral families was obtained using both sampling methodologies. Shannon index (H') when using AS indicates higher diversity of viruses, at the WWTP (H' = 4.8), than at the NH (H' = 4.1). Nevertheless, when applying NGS at building level, the diversity of viruses obtained using PS was higher than when using AS.

To our knowledge, this is the first study applying passive sampling to the successful genetic characterization of a wastewater virome, which could be of relevance in terms of viral emergence surveillance and key information for the corresponding targeted actions. The purpose of this study was to provide information that support the use of torpedo passive sampler as an useful affordable and practical tool to provide information on viral presence in the frame of

		WWTP		NURSING HOME	
FAMILY	GENUS	AS	PS	AS	PS
Adenoviridae	Mastadenovirus	31645	53138	50	46
Astroviridae	Mamastrovirus	231300	214	232	0
Caliciviridae	Norovirus	72606	138	0	0
	Sapovirus	11070	0	0	0
Circoviridae	Cyclovirus	3746	22	188	48
Coronaviridae	Alphacoronavirus	11359	181	0	0
	Betacoronavirus	390	0	0	0
Hepeviridae	Orthohepevirus	2805	0	316	20
Herpesviridae	Lymphocryptovirus	314	41	1852	232
	Simplexvirus	0	0	130	0
Influenzaviridae	Gammainfluenzavirus	32	0	0	0
Papillomaviridae	Alphapapillomavirus	0	210	0	116
	Betapapillomavirus	3813	286	5380	1506
	Gammapapillomavirus	567	0	720	0
	Mupapillomavirus	404	0	0	0
Parvoviridae	Bocaparvovirus	128080	8492	0	0
Picobirnaviridae	Picobirnavirus	206841	58	238	42
Picornaviridae	Cosavirus	2072	0	0	0
	Enterovirus	56633	422	0	0
	Hepatovirus	68	542	0	0
	Kobuvirus	109925	198	0	0
	Salivirus	241430	46	0	0
Polyomaviridae	Alphapolyomavirus	106	80	302	346
	Betapolyomavirus	5446	575	624	742
	Deltapolyomavirus	168	0	0	0
Reoviridae	Rotavirus	1121	56	0	0

1/6 positive samples
2/6 positive samples
3/6 positive samples
4/6 positive samples
5/6 positive samples
6/6 positive samples

Fig. 5 Total number of reads obtained for each viral family after mass sequencing using a target enrichment approach to test a pool of 6 wastewater samples for each category. Color gradation indicates the number of samples (out of 6 analyzed) presenting viral reads within each viral group.

wastewater-based epidemiology at building scale level. To obtain more detailed information about viral species some improvements in the protocol of the sequencing methodology should be conducted and will be the aim of further studies.

4. Conclusions

Passive sampling presents a low-cost, safe and power-free solution for smaller sewer catchments. Torpedo shaped devices fitted with electronegative membranes can be applied as an efficient method that is sensitive to a few infection cases residing in the same building. In the event of new epidemic outbreaks, the use of passive samplers can be used for targeted actions or to track back the excreted virome of specific facilities where reside or travel vulnerable population (*e.g.* schools, universities, or aged care facilities).

Conflicts of interest

There are no conflicts to declare.

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