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β-D-glucuronidase activity triggered monitoring of fecal contamination using microbial and chemical source tracking markers at drinking water intakes

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ABSTRACT

Intense rainfall and snowmelt events may affect the safety of drinking water, as large quantities of fecal material can be discharged from storm or sewage overflows or washed from the catchment into drinking water sources. This study used β-D-glucuronidase activity (GLUC) with microbial source tracking (MST) markers: human, bovine, porcine mitochondrial DNA markers (mtDNA) and human-associated *Bacteroidales* HF183 and chemical source tracking (CST) markers including caffeine, carbamazepine, theophylline and acetaminophen, pathogens (*Giardia, Cryptosporidium*, adenovirus, rotavirus and enterovirus), water quality indicators (*Escherichia coli*, turbidity) and hydrometeorological data (flowrate, precipitation) to assess the vulnerability of 3 drinking water intakes (DWIs) and identify sources of fecal contamination. Water samples were collected under baseline, snow and rain events conditions in urban and agricultural catchments (Québec, Canada). Dynamics of *E. coli*, HF183 and WWMPs were similar during contamination events, and concentrations generally varied over 1 order of magnitude during each event. Elevated human-associated marker levels during events demonstrated that urban DWIs were impacted by recent contamination from an upstream municipal water resource recovery facility (WRRF). In the agricultural catchment, mixed fecal pollution was observed with the occurrences and increases of enteric viruses, human bovine and porcine mtDNA during peak contaminating events. Bovine mtDNA qPCR concentrations were indicative of runoff of cattle-derived fecal pollutants to the DWI from diffuse sources following rain events. This study demonstrated that the suitability of a given MST or CST indicator depend on river and catchment characteristics. The sampling strategy using continuous online GLUC activity coupled with MST and CST markers analysis was a more reliable source indicator than turbidity to identify peak events at drinking water intakes.

1. Introduction

Variability in source water microbial quality is influenced by climate and hydrometeorological events (Leveque et al., 2021). During rainfall or snowmelt, wet-weather overflows (WWOs), including combined sewer overflows (CSOs), stormwater runoff, and sanitary overflows or bypasses are a primary cause of water quality impairments in urban watersheds (Ahmed et al., 2019; Marsalek and Rochfort, 2004; Olds et al., 2018). WWO events can contaminate drinking water resources with pathogenic protozoa, bacteria, and viruses (Sojobi and Zayed, 2022), which represents a public health risk because of the host specificity of many pathogens (Eisenberg et al., 2016; Unno et al., 2018). Agricultural runoff in rural catchments can also be a concern because livestock excreta contain many zoonotic pathogens (Mateo-Sagasta et al., 2017). Cann et al. (2013) discovered eighty-seven instances of waterborne outbreaks associated with severe water-related weather

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incidents in 29 different nations. The majority of these reported outbreaks occurred in North America, and it was determined that heavy rainfall and flooding were among the most frequent precursors of waterborne disease outbreaks. Dry weather overflows (DWOs) from water resource recovery facility (WRRF) by-passes might occasionally occur from sewer blockage or pumping stations and contribute to the microbiological contamination of drinking water supplies by introducing high levels of fecal pathogens (Ahmed et al., 2016). Additionally, due to less dilution compared to urban stormwater, these pathogens, as well as other fecal pollutants generally occur at higher concentrations in dry weather sewer overflows (Powers et al., 2020).

Assessing and monitoring the quality of water at drinking water intakes (DWIs) is required to determine their vulnerability to microbial contamination and it is a key component of source water protection (Besmer et al., 2017). An event-based survey is the process of identifying when a change in water quality has occurred, what is its origin and what are the subsequent requirements for managing the water treatment barriers in place. Currently, conventional monitoring strategies for source water microbial quality are typically based on low monitoring frequencies (e.g., bi-weekly or monthly)(MELCC, 2021). However, given the transient nature of WWOs and DWOs with respect to their short-time occurrence, the magnitude of flows and contaminant concentrations, there is a need for more frequent water sampling strategies to characterize short-term variations. The implementation of a water quality monitoring program that includes both routine and event-based water collection can contribute to a better understanding of aquatic contaminant dynamics (Mackay and Taylor, 2012; Thomas et al., 2022).

Fecal indicator bacteria (FIB) including *E. coli* along with turbidity are common indicators of water quality. More recently, near real-time *in situ* monitoring of β-D-glucuronidase (GLUC) activity has been made available to characterize fecal pollution temporal dynamics in environmental waters (Burnet et al., 2019b; Demeter et al., 2020; Sylvestre et al., 2020). Turbidity is regularly used as a primary indicator of microbial contamination levels, but it reflects the loading of organic and inorganic particles and cannot accurately inform on microbial contaminants (Tornevi et al., 2014). Enumeration of FIB in source water is used to identify fecal pollution and to indicate a possible occurrence of waterborne pathogens. However, FIB are able to survive and grow in various environments and they do not necessarily correlate with pathogens in sewage and water sources (Islam et al., 2017; McKee Anna and Cruz Marcella, 2021). Furthermore, FIB are not exclusively of human origin, they also originate in the feces of other warm-blooded animals (e.g., cow, dog, bird). They are thus, not specific to the source of fecal contamination. Without identifying possible pollution sources in the watershed, appropriate strategies and plans for source water protection and human health risk assessment are difficult to implement (Harwood et al., 2014; McLellan and Eren, 2014).

To identify the sources of fecal contamination, microbial source tracking (MST) methods imploy the use of host-associated markers, which may include a genetic or chemical marker that is unique to the feces of human and animal species (Griffith et al., 2013). A host-associated genetic marker refers to a unique nucleic acid (i.e., DNA) sequence that is exclusively or strongly associated with host sources. This sequence can be either a nucleic acid sequence of fecal cells (e.g., bacteria and viruses) or host cells (Zheng and Shen, 2018). Genetic markers for human-associated indicator bacteria, such as the human *Bacteroides* marker HF183 and host cells (mitochondrial DNA) are widely applied and used as indicators for human sewage (McGinnis et al., 2018; Tanvir Pasha et al., 2019). The chemical host-associated markers employed in chemical source tracking (CST) strategies are chemical compounds or waste-derived micropollutants (WWMPs) associated with waste from human sources. A variety of (CST) markers have been identified as potential indicators of sewage contamination, including caffeine, carbamazepine and other pharmaceuticals (Tran et al., 2015). Multiple lines of evidence or weight of evidence approaches to detect human sewage are now considered a more accurate method for determining the primary source of contamination and could thus be utilised to improve water quality monitoring (Codello et al., 2021; Kirs et al., 2017). Yet, the application of MST and CST techniques to assess the vulnerability of drinking water intakes to wet weather events has been explored in a few studies only (Edge et al., 2021). The aims of this paper were to: (i) better understand the short-term dynamics at DWIs for *E. coli*, human/animal markers, and pathogens related to recent rainfall events and WWOs in relation to β-D-glucuronidase activity (ii) investigate the usefulness of an MST toolbox to assess the contribution of wastewater or stormwater runoff to fecal contamination peaks at DWIs and (iii) evaluate the relevance of human markers in capturing the peak of pathogens after rainfall events for improved source water vulnerability assessments.

2. Materials and methods

2.1. Catchment description

The urban drinking water intakes (DWIs) takes their source from the Mille Île's river. The river is located in the Greater Montreal area in Quebec, Canada and it has an average water discharge of 286 m^3 /s. The water quality of the river is strongly influenced by urban discharges, although agricultural activities cover 45 % of the territory. The river receives periodic discharges of 194 CSOs (157 on the north shore and 37 on its south shore) and 14 WRRFs with nine outfalls located upstream of DWIs.

The DWI in the agricultural catchment is supplied by a small river with an annual average flowrate of 16 m3/s. According to the regional watershed protection plan, the area experiences intensive pig and cattle farming with more than 1500 animal units. Approximately 30 to 60 % of the land is devoted to agricultural activities. During the period from April to October, agricultural lands receive applications of cattle and swine manure.

2.2. GLUC activity and hydrometeorological event-based sampling conditions

The sampling strategy to capture sewage and/or agricultural runoff peak contamination in raw water was triggered by local meteorological conditions and/or short-term GLUC activity variations at both urban (A & B) and agricultural (C) drinking water intakes (Figs. S1–S3). Samples were gathered for event-based campaigns under specific circumstances: (i) when the cumulative rainfall exceeded 15 mm or the air temperature was higher than 5 °C within a 24 h period, and (ii) when there was a rise in GLUC activity of 5 modified Fishman Units per 100 mL (mMFU/100 mL) within one hour. The choice of the 5 mMFU/100 mL threshold for GLUC activity was informed by the observation of short-term increases during past hydrometeorological events at these DWTPs (Burnet et al., 2019a; Sylvestre et al., 2021a).

At DWI-A, five sampling events were conducted as previously described in (Sylvestre et al., 2021a). Two dry weather sampling events (baseline) and 3 targeted sampling events, two of which were during periods of heavy rainfall coupled with snowmelt (event A-1 on February 25 −26, 2017 (24 h event duration) and A-2 on April 4 −5, 2017 (21 h event duration), respectively (SI-Fig. S1). An additional event (A-3) was sampled on April 6 −10 at the onset of the major spring flooding experienced in southern province of Quebec in 2017 (SI-Fig. S1). This meteorological event was triggered by a combination of spring snowmelt, coupled with intense rain events that resulted in abnormally high precipitation in April and May. For example, within only 3 days from April 4 to 6, > 80 mm rainfall was recorded, and a total of 153 mm for the entire month (Environnement Canada, 2017). During targeted sampling events A-1 and A-2, 17 grab samples (5 to 40 L) were collected at a frequency of 3 to 4 h for approximately 24 h, whereas during event A-3, 8 samples were collected at a rate of one or more samples per day depending on GLUC activity dynamics.

At DWI-B (SI-Fig. S2), four sampling campaigns (10 samples in total) were conducted as described in (Sylvestre et al., 2021b), consisting in 3 event-based and 3 baseline sampling events. One targeted sampling event (Event B-1) was collected on February 7, 2018, during a planned raw sewage derivation/discharge (4 h duration) that was undertaken to maintain the main sewer system of a municipal water resources recovery facility (WRRF) located 5 kms upstream of DWI-B. The WRRF serves a population of 37,000 residents and treats an average of 28,000 m^3 raw sewage per day. Under normal conditions, the wastewater is treated using aerated lagoons. The second targeted sampling was conducted on February 20, 2018 (event B-2) when a 15 mm rainfall was recorded. The third targeted sampling (B-3, 5 samples) was performed during a gradual snowmelt event in absence of rainfall from February 28 to March 4, 2018. An influent and effluent sample was also collected at the upstream WWTP during event B-3.

At the agricultural DWI-C (SI-Fig. S3), 9 samples (1 L) in total were collected, 3 samples were collected in dry weather conditions on October 18, 23, and on December 2, 2017, and similarly to the sampling strategy adopted for drinking water intakes A and B, a targeted 20 h sampling event was conducted on October 30 and 31, 2017 following a 24 mm rainfall episode.

2.3. FIB analyses

The enumeration of *E. coli* and total coliforms was performed following the Colilert®/Quanti-Tray®/2000 system marketed by IDEXX Laboratories (Westbrook, Maine). The detection procedure involves adding Colilert-18 reagents to diluted or undiluted 100 mL water samples, followed by gentle mixing. The resulting solutions were introduced into Quanti-Trays 2000, sealed, and incubated at 35 ± 0.5 °C for 24 h. After incubation, Quanti-Tray wells that were yellow under ambient light indicate presence of total coliform bacteria. Yellow wells that exhibit blue fluorescence when viewed under ultraviolet light indicate the presence of *E. coli.* Enumeration is based on counting positive wells, and results are recorded as most probable numbers (MPN) using the provided IDEXX MPN charts. For quality control procedures, a sterile reagent water blank negative control was analyzed with each batch of sample and periodically, IDEXX- Colilert control comparator was used to monitor and confirm the performance of the Colilert assay procedures and identify any variations or issues.

2.4. Source tracking marker analyses

Water filtration and DNA extraction were described in (Villemur et al., 2015). All samples were assayed first by end-point or nested PCR for the HF183 marker and the human, bovine and porcine mtDNA markers (HumtDNA, BomtDNA, PomtDNA) (Kortbaoui et al., 2009). Those that were positive by these assays were assayed by qPCR with the respective markers (Ragot et al., 2023; Villemur et al., 2015); see supplemental documents for technical details and the list of primers). The limit of quantification (LOQ) in qPCR assays was determined by assessing the non-template control (NTC), which contains no target DNA, thus serving as a baseline reference for background signal. We estimated that qPCR results in average below 500 copies/100 mL (5–15 copies/reaction) were below the LOQ as we cannot distinguish marker signal from the background. Only results greater than LOQ were used in statistical analyses.

Five wastewater micropollutants (WWMPs) were selected for chemical source tracking: caffeine (CAF), theophylline (THEO), carbamazepine (CBZ), dihydro-carbamazepine (CBZ-2OH) and acetaminophen (ACET). WWMPs were analysed by an on-line solid-phase extraction combined with liquid chromatography electrospray tandem mass spectrometry with positive electrospray ionization (SPE-LC-ESI-MS/MS) first developed by (Viglino et al., 2008) and described in (Sauve et al., 2012). Detection limits (DLs) were estimated as three times the standard deviation of 5 replicate measurements of a field sample, and they were 0.5 ng/L for CAF, 0.2 ng /L for CBZ and CBZ-2OH, 2 ng/L for THEO and 10 ng/L for ACE. All samples were analyzed in duplicate. For each event, laboratory blanks and field blanks were analyzed for all parameters. All blank values were below DLs.

2.5. Pathogen analyses

For *Cryptosporidium* oocysts and *Giardia* cysts analysis, samples ranging from 10 to 40 L were collected and filtered on-site, using either Envirochek HV sampling capsules (Pall Gel-man Laboratory, Ann Arbor, MI, USA) in urban catchments or Hemoflow F80A hollow-fiber ultrafilters (Fresenius Medical Care, Lexington, MA, USA) in the agricultural catchment. The enumeration of *Cryptosporidium* oocysts and *Giardia* cysts, which were filtered using Envirochek HV sampling capsules, was performed in accordance with the USEPA method 1623.1. This method involved immunomagnetic separation (IMS) followed by immunofluorescence assay (IFA). For (oo)cysts filtered with Hemoflow ultrafilters, an adapted elution procedure was employed as described by (Sylvestre et al., 2021a). During this study, sample-specific analytical recoveries were not determined. However, ongoing precision recovery (OPR) samples were regularly conducted in line with standard USEPA method recommendations. These OPR samples involved tap water spiked with 98–100 flow cytometry-sorted, fluorescently labeled (oo)cysts (Colorseed, BTF, Australia). On average, the OPR samples exhibited yields of 43 % for *Cryptosporidium* and 44 % for *Giardia* respectively. all samples were analyzed at the centre d'expertise en analyze environnementale du Québec (CEAEQ). Virus concentration from water samples was performed as previously described in (Sylvestre et al., 2021b). Total nucleic acids were extracted from 200 μL of concentrated water sample and eluted with 50 μL RNase-free water using the MagaZorb® total RNA Prep kit (Promega, Madi-son, WI, USA) according to the manufacturer's instructions. Nucleic acid extracts were tested for Rotavirus, generic Adenovirus and Enterovirus. The quantification of viruses was performed by a one or two-step reaction (RT and qPCR) with the ABI PRISM 7500 Sequence Detection System (ABI) as previously described in (Pang et al., 2012; Qiu et al., 2016). RT-qPCR reaction was performed in a total volume of 10 μL containing 2 × TaqMan Fast Universal Master Mix (Applied Biosystems), 900 nM of each primer, 250 nM of specific probe, and 2.5 μL cDNA. Amplification consists of initial incubation at 95 °C for 20 s followed by 45 cycles of 3 s at 95 °C, 30 s at 60 °C. Quality control measurements of RT-qPCR were tracked with no template control values (NTC) and salmon DNA internal inhibition control. All NTC samples were negative with standard curves R-squared (0.997), efficiencies (96.37 %) and slopes (−3.34). The limit of detection (LOD) for the qPCR-based assays was established at one genome copy per PCR reaction, equivalent to 2–140 genome copies per 100 mL. The virus concentration procedure, sequences of primers and qPCR targets, standard curves details are all listed in the supplementary file.

2.6. Statistical analysis

All statistical analyses were performed using R (version 4.2.3, R Foundation for Statistical Computing, Vienna, Austria). The normality of the variables was tested with a Shapiro–Wilk test. Given that the raw and log10 transformed data were not normally distributed, non-parametric tests were performed. The Kruskal–Wallis and/or unpaired two-samples Wilcoxon statistical tests were used to assess differences between baseline and event-based marker concentrations and between influent and treated effluent. A nonparametric Spearman correlation test was also applied to describe relationships between, GLUC activity, *E. coli* and source tracking host-associated markers concentrations. We compared $\log_{10}$-transformed concentrations across the dataset with the expectation that positive correlations would be consistent with prediction of similar source. The significance level was set to alpha = 5 % for all statistical analyses.

3. Results and discussion

3.1. Short-Term dynamics in MST sewage markers at urban DWIs

Short-term fluctuations in MST sewage markers were studied during two snowmelt/ rainfall events in 2 sub-urban catchments and agricultural catchments. In this study, we enumerated the concentrations of culturable E. coli and two DNA sewage-associated marker genes including human Bacteroides marker HF183 and human mitochondrial marker (human mtDNA) to evaluate the impact of wet weather overflows (WWOs) and one dry weather overflow (DWO) on microbial water quality at drinking water intakes (DWI). Overall, E. coli and HF183 levels were higher in event samples, particularly for event A-1, compared to baseline samples (p-value = 0.039 -). During the snowmelt/rainfall event in February 2017 (event A-1, Fig. 1a), *E. coli* and HF183 concentrations in raw water reached a maximum of 2420 MPN/100 mL and 4.8 × 10^4 copies/100 mL, respectively. This is an increase of approximately 1.1 and 0.9 log, respectively, compared to baseline conditions, suggesting CSOs and/or WRRF by-passes were contributing to raw water sewage contamination. Among the 4 WRRFs and 37 CSO outfalls located upstream the DWI-A, one nearby CSO outfall and a WRRF by-pass discharged untreated sewage into the river upstream DWI-A (Burnet et al., 2019a). Peak *E. coli* and HF183 concentrations were synchronous during the contamination event at DWI-A, providing further evidence that human sewage inputs through WWOs was the primary source of fecal contamination during event A-1 (r = 0.631, p-value <.0.05).

In April 2017 (events-A2 and A-3, Figs. 1a, S4), intense rainfall

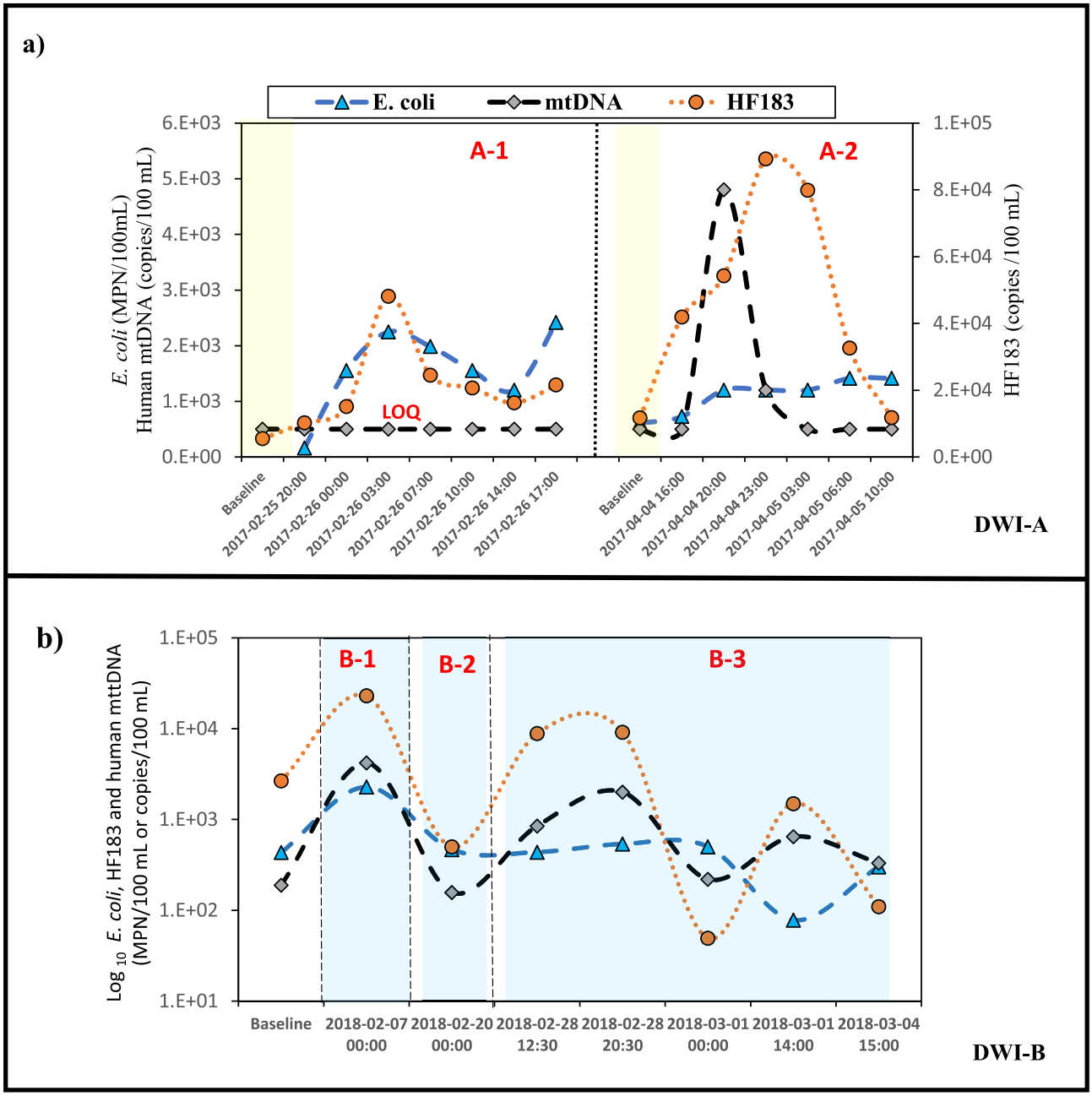

Fig. 1. Short-term variations in *E. coli*, human-associated Bacteroides marker HF183 and human mitochondrial marker (mtDNA) at DWI-A (a) for the first 24 h of two hydrometeorological events (snowmelt and rainfall) in February (Event A-1) and April (Event A-2) 2017 and at DWI-B (b) in February 2018 during event B-1 (WRRF by-pass) and March 2018 during event B-2 (rainfall) and event B-3 (snowmelt). Yellow rectangles (DWI-A) indicate baseline conditions preceding event conditions. Blue rectangles (DWI-B) indicate targeted events. During event A-1, mtDNA samples were below limit of quantification (LOQ). Dashed lines separate the events.

events associated with increasing river flowrates (up to 800 m^3/s, Fig. S4) were recorded. In addition to the WRRF by-pass upstream of DWI-A, CSOs occurred from the 4th to the 12th of April with a total duration of 192 h. Under such conditions, wastewater is diluted with a large amount of stormwater, and marker concentrations in surface water are expected to be diluted too, and to reach concentrations below dry weather ones. Whereas *E. coli* showed a lower amplitude peak (1414 MPN/100 mL) with an increase of only 0.4 log during the snowmelt events, HF183 marker exhibited a 0.9 log increase (peak concentration of 9.3×10^4 copies/100 mL). In addition, no synchronous peak of *E. coli* and HF183 was observed at event A-2 as the peak of *E. coli* was delayed and maintained during ~ 24 h at low concentrations of sewage markers (Fig. 1a). Intense rainfall may have also mobilised local non-point sources such as urban runoff and sediment resuspension as a source of *E. coli* (Unno et al., 2018).

At DWTP-B, two microbial contamination events (events B-1 and B-3) were captured based on short-term increases in GLUC activity. On February 7 (Fig. 1b, event-B1), 4 h after a planned WWRF by-pass (DWO) located 5 km upstream, a 0.7 and 0.9 log-increase in *E. coli* and HF183, respectively, confirmed that besides WWOs, DWOs should also considered as a period of high vulnerability to DWIs, especially because of the lower dilution potential of the river (Fig. S2).

During the March 2018 snowmelt event that lasted for approximatively 5 days (event-B3) in the absence of prior rain event, concentrations of *E. coli*, HF183 and human mtDNA of the water samples collected during the baseline and event-based conditions were similar (no statistically significant differences were observed, p-value = 0.17–0.885). No known WWOs were recorded upstream of DWI-B during the event sampling, suggesting that spring snowmelt not associated with WWOs might not be as critical for the drinking water intakes as compared to precipitation events during snowmelt. Despite this, GLUC activity gradually increased from 20 to 50 mMFU/100 mL over the five days (Fig. S2), which may result from a large proportion of non-culturable but metabolically-active *E. coli* cells carried away during spring snowmelt event and originating from other (diffuse) contamination sources in the catchment (Burnet et al., 2019a).

In comparison to the HF183 marker, human mtDNA was detected for all sampling events (Fig. 1b, SI-Fig.S4) confirming the sewage contamination. However, the concentrations were most of the time < LOQ except at event A-2 where the concentration reached a maximum of 4.8×10^3 copies/100 mL with a synchronous peak in HF183 concentration. Maximum concentrations of human Bacteroides and mtDNA recorded at event A-2 are therefore likely indicative of high levels of human fecal pollution. These results suggest that mtDNA marker gene while being highly host-associated (Malla and Haramoto, 2020), could not indicate the amplitude of the sewage contamination at the DWI. Therefore, it might be paired with HF183 if more evidence or confidence is needed to support the presence of contamination. The difference of concentrations and amplitudes observed between both markers may reflect different survivability/persistence of these sewage markers in the environment (Kapoor et al., 2013) or different analytical performances of the assays (Malla and Haramoto, 2020). Overall, compared to all other indicators, the HF183 marker was the most specific indicator of recent WWOs and DWOs, and it was a more sensitive indicator than human mtDNA across all periods. Similar results were found by (McGinnis et al., 2018) in urban creeks after WWOs and rainfall events.

3.2. Short-term dynamics in CST sewage markers at urban DWIs

The suitability of CAF, THEO, CBZ, CBZ-2OH and ACET as DWO (planned WRRF by-pass) and WWO markers was evaluated for the urban DWIs. Overall, differences in the amplitude and occurrences were observed between the source water intakes as ACET and CBZ-2OH were mostly detected at DWI-B. Moreover, the concentrations of CAF were 4 to 7 times higher at DWI-B compared to DWI-A. These results highlight the value of using multiple sewage markers to anticipate the shortcoming of low detection and the necessity to test the suitability of markers in different geographic locations and sub-catchments before choosing the best performing markers.

Short-term variations in concentrations of CST markers in baseline and event-based events are shown in Fig. 2. At DWI-A, similarly to MST markers, higher concentrations were observed during event conditions for wastewater-derived micropollutants compared with baseline concentrations (event-A1). Being specifically of human origin, the dynamics and amplitude of CST marker concentrations further support the contribution of municipal wastewater to fecal contamination peak measured at the intake. CAF, THEO and CBZ exhibited similar peak concentrations, but they did not share a similar trend/peak with *E. coli* and human DNA specific markers (Fig. 1). Indeed, the CST markers peaked 3 h earlier. As dissolved markers were analysed., CST markers rapidly reached the intakes suggesting that along with GLUC activity, they could be used as earlier indicators of CSO discharges warning that human sewage contamination is reaching the intakes. CST markers and FIB can be mass or flow limited, meaning that concentrations are influenced by the quantity of mass mobilized and dilution processes. *E. coli* has been observed to be less mass limited than CST markers in raw wastewater receiving stormwater during precipitation events (Tolouei et al., 2019), which could explain the earlier peak for the CST markers. However, further event-based sampling campaigns are needed to consolidate these results.

Furthermore, during events A-2 and A-3, CAF and THEO concentrations were lower particularly in the end of event in comparison with event A-1. This was different for CBZ that showed conservative behavior with an increase of its concentrations following the WRRF by-pass and the multiple CSOs (Figs. 2a, SI). As explained earlier, the duration of the rainfall/flood during events A-2 and A-3 events generated more runoff and WWOs characterized by more diluted domestic wastewaters. Consequently, CAF and THEO may not be suitable indicators during wet weather with high river dilution. The same behavior was observed at event B3 where a slow snowmelt (Figs. 2b, S4) resulted in high river flowrate and lower levels of all markers.

The raw and treated sewage of the nearest upstream WRRF from DWI-B was also monitored during baseline and event-based conditions to select the most suitable DWO/WWOs marker for event monitoring. Under baseline conditions, there was no statistically significant difference in CAF, THEO, CBZ, and CBZ-2OH concentrations except ACET which showed ~ 2 Log_{10} reduction between raw sewage and treated effluent (p-value = 0.033, Fig. S5). Thus, it was expected in a period of high dilution such as events A2, A3 and B3, that all marker concentrations would be similar to baseline conditions or even lower (Benotti and Brownawell, 2007; Poopipattana et al., 2021). Nevertheless, CBZ showed conservative behavior with an increase of concentration following the WRRF by-pass and the multiple CSOs. Given the high organic content of sewer deposits, it is likely that CBZ may be adsorbed to sewer deposits, and then desorbed when diluted with stormwater (Hajj-Mohamad et al., 2017; Madoux-Humery et al., 2013). Based on our observations during baseline and event conditions, CBZ was a better WWO/CSOs marker due to its conservative behavior and apparent high persistence, and it could indicate combined sewer sediment resuspension during wet weather events.

ACET was the most suitable marker for both wet (event B-2 and B3) and dry weather overflows (event B-1). It was only detected after overflow events indicating sewage contamination. ACET showed similar behavior with *E. coli* and sewage DNA markers, suggesting a potential use as chemical fecal indicator. Interestingly, ACET was detected along event 3 while all the markers were below dry weather concentrations indicating the possible presence of an additional upstream sewage source. ACET has been reported as a suitable marker of untreated wastewater discharge due to its high removal during wastewater treatment (Munro et al., 2019). DWI-B is located at the end of the river downstream of all WRRF and CSO outfalls, consequently cumulative sewage contamination might reach the intakes (Madoux-Humery et al.,

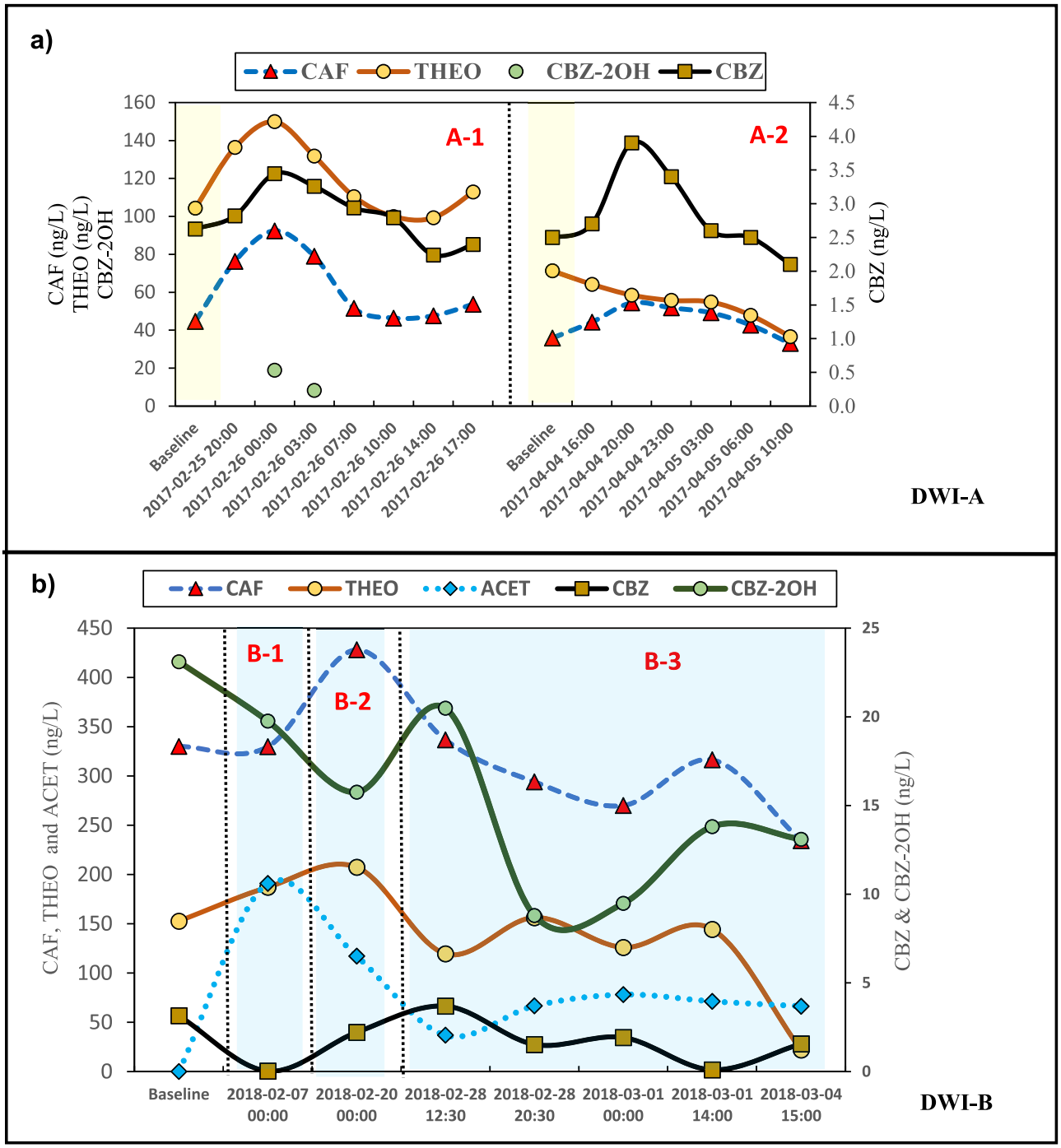

Fig. 2. Short-term variations in caffeine (CAF), theophylline (THEO), carbamazepine (CBZ), dihydro-carbamazepine (CBZ-2OH) and acetaminophen (ACET) at DWI-A (a) for the first 24 h of two hydrometeorological events (snowmelt and rainfall) in February 2017 (Event A-1) and April 2017 (Event A-2) and at DWI-B (b) in February 2018 during event B-1 (WRRF by-pass) and March 2018 during event B-2 (rainfall) and event B-3 (snowmelt). Yellow rectangles indicate baseline conditions preceding event conditions. Blue rectangles indicate targeted events. During event A-1, ACET was not analysed. Dashed lines separate the events.

2016).

3.3. Short-term dynamics in human and animal-associated markers at the agricultural DWI

Since the probability of finding levels of fecal contamination from diverse sources in the agricultural catchment is higher (Villemur et al., 2015), we used a mixed "toolbox" representative of the land use and composed of human and animal associated DNA markers. Thus, along with *E. coli*, human *Bacteroidales* HF183 and mitochondrial markers targeting human-derived pollution, two livestock (bovine/porcine) markers were tested to define the origins of fecal contamination and potentially assess the agricultural runoff contribution to peak contamination during a 20 h rainfall-induced contamination event. Although fewer samples were collected in baseline conditions, the host-associated microbial investigations unveiled that fecal contamination reaching the agricultural DWI was both from human and bovine origins ($r = 0.712$, p-value < 0.05) (Fig. 3). The concentrations of bovine mtDNA were approximately 3.5 times higher (average 7168 copies/100 mL) than the concentrations of human mtDNA (average 2050 copies/100 mL).

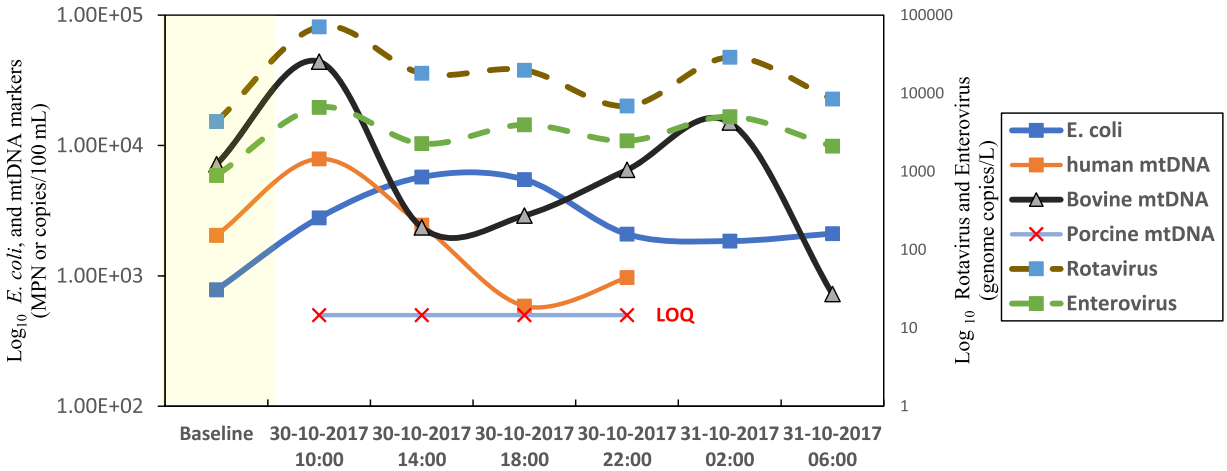

Fig. 3. Short-term variations in *E. coli*, enteric viruses and human, bovine, porcine (mtDNA) markers in October 2017 at agricultural DWI-C. Yellow rectangles indicate targeted baseline event. LOQ = MST markers limit of quantification. The porcine mtDNA was not detected (negative result) at the baseline and the end of event.

Moreover, the human-associated Bacteroides marker HF183 was not detected in any baseline samples suggesting lower incidence of sewage contamination.

The event-based sampling highlighted the impact of agricultural runoff, as it caused an increase (in comparison to baseline conditions) of 0.9-log (p-value = 0.02014), 0.8-log (p-value =0.03556), and 0.6-log (p-value = 0.020), in *E. coli*, bovine mtDNA levels and human mtDNA, respectively, corresponding to peak concentrations of 5.3×10^3 MPN/100 mL, 4.4×10^4 copies/100 mL and 7.9×10^3 copies/100 mL, respectively (Fig. 3).

The porcine mtDNA marker was not detected in baseline conditions and although it was detected during the first 12 h of the rainfall-induced event, all positive samples were below the limit of quantification (Fig. 3). Both human and animal mtDNA markers exhibited a synchronous trend and peaked at the beginning of the rainfall event and rapidly decreased as the precipitation and surface runoff subsided, suggesting a flashier runoff response to heavy precipitation. In contrast, the *E. coli* peak concentration was measured 4 h later and was relatively stable over the duration of the event. This result indicates that other sources of fecal contamination than bovine or porcine animals might contribute to the elevated *E. coli* concentrations observed. Non-point sources from dogs, chicken and other livestock's including sheep, horses, and goats commonly present at low density in agricultural catchments might also be drained by rainfall-runoff from the whole catchment.

Human mtDNA marker could be attributed to the discharge of treated effluents from municipal WRRFs and CSOs (McGinnis et al., 2018) as well as leaking on-site septic tanks frequently present in agricultural catchment (Villemur et al., 2015). One municipal WWRF and four CSO outfalls are located 10 km upstream from the drinking water intake. Interestingly, HF183 marker was not detected during the rainfall event. Considering the high environmental concentrations of HF183 marker in comparison to human mtDNA observed at the urban DWIs, we were expecting concurrence between both markers. While variations in marker stability and persistence in the environment likely played a role in the varying detection and amplitude of both human markers, it was found that human-associated mtDNA markers could cross-react with cow, pig, and poultry fecal-source samples (Ballesté et al., 2010). Consequently, it may be difficult to establish if human feces or sewage are present or absent from samples testing negative for the HF183 marker but positive for human mtDNA. Furthermore, there is a low possibility that animal-associated mtDNA markers, particularly bovine mtDNA, could also be detected in wastewater (Malla and Haramoto, 2020). Caldwell et al. (2007) showed that bovine mtDNA was detected in feces of 2 humans out of sixteen who consumed beef. Nevertheless, the high concentrations of bovine marker observed during the dry or rainfall event strongly confirm the dominant bovine contamination at the DWI.

The results highlight the utility of using an MST toolbox markers approach including more than one marker for each potential source. Although not performed for DWI-C, the use of CST markers would be valuable to confirm the sewage contamination with more confidence at this site. For bovine sources, an ideal candidate for further investigation is the Bac-Bovine marker, which can reportedly be detected at lower concentrations (Lee et al., 2010).

3.4. Relationships between turbidity, pathogens and MST markers

Turbidity is a valuable indicator that can indicate the occurrence of impaired water quality in drinking water sources (World Health Organization, 2017). Moreover, it has been well-documented that rainfall-runoff can lead to significant increases in turbidity, fecal indicator and pathogen concentrations (Dorner et al., 2007). Sylvestre et al. (2021b) observed that turbidity did not simultaneously peak with virus and protozoan concentrations during snowmelt events at the intake of a treatment plant supplied by a large urban river. In contrast, GLUC activity level was a better surrogate than turbidity to identify transient peak contaminations in source water. Similarly, our results showed similar dynamics between sewer markers and GLUC activity, meanwhile no relationship with turbidity was observed (Fig. 4).

Here, we also explored the relationship between MST markers and some pathogens during the event-based sampling. Similar dynamics were observed in DWI-A during peak event A-1. Human MST marker HF183 exhibited synchronous peak and dynamics with Adenovirus (Fig. 5C), *Giardia* (Fig. 5B), and to some extent with *Cryptosporidium* (Fig. 5A). While further events and analyses mut be done, the results obtained here suggest that an increase in HF183 marker concentrations can, under certain conditions, indicate or predict the presence of protozoa and viruses. Alternative indicators, such as source-associated MST markers have been used as possible surrogates to better approximate pathogen abundance. Marti et al. (2013) studied the relations between Bacteroides, FIB and pathogens, and they concluded that presence and absence of the Bacteroides marker were not correlated to FIB, but positively related to pathogens such as *Cryptosporidium* and *Giardia*.

At the agricultural DWTP, porcine mtDNA was detected during the beginning of the event, human and bovine mtDNA exhibited similar dynamics and peaked with both enterovirus and rotavirus (Fig. 3) suggesting that the enteric viruses might be mostly of animal origin and to some extent of human origin. Detailed investigations and additional

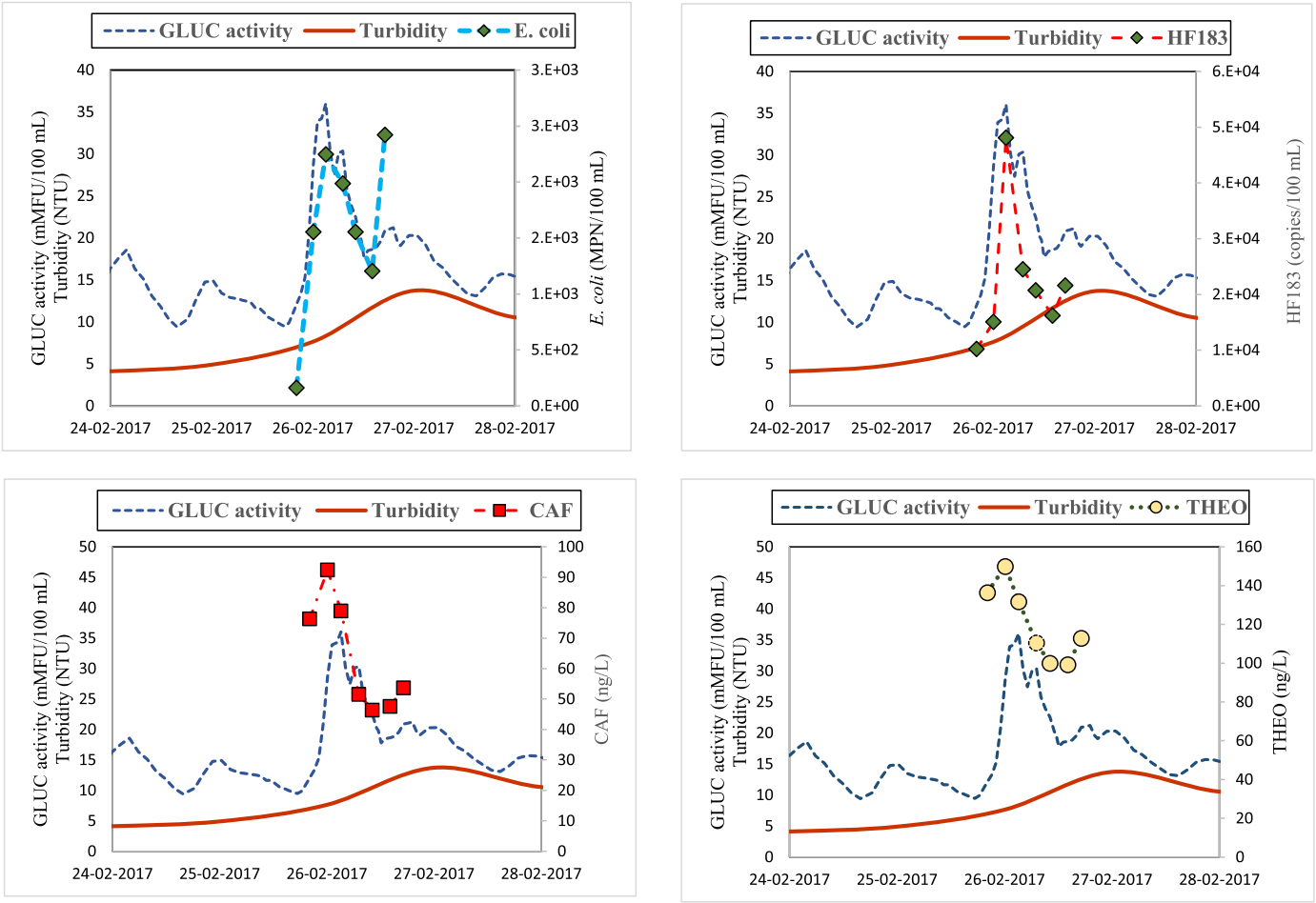

Fig. 4. Short-term variations of GLUC activity, turbidity, *E. coli* and human-associated source tracking markers at DWI-A during event-A.

event-based sampling campaigns confirming these findings in other areas and under different environmental conditions are needed to generate meaningful and accurate MST outputs for waterborne pathogens. However, these results suggest not only that host-associated markers can be successful predictors of the presence of some pathogens, but they may give insight into which host-associated pathogens are most likely to be present.

4. Conclusions

This study investigated microbial contamination during snowmelt and rainfall events at three drinking water intakes located in urban and agricultural catchments. The following conclusions can be drawn:

- MST analyses matched the land use in the watershed allowing an accurate assessment of the main hazards and sources of pollution in this case mainly human/sewage following WWOs, and bovine-related pollution following agricultural runoff.
- The use in tandem of both chemical am microbial human markers confirmed the presence of untreated/recent sewage contamination of human origin
- HF183 with CBZ were the best performing human wastewater-associated markers.
- CAF and CBZ were more sensitive to dilution effects after WWOs during snowmelt than rainfall.
- Mitochondrial DNA may prove to be a good option to be used in combination with *E. coli* in agricultural watersheds to distinguish sources of contamination. Bovine mtDNA has potential as an indicator of enteroviruses and rotavirus in agricultural catchments.
- Rainfall and snowmelt were a driver of human and animal fecal pollution in urban and agricultural catchments. Snowmelt alone was not as important as a driver of fecal pollution, likely because the sources of contamination were aged.
- Source water quality sampling programs should be designed to intensively sample during and/or following events to determine what critical rain fall/snowmelt amounts have the potential to negatively impact drinking water intakes.
- GLUC activity monitoring was a valuable tool for planning event sampling and interpreting the results because of its high temporal resolution measurement capacities.
- GLUC activity was a more reliable indicator than turbidity to identify peak events at urban drinking water intakes.

CRediT authorship contribution statement

Mounia Hachad: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. **Jean-Baptiste Burnet:** Writing – review & editing, Validation, Visualization. **Émile Sylvestre:** Methodology, Writing – review & editing, Data curation. **Sung Vo Duy:** Methodology, Visualization. **Richard Villemur:** Methodology, Writing – review & editing. **Sébastien Sauvé:** Visualization. **Michèle Prévost:** Funding acquisition, Validation, Visualization. **Judy Y. Qiu:** Methodology, Writing – review & editing. **Xiaoli Pang:** Methodology, Writing – review & editing. **Sarah Dorner:** Funding acquisition, Supervision, Validation, Writing – review & editing, Project administration.

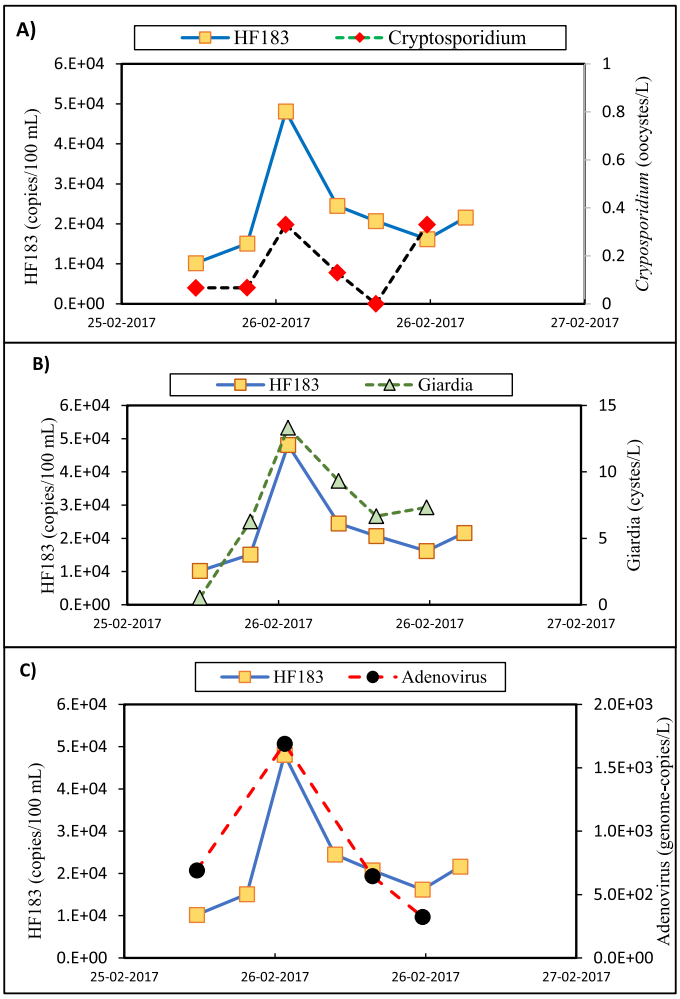

Fig. 5. Relationship between HF183 and A) *Cryptosporidium*, B) *Giardia* and C) Adenovirus at event A-1.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.121374.

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