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Levels of human Rotaviruses and Noroviruses GII in urban rivers running through the city mirror their infection prevalence in populations

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HIGHLIGHTS

• Correlation of enteric virus in water matrices and clinical prevalence were analyzed.
• The virus abundance in wastewater ef-fluents has no correlation with local prevalence.
• HRVs and HuNoVsGII in urban rivers positively correlated with clinical prevalence.

GRAPHICAL ABSTRACT

ABSTRACT

Enteric viruses exposed to water pose a huge threat to global public health and can lead to waterborne disease outbreaks. A sudden increase in enteric viruses in some water matrices also underpins the prevalence of corresponding waterborne diseases in communities over the same time period. However, few efforts have focused on water matrices whose viral pollution may best reflect the clinical prevalence in communities. Here, a one-year surveillance of human enteric viruses including Enteroviruses (EnVs), Rotaviruses (HRVs), Astroviruses (AstVs), Noroviruses GII (HuNoVsGII) and Mastadenoviruses (HAdVs) in four representative water matrices: an urban river (UR) running through city, effluent from Wastewater Treatment Plant (EW), raw water for Urban Water Treatment Plant (RW), and tap water (TW) were performed by qPCR. The relationship between the virus detection frequency at each site and their prevalence in clinical PCR assay was further analyzed. We found that the detection frequencies of HRVs, HuNoVsGII, and AstVs in stools peaked in winter, while EnVs peaked in autumn. For UR, all types of enteric viruses could be detected and the levels of acute gastroenteritis viruses (HRVs, HuNoVsGII, AstVs, and HAdVs) were highest in autumn or winter, whereas EnVs peaked in summer. In terms of correlation analyses, only HRVs and HuNoVsGII levels in UR showed a strong positive correlation with their prevalence in clinical stool samples. This study indicated that HRVs and HuNoVsGII levels in URs may mirror the local virus prevalence, thereby implying the possibility of revealing their local epidemiology by monitoring them in the URs.

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1. Introduction

Human enteric viruses transmitted by the fecal–oral route cause millions of infections each year and result in various diseases such as gastroenteritis, meningitis, hepatitis, and encephalitis (Okoh et al., 2010). Generally, they circulate continuously between an infected population and the environmental water. After propagation in infected individuals, they are released into the surrounding environment via excrement (up to \(10^7\) viral particles/g stool) (Teunis et al., 2015). Since they cannot be removed from wastewater by traditional sewage treatment processes and they have strong resistance to unfavorable conditions, they may survive in various water matrices for an extended period once discharged into wastewater (Lopman et al., 2012; Okoh et al., 2010), leading to the infection of the exposed population and a large-scale outbreak or epidemic. Therefore, exposure to enteric virus-containing water—such as drinking water and environmental water—is widely believed to be responsible for most outbreaks of waterborne diseases, which pose a great threat to public health.

Conversely, considering viruses’ inability to propagate in water, the sudden increase in viral load in wastewater or environmental water that receives wastewater or treated effluent may support the prevalence of viral waterborne disease in cities over the same period. Therefore, the occurrence of enteric viruses in some water matrices may be used as an indicator to mirror the disease’s prevalence among the local population. Nowadays, there is increasing evidence that human enteric viruses can be independently detected in almost all types of water, such as wastewater (Bissequx et al., 2018; Farkas et al., 2018; Jahne et al., 2019; Simhon et al., 2019) and city surface water (Goh et al., 2019; Keller et al., 2015; Masachessi et al., 2018; Pang et al., 2019; Sassi et al., 2018; Sedji et al., 2018; Tandukar et al., 2018). Wastewater-based epidemiology can be used to capture a near real-time picture of the viral disease burden within a community (Bissequx et al., 2018; McCall et al., 2020). However, few studies (Prevost et al., 2015) have linked the dynamics of enteric virus in other water matrices to the epidemiology of viral infections over the same period. The correlation between virus occurrence in various water matrices and clinical prevalence remains unclear.

In this study, surveillance of human enteric viruses was performed from September 2014 to August 2015 in major urban water matrices together with virus tests on stool samples from hospital patients. Five kinds of human enteric viruses, namely Enteroviruses (EnVs), Rotaviruses (HRVs), Astroviruses (AstVs), Noroviruses GII (HuNoVsGII), and Mastadenoviruses (HDaVs) were tested in four representative water matrices: urban rivers running through a city (UR), disinfected final effluent from Wastewater Treatment Plant (EW), raw water for UWTP (RW), and tap water (TW). Then, the relationship between virus occurrence in water and the prevalence in clinical samples was further analyzed to determine the water matrices whose viral contamination was epidemiologically the most closely related to the virus infection. To our knowledge, this was the first study to reveal the relationship between enteric virus levels in urban representative water matrices and the local prevalence. This study implied the applicability of monitoring enteric virus in environmental water for the aim of helping reveal the local epidemiology of waterborne disease outbreaks.

2. Materials and methods

2.1. Sample collection

Fig. 1 indicates the sampling sites on the map. In total, 180 samples were collected monthly from September 2014 to August 2015 in Tianjin City (China) from surface water of the Urban Rivers (UR) flowing through the urban central area, including Haihe (n = 36, replicate samples in each month) and Jinhe Rivers (n = 36), disinfected final effluent (EW, n = 36) from a Wastewater Treatment Plant (WWTP), raw water (RW, n = 36) for an Urban Water Treatment Plant (UWTP), and terminal tap water (TW, n = 36) in a pipeline. Meanwhile, 1906 fecal specimens were collected from patients at Tianjin Children’s Hospital over the same period. The study was approved by the local ethics committee, and consent was obtained from the parents of the children. All samples were transported to the laboratory in refrigerated conditions within approximately 3 h. According to the Chinese Meteorological Administration, March–May is defined as spring (10–20.7 °C), June–August as summer (22.3–30.7 °C), September–November as autumn (11–19.3 °C), and December–February as winter (−3–4.3 °C).

2.2. Water quality measurement

The biological and physicochemical parameters of various representative water samples were assayed (Table 1). According to the standard membrane filter procedure (Jin et al., 2014), heterotrophic plate counts (HPC) and total coliforms (TC) were assayed on Luria–Bertani agar and M-Endo (BD Difco, USA), respectively. The physicochemical parameters turbidity and conductivity were measured with a portable turbidity meter (HACH 1900C, USA) and a conductivity meter (HACH sension5, USA), respectively. Chemical oxygen demand (CODmit) and ammonium content (NH4-N) were measured according to standard methods (APHA, 1998).

2.3. Virus recovery from water samples with electropositive granule media (EGM) filter

Fifty liters of water collected from each sampling site was quickly transported to the lab under refrigerated conditions for further virus concentration tests. EGM filters were prepared to concentrate the virus from water samples as described previously (Jin et al., 2014). Briefly, after each 50 L water sample was flowed through an EGM filter, the filter was eluted with 3 L of elution buffer (2% sodium hydroxide, 0.375% glycin, 1.5% sodium chloride, 3% tryptone, and 1.5% beef powder). Then, 0.1 mol/L HCl was added to adjust the pH of the eluate to 7.0 ± 0.2 immediately after collection and 10% polyethylene glycol (PEG) was added to the eluate before overnight incubation and centrifugation (15,000 rpm for 30 min at 4 °C). Then, the pellets were resuspended in 40 mL PBS and stored at −70 °C for further analysis. To evaluate the virus recovery efficiency, 10⁶ PFU of bacteriophage MS2 cultivated by confluent lysis on its host strain E. coli (ATCC 15597) was added to water samples as an indicator and detected using the double-layer plaque assay (Hornstra et al., 2011). Virus recovery, all of which were demonstrated above 90%, was calculated using the following Eq. (1):

\[
\text{Virus recovery (\%) = (B-C)/A} \times 100\%
\]

where A is the number of MS2 added into the water samples, B is the number of MS2 in the final buffer concentrate, and C is the number of background MS2 measured from the final buffer concentrate samples without MS2.

2.4. Virus RNA/DNA extraction

Virus RNA was extracted from the concentrated virus suspension using the QIamp viral RNA mini kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. The UNIQ-10 viral DNA kit (Sangon Biotech) was used for viral DNA extraction. The purity and concentration of the extracted RNA and DNA was determined by a GeneQuant1300 system (GE Healthcare) and the samples that met the purity standards (A260/A280, 1.8–2.0) were used for further analysis. The nucleic acid extraction recovery was evaluated by the addition of internal control (IC) RNA to the lysis buffer according to the manufacturer’s instructions (QIAamp Viral RNA Mini Handbook, Qiagen, Hilden, Germany).
2.5. Quantification of enteric viruses in water by (RT-)qPCR

Quantification of viruses by (RT-)qPCR was performed as previously described (Miao et al., 2018). Briefly, virus RNA was first reverse-transcribed using a cDNA first-strand synthesis system (Thermo Fisher Scientific, Waltham, MA). According to the instructions, the RNA template was first mixed with Random Hexamer primer, incubated at 65 °C for 5 min, and then chilled on ice. Then, the reaction mixture was added to the samples, and the reaction was performed in a thermocycler (Applied Biosystems, USA) to synthesize cDNA. The qPCR reaction was performed in an ABI 7300 sequence detection system (Applied Biosystems, USA). Two microliters of DNA or cDNA samples were added to a 20 μL reaction mixture containing 10 μL PCR SuperMix-UDG (Platinum PCR SuperMix-UDG, Invitrogen, USA), 0.5 μL primer (10 μmol/L), 0.5 μL Taqman probe (5 μmol/L), and 6.5 μL nuclease-free water. The reaction conditions were 95 °C for 30 s followed by 40 cycles of 95 °C for 30 s and 60 °C for 1 min. All qPCR analyses were performed in triplicate with positive controls for each target and DEPC-treated water as the negative controls. Table S1 shows all primers and probes (Jin et al., 2014; Kageyama et al., 2003; Le Cann et al., 2004; Miao et al., 2018; Xagoraraki et al., 2007) labeled with FAM detector dyes and TAMRA quencher dyes (Invitrogen, Shanghai, China). Table S2 shows the standard curves for the quantification of virus.

2.6. Inhibition control

To avoid inhibition during RT-qPCR, HCV RNA IC was added to 2 μL of nucleic acids extracted from the samples (diluted 10-fold or 50-fold or undiluted) or DEPC water (blank control) at a concentration of 10^5 genome copies (GCs) per reaction before RT-qPCR. If the threshold cycle value (Ct) of the HCV RNA IC detected in the blank control was one cycle fewer than that in the sample nucleic acid extracts, the reaction’s inhibition had occurred and the nucleic acid extracts were diluted before RT-qPCR until no inhibition was observed. As background control, all samples were verified as being free of HCV using RT-qPCR prior to inhibition testing.

The quantification of HCV RNA IC was carried out using the same RT-qPCR conditions as virus detection with the Primers and TaqMan probe.

Table 1

| Water samples              | HPC (CFU/ml) | TC (CFU/ml) | T (°C) | pH  | Turbidity (NTU) | COD_mn (mg/L) | NH4-N (mg/L) | Conductivity (μs/cm) |
|----------------------------|--------------|-------------|--------|-----|-----------------|---------------|--------------|---------------------|
| Haihe River                | 24,295.83    | 99.17       | 15.69  | 6.99| 16.02           | 8.95          | 1.43         | 674.25              |
| Jinhe River                | 54,812.50    | 158.31      | 15.84  | 6.94| 9.33            | 9.31          | 2.07         | 732.83              |
| Effluent from WWTP         | 441.63       | 1.97        | 18.37  | 6.96| 1.41            | 4.69          | 1.44         | 1891.08             |
| Raw water for UWTP         | 346.75       | 4.00        | 16.57  | 7.70| 3.29            | 4.00          | 1.57         | 560.08              |
| Terminal tap water         | 91.83        | 0.00        | 18.32  | 7.32| 0.53            | 3.63          | 0.50         | 618.08              |

Abbreviations: HPC heterotrophic plate counts, TC total coliforms, T water temperature, NTU nephelometric turbidity unit, COD_mn chemical oxygen demand, NH4-N ammonium nitrogen content.
sequences listed in Table S1. The equation for calculating the sample inhibition is:

\[
\text{inhibition} \% = \left( \frac{A - B}{A} \right) \times 100 \%
\]

where \( A \) is the GCs of IC per reaction in the blank control and \( B \) is the measured GCs of IC per reaction mixed with the nucleic acid extracts in the tested water samples.

2.7. Calculations for virus concentration

The standard curves for the quantification of HRVs, HuNoVsGII, AstVs, EnVs, and HAdVs were obtained by analyzing 10-fold serial dilutions of viral RNA or DNA standards (Jin et al., 2014). Virus concentrations in all water samples were calculated using the Eq. (3) or Eq. (4):

\[
\text{Virus (GC}\,\text{s}) = \frac{\text{GC}\,\text{s/r} \times 80 \mu\text{L}/2 \mu\text{L}}{\text{Ve} / 140 \mu\text{L}} \times N
\]

\[
\text{Virus (GC}\,\text{s}) = \frac{\text{GC}\,\text{s/r} \times 50 \mu\text{L}/2 \mu\text{L}}{\text{Ve} / 200 \mu\text{L}} \times N
\]

where 2 \( \mu\text{L} \) was the volume of each sample per reaction tube, and 140 and 80 \( \mu\text{L} \) are the volumes of the sample extracted and the RNA extract volume of HRVs, HuNoVsGII, AstVs, and EnVs, respectively. The 200 and 50 \( \mu\text{L} \) are the volumes of the extracted sample and the DNA extract volume of HAdVs, respectively. Ve is the volume of the final buffered concentrate (\( \mu\text{L} \)) and \( N \) represents the dilution of the nucleic acid extract.

2.8. Detection of enteric viruses in stool by polymerase chain reaction (PCR)

Viral RNA and DNA were extracted from stool supernatants using QIAamp viral RNA Mini kits and UNIQ-10 viral DNA kits according to the manufacturer’s instructions, respectively. Then, nucleic acid samples were tested for the presence of HRVs, HuNoVsGII, AstVs, EnVs, and HAdVs using PCR as previously described (Ouyang et al., 2012). The primers used to detect above viruses are listed in Table S3.

2.9. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics (Version 20.0, IBM Corp) and R Studio (Version 1.2.1335, RStudio, Inc.). The average concentration of human enteric virus throughout the year was compared using the non-parametric Kruskal–Wallis test, and students’ \( t \)-test was used to analyze the differences in viral concentrations between seasons. The Pearson test was calculated using R Studio to measure the associations of enteric virus concentrations between different water matrices. This was followed by a Student–Newman–Keuls–q test to analyze the correlation between these variables.

3. Results

3.1. Occurrence of enteric viruses in the representative water samples

Table 2 shows the detection frequencies of enteric viruses in the samples from representative water matrices. It presents the diversity of enteric viruses in different water matrices with the exception of HAdVs, which were found in all tested water samples with a 100% detection rate. The UR, including the Haihe and Jinhe Rivers, had abundant virus types, and all five tested human enteric viruses were detected in them; all detection rates were > 75%. In addition, the UR showed the highest detection frequencies—83.33% for both HuNoVsGII and AstVs and 91.67% for EnVs—among all observed water matrices. However, for EW, RW, and TW, four types of acute gastroenteritis viruses (HAdVs, HRVs, HuNoVs GII, and AstVs) could be detected positively.
but no EnVs were detected throughout the whole year. Above all, HRVs were the only viruses to show a higher detection rate there than in the Haihe and Jinhe Rivers. The detection rate of HRVs in EW reached 100%, followed by RW and TW, both of which were 91.76%. In addition, the detection rates of HuNoVsGII and AstVs in RW and TW were < 35%, significantly lower than those in EW and UR ($P < 0.05$). Based on further analysis of the detection frequency's seasonal distribution (Table S4), EnVs in UR presented the lowest frequency in winter but always occurred in the water samples from other seasons. HRVs, HuNoVsGII, and AstVs could be found in all samples from UR and EW collected in spring and winter, but AstVs only appeared in the RW and TW samples collected in winter.

No inhibition to qPCR was found in the concentrates of EGM filter from all the observed water matrices. Fig. 2 shows the average concentration of human enteric virus throughout the year in each representative water sample. For samples collected from the Haihe and Jinhe
Rivers, EnVs showed the highest average concentration, reaching \(1.39 \times 10^6\) GC/L and \(4.34 \times 10^5\) GC/L, respectively, followed by AstVs with \(4.11 \times 10^5\) GC/L and \(4.20 \times 10^5\) GC/L. However, in other water matrices, including EW, RW, and TW, AstVs were always the richest, reaching \(1.25-6.62 \times 10^5\) GC/L. Among all the water matrices, the maximal concentrations of HuNoVs GII and AstVs, in the range \(3.56-6.62 \times 10^5\) GC/L, occurred in the EW, while HAdVs, HRVs, and EnVs had the highest detection levels in UR. RW and TW presented lesser virus abundance and all target viruses remained at a lower level than that of the other water matrices \((P < 0.05)\). Furthermore, in comparison with RW, there was significant declination in the concentration of all viruses in TW after chlorination \((P < 0.05)\).

Fig. 3 demonstrates the seasonal changes in the concentration of human enteric viruses in observed representative water matrices. Significant seasonal distribution of human enteric virus in UR can be found and the concentrations of HRVs, HuNoVs GII, AstVs, and HAdVs mainly peaked in autumn or winter, reaching the minimum in summer. However, the concentration of EnVs peaked in summer and was at its minimum in winter. Additionally, for EW, RW, and TW, only HuNoVs GII and AstVs concentrated in winter while HRVs and HAdVs showed no significant seasonality.

3.2. Detection of enteric virus in clinical stool samples

Enteric viral infections were found in 775 of the 1906 cases \((40.66\%)\) with HRVs showing the highest detection rate \((19.88\%)\), followed by HuNoVs GII \((11.91\%)\), HAdVs was only positive in 2.36% of stool samples. Therefore, HRVs and HuNoVs GII were the main epidemic EnVs in Tianjin in September 2014–August 2015 \((Table 5)\).

Notably, significant seasonal detection of all observed virus groups was also found in stool samples \((Figs. 4 and S2)\). Detection frequencies of HRVs, HuNoVs GII, and AstVs peaked in winter, while EnVs peaked in autumn \((Kendall’s Tau-b, P < 0.05)\). According to Fig. 4, which shows the monthly detection frequency of human enteric viruses in stool samples, the positive rates of HRVs, HuNoVs GII, and AstVs peaked in November \((40.27\%, n = 221)\), December \((25.41\%, n = 244)\), and January \((17.8\%, n = 213)\), respectively. EnVs infection was concentrated in September with the highest detection rate of 21%.

3.3. Correlation between viral concentrations in various water samples and clinical viral detection

Fig. 5 shows the correlation of monthly virus detection between different water matrices. The Haihe and Jinhe Rivers showed significant positive correlation with each other regarding the concentrations of all five kinds of human enterovirus \((P < 0.01)\), and a significant positive correlation was found with EW for the concentration of AstVs. However, for the RW, only the concentrations of AstVs and HRV were positively correlated with those in EW. For the TW, only the concentrations of AstVs and HAdVs were positively correlated with those in RW and the concentrations of HRVs were positively correlated with those in EW.

Fig. 5 also presents the correlation between monthly virus detection in water matrices and clinical stool samples. No significant relationship was found between the concentrations of enteric virus in the EW or TW and their prevalence in clinical stool samples. However, the concentrations of HRVs and HuNoVs GII in both the Haihe and Jinhe Rivers and AstVs in RW were significantly positively correlated with their prevalence in clinical stool samples \((P < 0.05)\).

4. Discussion

Enteric viruses can cause water-borne disease outbreaks throughout the year \((Kotwal and Cannon, 2014)\). Due to their transmission via the fecal–oral route, including in contaminated food and water, the circulation of viruses between contaminated environmental water and human populations poses a health hazard for humans and is becoming a global concern \((Haramoto et al., 2018)\). To take better prevention and control measures, it is essential to clarify the levels of enteric viruses in various environmental waters and reveal their impact on clinical prevalence. Here, after one-year surveillance of human enteric viruses in different water matrices and their correlation analysis to the prevalence of viral infections over the same period. The results revealed that the concentrations of HRVs and HuNoVs GII in UR running through a city are significantly positively correlated with their clinical prevalence, indicating that only the contamination of HRVs and HuNoVs GII in UR mirrors their clinical prevalence. Correspondingly, the analysis of HRVs and HuNoVs GII in UR could serve as a proxy for monitoring the prevalence of human enteric viruses in local populations. Currently, wastewater-based epidemiology is considered a powerful tool to understand the actual incidence of human viruses in a community, such as enteric viruses and Aichi virus \((Cuevas-Ferrando et al., 2019; Lodder et al., 2013; McCall et al., 2020; Rimoldi et al., 2020)\). However, due to its poor biosafety and inconvenient collection of sewage samples that entered WWTP through the fully closed pipeline, it was not a wise approach to monitor the virus occurrence in the raw sewage. Previous studies detected severe acute respiratory syndrome coronavirus 2 \((SARS-CoV-2)\) RNA in secondary-treated wastewater when the cases peaked in the community \((Haramoto et al., 2020; Randazzo et al., 2020)\). However, in this study, we did not find the correlation between viral concentrations in the EW and their clinical prevalence even if they both peaked in winter; this also contradicts previous studies. Prevost et al. found a close relationship between the health status of inhabitants and the viral contamination of WWTP effluents and consequent surface water contamination in the Paris urban area \((Prevost et al., 2015)\). They also suggested that monitoring viruses in wastewater and environmental water matrices that receive effluents from WWTPs could be an appropriate method to determine the actual prevalence and molecular epidemiology of gastroenteritis viruses in a given geographical region, rather than clinical studies \((Haramoto et al., 2018; Kazama et al., 2016)\). Considering that Prevost did not describe the disinfection process in the WWTP for the Paris urban area \((Prevost et al., 2015)\), a possible reason for this contradiction may be the chlorination process of final effluent in WWTP in China, which would kill sensitive viruses but allow the disinfection-resistant viruses to survive \((Jin et al., 2013; Xue et al., 2013)\). This has led to a huge microbial community shift and variations of microbial diversity in water after the chlorination process \((Potgieter et al., 2018; Zhang et al., 2017)\). However, the Haihe and Jinhe Rivers, as UR running through main residential districts in Tianjin city, inevitably receive untreated domestic wastewater along the river banks, where a large amount of excrement from patients may be discharged. Subsequently, viral contamination in urban rivers running through the city should be
a better candidate to mirror the epidemiology of viral infections than raw sewage or disinfected final effluent from WWTP.

However, not all enteric viruses in urban rivers mirrored the epidemiology of viral infections. HAdVs, AstVs, and EnVs concentrations in rivers showed no correlation with their clinical prevalence. This may have resulted from their strong resistance to the environment, which has allowed them to survive in water for long periods of time. For example, among the human enteric viruses, only HAdVs and AstVs were positively detected in the swimming area of recreational marine water at bathing beaches (Wei et al., 2020). Above all, HAdVs is often used as a viral indicator of water quality (fecal contamination) (Albinana-Gimenez et al., 2009; Lin and Ganesh, 2013; Rames et al., 2016; Rames and Macdonald, 2019) as this pathogen has been shown to be up to 60 times more resistant to UV irradiation than RNA viruses such as EnVs and hepatitis A virus (Fong and Lipp, 2005). Furthermore, due to its stability and persistence, HAdVs were the most frequently detected viruses in a range of water matrices such as wastewater, rivers, and drinking water, using PCR or qPCR methods (Katayama et al., 2008; Simmons and Xagoraraki, 2011). It is prevalent in untreated domestic wastewater all year round unlike EnVs and HuNoVs, which are usually only detected in certain seasons (Rames et al., 2016).

HRVs and HuNoVs are the leading etiologic agents of water-foodborne disease and gastroenteritis outbreaks worldwide (Banyai et al., 2018; Patel et al., 2008). HRVs primarily affect young children, accounting...
annually for almost 40% of hospital admissions for diarrhea and 200,000 deaths worldwide. The majority of which occur in developing countries (Banyai et al., 2018). HuNoVs affect people of all ages, accounting for 48% of all foodborne outbreaks in the United States (Hall et al., 2014) and 22% of diarrhea-related diseases among infants in African and Asian countries (Rouhani et al., 2016). Generally, HRV and HuNoVs molecular epidemiology data comes from laborious actual clinical assays and time-consuming statistical analyses, resulting in delayed epidemiological information on waterborne disease outbreaks. Wastewater-based epidemiology was considered a useful tool for the surveillance of HRVs and HuNoVs transmission in the population and their molecular epidemiology (Santiso-Bellón et al., 2020). However, considering the inconvenient and poor biosafety of wastewater sampling, monitoring HRVs and HuNoVs in urban rivers to understand human enteric virus prevalence in local populations both timely and rapidly would be a better approach.

5. Conclusions

Previous studies limitedly revealed the existence of a close relationship between inhabitants’ health status and the viral contamination of WWTP effluents. However, our study is the first to demonstrate that viral abundance in WWTP effluents has no correlation with local prevalence, but the concentration of HRVs and HuNoVs GII in UR running through a city significantly positively correlates with their occurrence in clinical prevalence. Considering HRVs and HuNoVs are leading etiologic agents of gastroenteritis outbreaks worldwide, our findings gave an indication that the analysis of HRVs and HuNoVs GII in UR could be used as an indicator to monitor their prevalence in local populations, which would help reveal the viral epidemiology in a timely manner and take measures to control disease outbreaks.

CRediT authorship contribution statement

Danyang Shi: Conceptualization, Validation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. Hui Ma: Methodology, Investigation, Resources. Jing Miao: Methodology, Formal analysis, Investigation, Writing – original draft. WeiLi Liu: Methodology, Investigation. Dong Yang: Methodology, Investigation, Validation. Zhigang Qiu: Methodology, Investigation, Supervision. Shigang Chen: Methodology, Investigation, Supervision. Jing Yin: Methodology, Investigation, Project administration. Zhongwei Yang: Methodology, Investigation, Project administration. Junwen Li: Conceptualization, Methodology, Validation, Investigation, Supervision, Funding acquisition. Min Jin: Conceptualization, Methodology, Validation, Investigation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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.

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Appendix A. Supplementary data

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