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RESEARCH ARTICLE

Enteric pathogens in flood-related waters in urban areas of the Vietnamese Mekong Delta: a case study of Ninh Kieu district, Can Tho city

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ABSTRACT
This paper investigates the contamination of floodwaters in the urban center of Can Tho city, Vietnam. We sampled water from sewers, surface water bodies, and flood, before, during, and after specific flooding events. Total nucleic acid was extracted from the samples and subjected to a quantitative polymerase chain reaction (qPCR) to detect specific enteric pathogens. The difference between pathogen concentrations in floodwater and sewer water was compared by using the Mann Whitney U test. Correlations between the different pathogens were determined using the non-parametric Spearman test. *E. coli* and Rotavirus-A were the most prevalent pathogens in floodwater. We observed a weak association between *E. coli* and Rotavirus in flood-related waters ($r < 0.5$). Floodwater quality showed no difference to sewer water quality in terms of the *E. coli* and Rotavirus A concentrations ($p > 0.05$). Our results indicate that floodwater poses a significant urban public health risk due to the presence of enteric pathogens.

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Floodwater quality; health risk; microbial pathogens; urban flood

Introduction

Water pollution due to extreme weather events is a major challenge for communities living in urban areas. Waterborne pathogens in surface water and floodwater have been determined to be a major source of enteric disease (Fewtrell, Kay, and Ashley 2008a; Katukiza et al. 2013; Pandey et al. 2014). For example, floodwaters in Bangladesh were screened for *Vibrio cholera* because of the potential of this organism to induce major disease outbreaks (Mark et al. 2015). Pathogens such as *E.coli* (O157:H7), *Salmonella*, and *Campylobacter jejuni* have also been commonly found in high concentrations in polluted urban water (Cann et al. 2013; Bitton 2011). *Cryptosporidium parvum* and *Giardia lamblia* are also associated with diarrheal disease and can be transmitted through contaminated water (Efstratiou, Ongerth, and Karanis 2017). The principal sources of floodwater pollution have been attributed to the mixing of sewer water and the contamination of water bodies like rivers. For example, the presence of microbial pathogens in floodwater has been traced to sewer water in the United Kingdom (Fewtrell 2011) and the Netherlands (De Man et al. 2014). Multiple studies have shown a correlation between flooding events and waterborne disease, including studies originating from Vietnam and Cambodia (Phung et al. 2014; Davies et al. 2015; Thompson et al. 2015). This association indicates a potential breakdown in sanitation, whereby pathogens can enter flood and surface water (Fewtrell et al. 2008a). However, few studies have investigated the real time presence of enteric pathogens in floodwaters due to difficulties in sampling floodwaters (i.e. anticipating and preparing materials for flood events), especially in low-to-middle income countries (LMICs).

A lack of floodwater sampling means we lack data regarding microbial pollutants in urban floodwaters, which is essential for assessing the risk of disease and providing information for suitable public health interventions. A previous study conducted a primary assessment for flood-related waters in the Mekong delta; however, this investigation was limited to physical/chemical parameters (Nguyen et al. 2017) Here we conducted a study was conducted to measure the occurrence, magnitude, and potential correlation between key enteric pathogens by sampling surface water bodies, sewers, and inundated water before, during and after flood peaks in Can Tho city in the Mekong delta region of Vietnam.

Study area

We selected Ninh Kieu District in Can Tho City (Vietnam) located on the Western side of the Hau (Mekong) river (Figure 1). This urban area of the Mekong delta has a large population (approximately 1.25 million people) compared to other Vietnamese provinces. Ninh Kieu district, the urban center, has an average population density of approximately 8,800 people/km^2 (Can Tho city Statistical Office 2014). Urban areas in Can Tho are frequently flooded from April to October due to extreme rainfall and high tide (Kingston, Thompson, and Kite 2011; Van et al. 2013). Ninh Kieu experienced approximately 25 flooding events between 2013 and 2017 with floodwater levels varying from 10

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Supplementary data for this article can be accessed here.

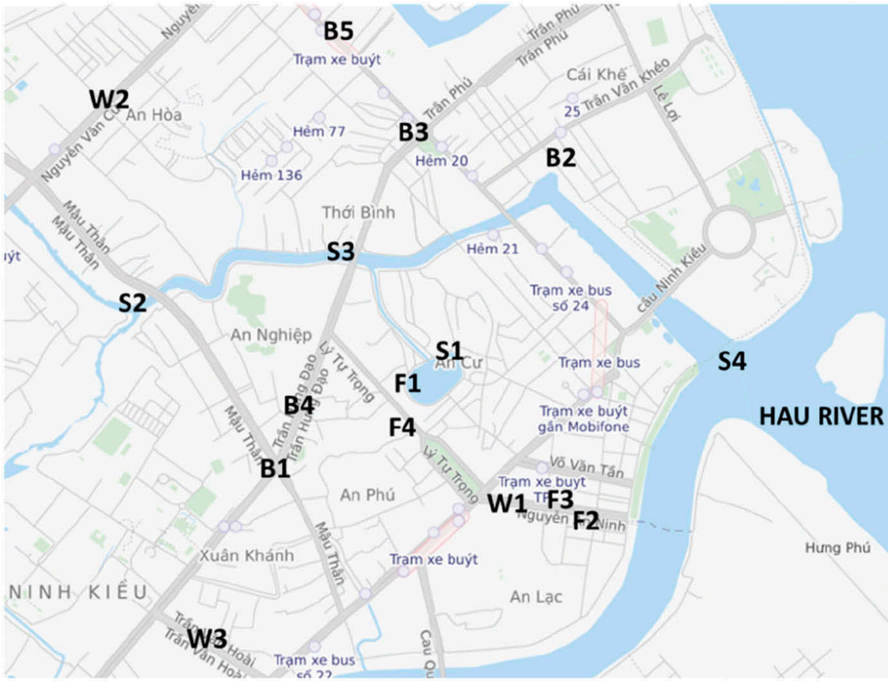

Figure 1. Water sampling sites in Ninh Kieu district, 2016. S1, S2, S3, and S4: Surface water sampling sites. W1, W2, and W3: Sewer water sampling sites. F1, F2, F3, and F4: Flood water sampling sites. B1, B2, B3, B4, and B5: Both flood water and sewer water were sampled at these places.

to 40 cm and flooded duration of 0.5–2.5 hours (Can Tho Drainage and Sewage Company 2017).

Water pollution is an ongoing challenge in Can Tho. The current drainage system mainly consists of combined sewers, which are of inadequate capacity for collecting untreated domestic wastewater mixed with stormwater (Leloup et al. 2013). In addition, almost all wastewater in Can Tho is discharged into the Hau river, untreated (Yen-phi 2010). The main sources of surface water pollution are domestic wastewater, industrial wastewater (from fisheries, aquaculture/agricultural production), and solid waste (Nguyen and Loan 2010). Recently, surface water in Can Tho city was reported to have substantial microbial pollution, for example: at the Tham Tuong canal, total Coliforms exceeded national water quality standards around 30 times (Can Tho Department of Natural Resources and Environment 2015). *E. coli* concentration ranged from 3,000 to 83,000 MNP/100mL in surface water near residential areas in Ninh Kieu district (Baino-Salingay et al. 2017). The tidal periods also influence urban water quality in Can Tho (Ozaki et al. 2014). During the flooding periods, untreated wastewater from the sewer system surges onto the streets and enters the surface water (Nguyen et al. 2017). As a result, microbial pollutants in urban floodwaters, such as *E. coli* can be found in high concentrations, often exceeding the national standard for surface water quality of Vietnam by 5 to 50 times (Salingay et al. 2014).

Materials and methods

The study design included: (a) Anticipating flood events in the study area, (b) Collecting water samples before, during and after flood events from the surface, sewer and floodwaters, and (c) molecular analysis of samples to detect enteric-pathogens.

Sampling strategy and flood event

Anticipating floods and logistical hardships such as preparing materials and labours within usually a few hours' notices were the major challenge in sampling campaigns. The months of September and October (2016) were selected as the potential sampling period, as this is the period with the highest probability of flooding (Birkmann et al. 2012). Basic logistics of sampling, including support personnel, were organized, anticipating floods. We were able to sample during two flood events.

The first flooding event was caused by heavy rain on 11th September during a low tide period. The second flooding event lasted four days (from 16th to 19th October) during high tide periods. In this latter case, flood occurred in the morning and afternoon-evening coinciding with peak river-level (Figure S2, supplementary). Heavy rainfall event occurred in the early morning on 17th October. On this day, the water level in Can Tho River was recorded as the highest value with 2.03m (Figure S2, Supplementary). All flooded sites were associated with the combined sewer system. Some inundated roads are shown in Figure S3, Supplementary. Details of the characteristics of floodwater at some flooded streets, for example, rising time, peak, and receding time are shown in Table S2 (Supplementary)

Water sampling

We collected 94 water samples from sixteen sampling sites for sewer water (31), surface water (26), and floodwater (37) around the occurrence of the two flood events (Figure 1). The details of this sampling are provided in Figure 1 and Table S1 (Supplementary).

Sewer water was collected from selected manholes before, around the peak, and after flooding. Floodwater samples were also collected during these three phases of the flood.

All water samples were collected in 0.5L sterile glass bottles. These bottles were stored in iceboxes, unexposed to light, and transported to the laboratory within 24 hours for analysis (APHA 2012).

Molecular analysis

Quantitative real-time Polymerase Chain Reaction (qPCR) was applied to detect and qualify the presence of various enteric pathogens(Pestana et al. 2009). These pathogen were *E. coli, Salmonella, Campylobacter spp., Shigella/EIEC*, Giardia spp., Cryptosporidium spp., Norovirus and Rotavirus, specific primers in the qPCR were used instead of universal ones. PCR amplification was performed in the Laboratory of Oxford University Clinical Research Unit (OUCRU) in Ho Chi Minh City, Vietnam. Details regarding the qPCR analysis are provided in the supplementary material (Section S1).

Statistical analysis

We employed the Mann-Whitney U test to identify differences between flood-related waters during flooding time in terms of the enteric pathogens. Spearman's rank correlation was applied to determine the association among the pathogens of flood-related waters. All analyses were performed in R software (Dalgaard 2008).

Results

The prevalence of enteric pathogens in water bodies

E. coli and Rotavirus A were the most prevalent enteric pathogens found in water bodies (i.e. sewer water, surface water, and floodwater) in flood events (Table 1).

E. coli was identified in more than one-third of the floodwater (13/37, 35%) and surface water (13/30, 41%) samples. The mean concentration of *E. coli* in floodwater was the same order of magnitude in surface water with 7×10^5 CFU/100mL and 4×10^5 CFU/100mL, respectively. Approximately 97% (30/31) of sewer water samples were positive for *E. coli*, with a mean concentration of 7.8×10^6 CFU/100mL, which was higher than floodwater and surface water by an order of magnitude.

Rotavirus A was detected in approximately half of the floodwater samples (21/37, 57%), the sewer water samples (15/31, 48%) and almost one-third of the surface water samples (8/26, 30%). The mean concentration of Rotavirus A in floodwater was 5.9×10^7 gc/100mL, which was similar to the mean concentration of sewer water ($6.1x10^7$ gc/100mL) and higher than mean value in surface water ($3.2x10^7$ gc/100mL) (Table 1).

Approximately 10% of surface water and sewer water samples tested positive for Norovirus *Salmonella* and Norovirus were negative in floodwater samples. *Campylobacter spp., Shigella/EIEC*, Giardia spp., or Cryptosporidium spp., were not detected in any of the samples.

Floodwater quality

The difference between floodwater and sewer water quality (in terms of *E. coli* and Rotavirus A concentration) was not significant ($p > 0.05$, Mann-Whitney U test). The p-values for *E. coli* and Rotavirus were 0.09 and 0.2, respectively. The results showed an equivalent microbial concentration in flood and sewer water. The concentration of *E. coli* contamination was comparable between them on flooding days 11/9/2016 and 17/10/2016 (Figure 2). During flooding time, at peak stage in the morning (6:00) and afternoon (17:00–18:00), *E. coli* and Rotavirus concentrations in the floodwater were close to thoes in sewer water, which were sampled before flooding. The mean log10 concentration of *E. coli* and Rotavirus in floodwater were equivalent to sewer water with 6.0 and 7.5, respectively (Figure 3).

Table 1. Microbial concentrations in flood water, surface water and sewer water in flooding days in Ninh Kieu district, 2016.

	Range of concentrations Mean (Min – Max) (SD,[a] positive samples)		
Parameters	Flood water	Surface water	Sewer water
Bacteria (10^6 CFU/100mL)[b]			
E. coli	0.7 (0.01–3.7) (1.2, 13/37)	0.4 (0.005–3.7) (1.1, 12/26)	7.8 (0.005–62) (15, 30/31)
Salmonella spp.	ND[c]	ND	0.3 (0.2–0.4) (0.14, 3/31)
Campylobacter spp.	ND	ND	ND
Shigella/EIEC	ND	ND	ND
Viruses (10^6 gc[d]/100mL)			
Rotavirus A	59 (0.2–296) (84.6, 21/37)	32 (0.3–71) (24, 8/26)	61 (5.9–258) (65, 15/31)
Norovirus GII	ND	0.004 (0.004–0.005) (1E-04, 2/26)	0.007 (0.003–0.01) (0.004, 4/31)
Parasites			
Cryptosporidium spp.	ND	ND	ND
Giardia spp.	ND	ND	ND

[a]SD: standard deviation.
[b]CFU: Colony forming-unit.
[c]ND: not detected.
[d]gc: genomic copies.

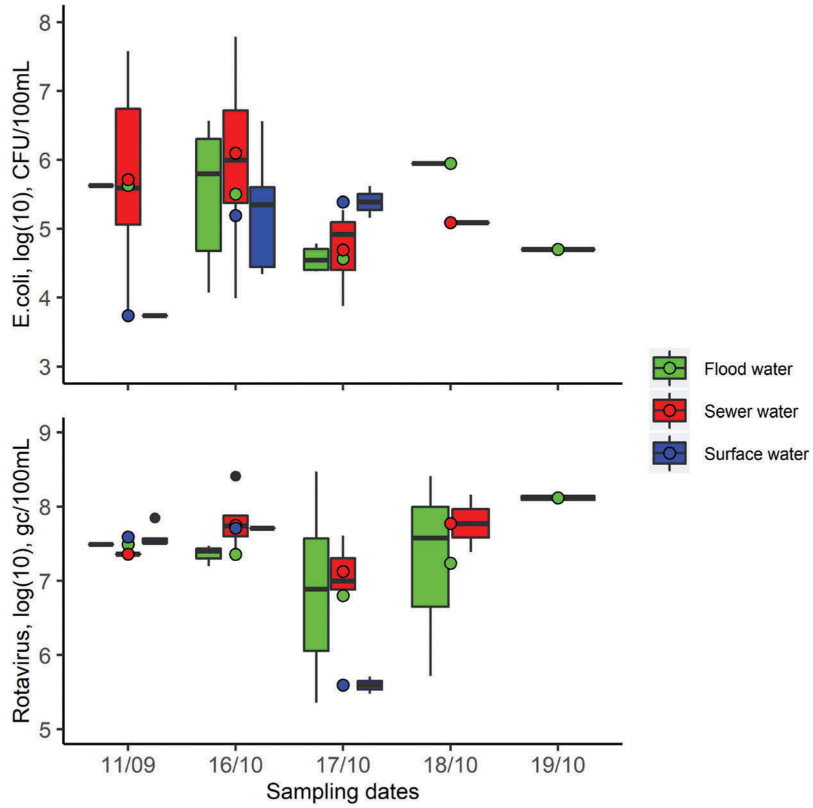

Figure 2. Box plots of *E. coli* and Rotavirus A in flood water, sewer water and surface water samples during the flooding days. In each sampling day, from left to right, the green, red and blue box plots indicate positive results of flood water, sewer water, and surface water samples. The green, red, and blue points represent the mean values and are arranged in the middle line for easier to compare among their values. The black points are outliers.

Surface water quality during the floods

E. coli in surface water was at a high concentration on the heavy flooding day (17/10/2016) (Figure 2). On this day, the mean *E. coli* concentration in the surface water was 2.8×10^5 CFU/100mL, which was three times higher than sewer water ($9x10^4$ CFU/100mL) and almost an order of magnitude (i.e. one log) greater than floodwater ($4x10^4$ CFU/100mL)floodwater. Additionally, *E. coli* concentrations at S1 (Xang Thoi Lake), S2 (Rach Ngong canal), and S3 (Cai Khe canal) which are located near to a residential area were an order of magnitude higher than at S4 (Hau River), which is located further from the residential area (Figure 3). Furthermore, Rotavirus was not detected at S4, but identified at S1, S2, and S3 with mean concentrations log10 from 6 to 8 (Figure 4).

Correlations of enteric pathogens concentration in water samples

We identified a weak correlation (Spearman r) during flooding periods between *E. coli, Salmonella spp.*, Rotavirus A, and Norovirus GII in flood-related waters ($r < 0.5$, Spearman correlation), the same was not observed for *Salmonella* – Norovirus (Figure S1, Supplementary). The correlation coefficients (r) between *Salmonella* and Norovirus was 0.7 ($p < 0.001$). The correlation of *E. coli* – *Salmonella, E. coli* – Rotavirus and *E. coli* – Norovirus in flood-related waters were 0.26 ($p < 0.05$), 0.19, and 0.13, respectively.

Discussion

Highly polluted floodwaters

Surface water and floodwater were highly polluted with *E. coli* and Rotavirus A during flooding time in Ninh Kieu district. According to previous studies, *Campylobacter* spp., *Shigella/EIEC, Giardia* spp., and *Cryptosporidium* spp. in surface water and floodwater were found to pose a risk to human health (Fewtrell et al. 2011; Ten Veldhuis et al. 2010; Sales-Ortells and Medema 2015). However, we did not detect these pathogens in the flood-related water samples. Alternatively, we commonly found *E. coli* and Rotavirus A in flood-related water in this environment. The concentration of *E. coli* in this study was up to three orders of magnitude (i.e. three log) higher than values recorded from other urban flood events in Can Tho city (Vietnam) after a flooding event in 2013 (Nguyen et al. 2014), Jakarta (Indonesia) (Phanuwan et al. 2006), and The Hague (the Netherlands) (Sterk et al. 2008).

Additionally, the concentration of Rotavirus in surface water was two to three orders of magnitude higher than values reported from the river Negro in Brazil (Vieira et al. 2016). The occurrence of Rotavirus in floodwater has been reported previously (Fewtrell, Smith, and Kay 2010). For example, Rotavirus A was detected in 9 out of 100 floodwater samples in Thailand (Ngaosuwankul et al. 2013).

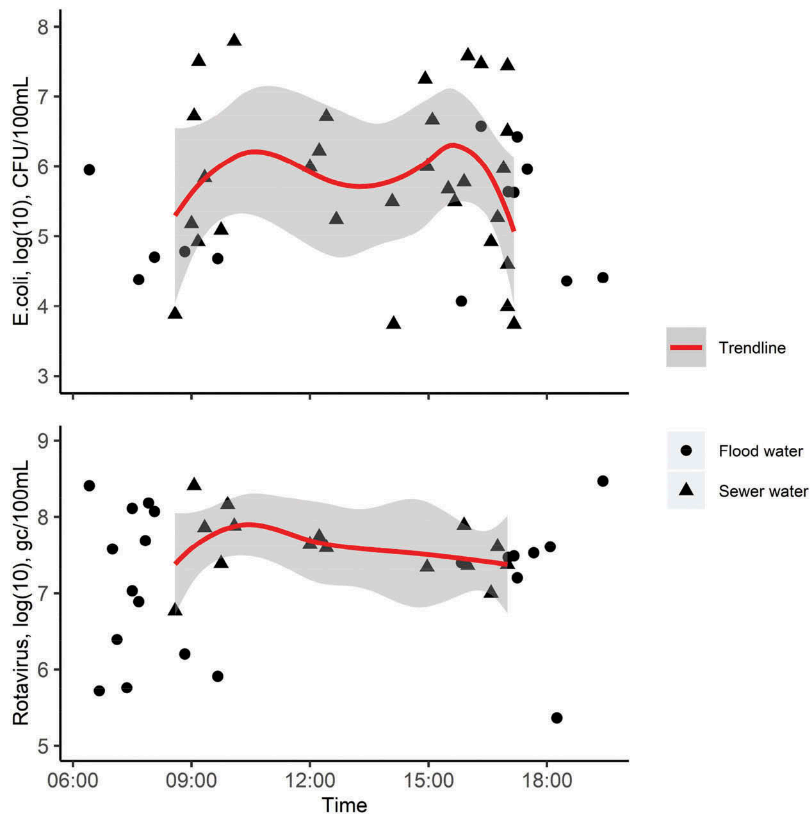

Figure 3. *E. coli* and Rotavirus a concentrations in flood water and sewer water. The red line is the moving average of *E. coli* and Rotavirus in sewer water with standard deviation (grey area).

No correlation between E. coli and Rotavirus A

In the previous studies, due to the difficulty of detecting viruses, often an assumed correlation ratio between *E. coli* and Rotavirus A has been used to estimate Rotavirus concentration in drinking water (Lulani, van der Steen, and Vairavamoorthy 2008; Mara et al. 2007; Howard et al. 2007; Machdar et al. 2013). This approach, however, has not been previously applied to floodwater. Our results indicate that care should be taken in using this ratio for future research on floodwater because a significant correlation was not found between *E. coli* and Rotavirus A in flood-related waters.

Flooding may connect additional sources of pollution

Our data suggest that surface water may receive additional contamination sources with respect to faecal pollutants: For sewer water and floodwaters, the mean *E. coli* concentrations on the high flooding day (17 October 2016) was lower than other days (Figure 2). This is an expected result for sewer water and floodwater, as extreme rainfall and a high tide may dilute the concentrations. In contrast, for surface water, the mean *E. coli* concentration was observed at high concentrations on this day. Moreover, *Salmonella* spp. and Norovirus GII in surface water were not detected, which may be attributed to dilution associated with rain and high tide. This result leads us to suspect that the concentration of *E. coli* in surface water on high flooding days was likely impacted by other reasons beyond the dilution factor, which compensates for the expected dilution effect. Additionally, the concentrations of *E. coli* and Rotavirus near residential areas (S1, S2, and S3) were found to be higher than those in S4. This observation indicates that there are other potential pollutant sources than sewer water. The probable contamination sources which contain a high concentration of faecal pollutant could originate from septic tanks of local households which are located near river/canal. Due to the high floodwater level, these contaminated sources may connect directly into surface water.

Confirms previous results

The present study confirmed some results of a previous study by Nguyen et al. (2017). Firstly, Nguyen et al. originally observed that floodwater, wastewater, and surface water quality in fluvial flood event in Ninh Kieu district in 2013 were not significantly different in their contamination because most sewer water and floodwater originated from surface water. The current study indicates a high likelihood of a large volume of sewer water entering floodwater, as there was no significant difference between *E. coli* and Rotavirus concentrations in flood and sewer water. Secondly, Nguyen et al. previously reported that floodwater quality deteriorated as the floodwater level increased. Similarly, in the current study, the concentration of pathogens was highest at the peak stage of the flooding. The mean concentrations of *E. coli* and Rotavirus at the peak stage of floodwater were higher than those at the peak stage of sewer water before flooding (Figure 3).

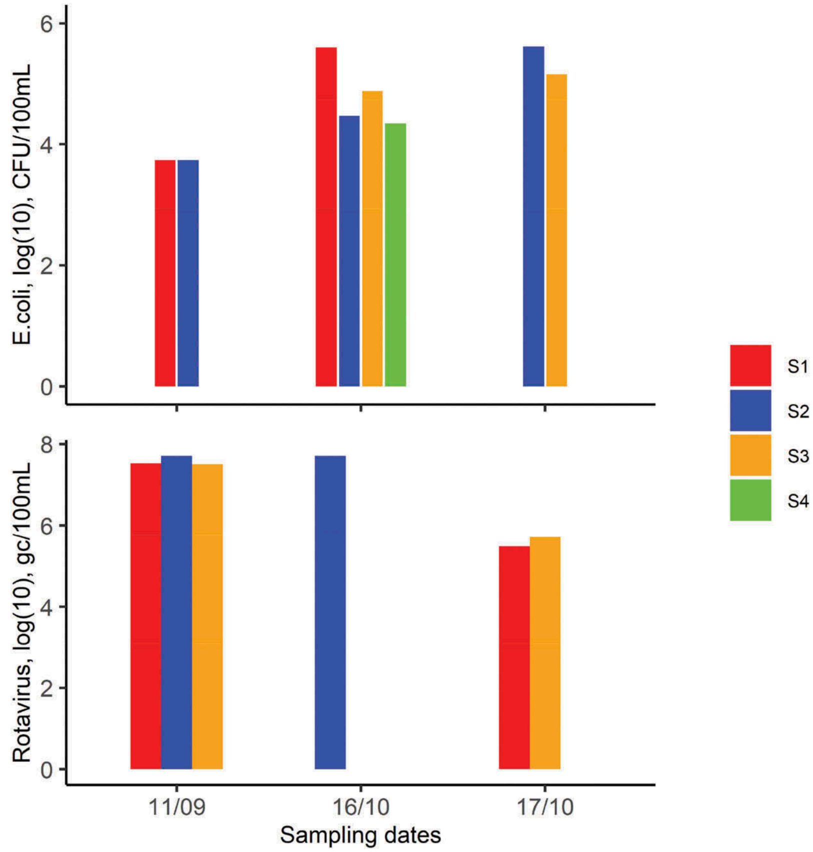

Figure 4. *E. coli* and Rotavirus mean concentrations for each sampling day at surface water sites: S1 (Xang Thoi lake), S2 (Rach Ngong canal), S3 (Cai Khe canal), and S4 (Hau River).

Potential health risk

High concentrations of pathogens in floodwater may lead to potential health risks associated with waterborne diseases to the exposed population in LMICs (Nguyen et al. 2001; Bich et al. 2011; Fuhrimann et al. 2017). Additionally, it has been reported that cases of diarrhoea increased when *E. coli* and Rotavirus were found in flood events (Ahern et al. 2005). In our study, the variation in pathogen concentration, especially at first stage of flood event, may pose a specific risk when it coincides with rush hour of traffic, for example, morning (5:30–7:00) and afternoon-evening (17:00–19:00) in Can Tho city (Figure S2 – Supplementary). More people may be exposed to floodwater through transportation activity. Therefore, citizens of Ninh Kieu face not only 'too much' but also 'too dirty' waters during flooding periods.

Further investigations

This study identifies several areas for future investigation. In order to determine which source causes high *E. coli* concentration in surface water and floodwater during high flood events, other potential contamination sources, such as runoff from businesses like markets, landfills, or neighbourhoods with pit latrines have to be investigated. Floodwater quality modeling may aid in understanding the transportation dynamics of pathogens at different spatial and temporal scales. Climate change and increasing urbanization are likely to affect flooding in Can Tho (Huong and Pathirana 2011). The model-based analysis will enable an understanding of the influence of different future scenarios on floodwater quality. A further important area of investigation is the citizen's awareness of the reality of floodwater quality and the impact of exposure on health.

Conclusions

This study attempted to address a lack of observational evidence for microbial pathogen contamination (and their concentration) during flooding events in LMICs. Our findings provide evidence regarding which waterborne pathogens may represent a hazard to human health during flooding period. To our knowledge, this is the first study to detect the human enteric virus Rotavirus A in floodwater in Vietnam. Floodwater in Can Tho showed the prevalence of *E. coli* and Rotavirus A. Furthermore, the concentrations in of these pathogens in floodwaters were not statistically different from those in sewer water, indicating highly polluted floodwaters, which may pose a serious risk to health hazard.

Due to the difficulty in testing for viruses, some previous studies assumed a correlation of viruses with (an easily detectable) *E. coli*. The current findings may be used as input for health risk assessments of waterborne diseases during flooding time. While more evidence is required regarding the health risk of flooding, it is important to advocate for more efficient approaches to reduce faecal pollution during flood events. In addition, we need to raise the awareness of the potential risk from common waterborne pathogens in order to protect residents from waterborne diseases.

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Author contributions

Thi Thao Nguyen Huynh, Hong Quan Nguyen, and Assela Pathirana conceived and designed the study. Thi Thao Nguyen Huynh and Hong Quan Nguyen performed the study. Stephen Baker and Phat Voong Vinh were responsible for microbial analysis. All authors contributed to writing the paper.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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