Monitoring of human enteric viruses and coliform bacteria in waters after urban flood in Jakarta, Indonesia

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Abstract Floodwaters in Kampung Melayu village, Jakarta, Indonesia, as well as river water and consumable water (including groundwater and tap water) samples in flooded and non-flooded areas, were quantitatively analysed to assess occurrence of viruses and total coliforms and *E. coli* as bacterial indicators after flooding event. High numbers of enterovirus, hepatitis A virus, norovirus (G1, G2) and adenovirus were detected at high concentration in floodwaters and waters sampled from Ciliwung River which runs across metropolitan Jakarta and is used widely for agriculture and domestic purposes by poor residents. One out of three groundwater wells in the flooded area was contaminated with all viruses tested while no viruses were found in groundwater samples in non-flooded areas and tap water samples. The results revealed that human enteric viruses, especially hepatitis A virus and adenovirus, were prevalent in Jakarta, Indonesia. This study suggested that flooding posed a higher risk of viral infection to the people through contamination of drinking water sources or direct contact with floodwaters.

Keywords Coliform bacteria; *E. coli*; health risk; human enteric viruses; Jakarta; urban flooding

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Introduction

Rapid urbanisation in developing countries brings about not only the economic development of the cities, but also a negative side impact to people's health and environment. Uncontrolled urbanisation intensifies the frequency and severity of flooding which is one of the big problems in downtown areas of Asian cities. Jakarta is the capital city of Indonesia and is located in the western corner of the Java Island. It has a tropical climate with an average annual temperature of 27 °C and an average annual precipitation in Jakarta of 2,000 mm with the highest in January. Most of Jakarta lies in low land, 0–10 m above the mean sea level. Ciliwung River is one of the major rivers which used to be the source for water supply in Jakarta. One of the most serious problems in Jakarta is the lack of sewerage systems in urban areas; less than 3% of Jakarta's population is connected to a sewer system (World Bank, 2002). Jakarta is frequently flooded due to low elevation and the absence of an adequate drainage system. Floods in Jakarta peak every year in January and February. More than 70 areas have been identified as prone to flooding while the worst affected area is called Kampung Melayu in the Jatinegara district in East Jakarta.

Waterborne diarrhoeal disease is a major concern of public health problem impacted by flooding. In 2002, more than 35,000 people in Jakarta suffering from diarrhea disease were recorded in the event of the worst flood in recent history (www.urbanpoor.or.id on 1 July 2004), but the agents causing diarrhoea were unknown. Enteric viruses are the major cause of the non-bacterial waterborne diseases which are transmitted mainly by the faecal-oral route via contaminated food or water. A recent study reported that a high incidence of enteric virus-related infections was found among acute diarrhoeic patients

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in Jakarta, Indonesia (Subekti *et al.*, 2002). Flooding can possibly increase the risk of enteric virus infection; however, there are only a few scientific studies on the occurrence of the enteric viruses associated with flooding events. In addition, floodwater or surface water contaminated with raw sewage contains useful information on the prevalence of specific pathogens in the flooded area. Therefore, an understanding of viral occurrence in flooding is needed for an assessment of the risk of pathogenic viruses and for proper management to protect public health from viral waterborne diseases. The objective of this study was to compare the occurrence of viruses in various waters sampled from the flooded and non-flooded areas in Jakarta.

Materials and methods

Samples collection

The selected study area was Kampung Melayu village, which is a city-centre slum located on the bank of the Ciliwung River in Jatinegara District, East Jakarta (Figure 1).

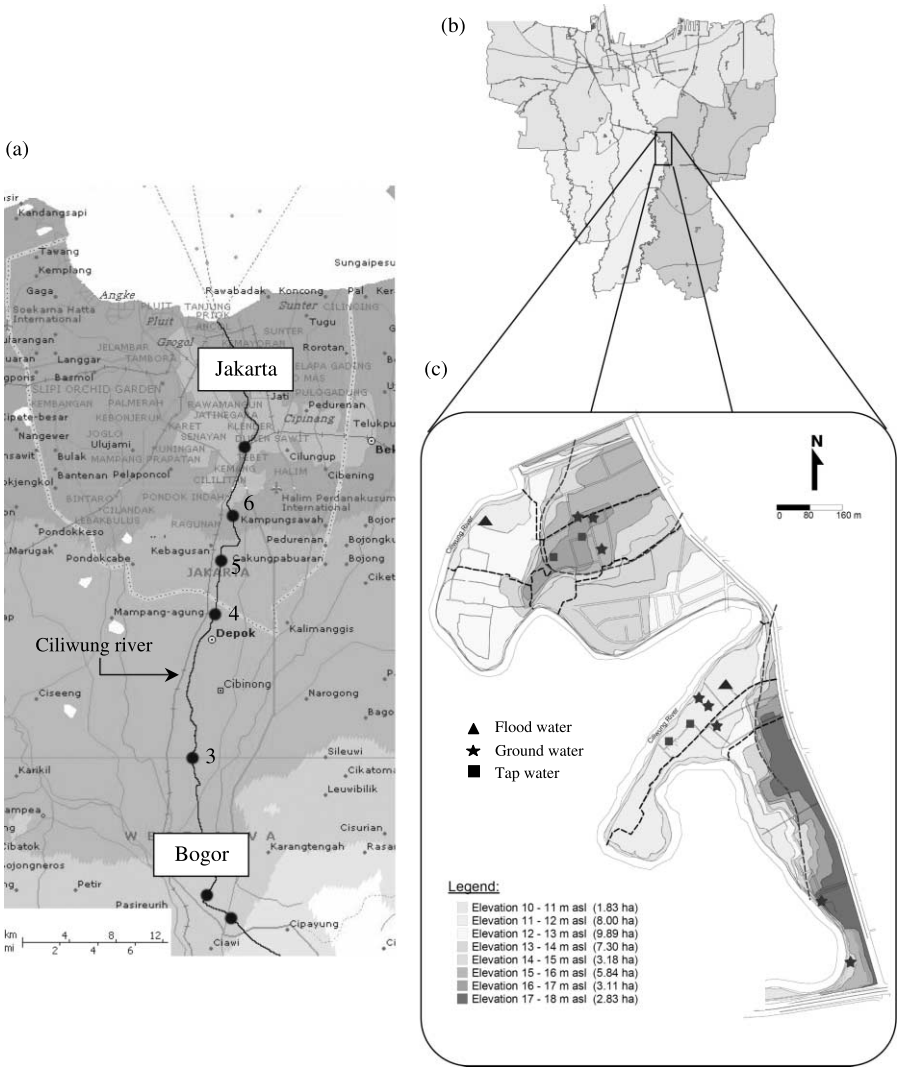

Figure 1 Map of study area and sampling points: (a) sampling points along Ciliwung River from Bogor city (upstream) to Jakarta city (downstream), (b) Jakarta map and (c) study area and sampling points in Kampung Melayu village, East Jakarta

The village suffers from occasional flooding every year. A severe flooding event occurred on January 19 2005 which inundated nearly half of the village. Two floodwater samples were collected on January 23 2005. During January 23–27 2005, three groundwater and two tap water samples were collected from the flooded area and five groundwater and two tap water samples were collected from the non-flooded area. Seven river samples were collected from the Ciliwung River, which runs through the village. The upstream is located in Bogor city and runs through Jakarta. There is a residential area along the river. Sampling points along the Ciliwung River from upstream to downstream are shown in Figure 1. At each sampling point, approximately 2 L of samples analysis were collected into a sterile plastic bag and microbial analyses carried out on the same day.

Physicochemical parameters

Temperature, pH, conductivity, turbidity, dissolved oxygen (DO), salinity, total dissolved solids (TDS) and oxidation reduction potential (ORP) were determined at each sampling site by Multi-Probe W23XD system (Horiba, Japan). Free and total chlorine, iron (Fe(II)) and ammonia nitrogen (NH_3-N) were measured using a DR/890 Colorimeter (Hach, USA).

Microbial analysis

Indicator bacteria. Total coliforms and *E. coli* were determined on the same day of sampling by a membrane filtration technique using m-ColiBlue24 Broth (Millipore), according to the protocols described by the manufacture, and incubated at 37 °C for 24 h. *E. coli* was identified as blue coloured colonies while other coliforms were red. Total coliforms were the sum of the two coloured colonies.

Virus concentration process upon sampling. Viruses were concentrated by a modified method following Katayama *et al.* (2002). Briefly, $MgCl_2$ was added to the sample (final 25 mM) and filtered (47 mm, 0.45 μm sterile type HA negatively charged membrane; Millipore, Tokyo, Japan). The sample volumes filtered were 50 mL for river water and floodwater and 2 L for groundwater and tap water. The membrane filter was rinsed with 200 mL 0.5 mM H_2SO_4 to remove cations on the membrane filter, followed by elution of viruses from the membrane with 5.0 mL 1 mM NaOH (pH 10.5–10.8). The filtrate was recovered in a tube containing 25 μL 100 mM H_2SO_4 and 50 μL 100 × TE buffer (pH 8) for neutralisation. The eluates were kept cool with gel ice and transported to the laboratory at the University of Tokyo.

Virus detection. The eluates were further concentrated using Centriprep YM-50 ultrafiltration (Millipore) to obtain a final volume of 1 mL. These concentrates were processed for RNA/DNA extraction by using a QIAamp viral RNA/DNA mini kit (Qiagen, Japan). The RNA viruses were processed for a reverse transcription step using the GeneAmp PCR system 9600 (Applied Biosystems, USA) and the concentration of enterovirus, norovirus (G1, G2), hepatitis A virus and adenovirus were quantified by TaqMan PCR using the ABI PRISM 7000 (Applied Biosystems, USA) method described previously (Haramoto *et al.*, 2005). Briefly, 5 μL of each DNA or cDNA sample was mixed with 45 μL of a reaction mixture containing 25 μL 2 × TaqMan universal PCR master mix (Applied Biosystem, USA), 400 nM of each primer and 300 nM TaqMan probe. The primers and the TaqMan probes used for all viruses, except hepatitis A virus, followed the method previously described by Haramoto *et al.* (2005). For hepatitis A virus, sense primer was 5′-AGGGTAACAGCGGCGGATAT-3′, antisense primer was 5′-ACAGCCCTGACARTCAATYCACT-3′ and Taqman Probe

was 5′-FAM-AGACAAAAACCATTCAACRCCGRAGGAC-TAMRA-3′ (Nishio Osamu, National Institute of Infectious Disease, Japan, pers. comm.). The amplification included: (a) incubation at 50 °C (2 min) and heating at 95 °C (10 min); (b) 50 cycles at 95 °C (15 s) and at 56 °C (1 min) for norovirus G1 and G2, at 95 °C (15 s) and 60 °C (1 min) for enterovirus and hepatitis A virus, at 95 °C (3 s), at 55 °C (10 s) and at 65 °C (1 min) for adenovirus. The amount of each virus was calculated as a PCR detection unit (PDU) according to the end point dilution of detection limit of the positive control.

Results and discussion

Occurrence of enteric viruses and bacterial indicator in floodwater and river

The concentrations of enteric viruses, total coliforms and *E. coli* are summarised in Table 1. All viruses tested were found in floodwater samples. The most abundant virus in floodwater was Hepatitis A virus with a geometric mean of 79 PDU/mL, followed by adenovirus (55 PDU/mL), Enterovirus (30 PDU/mL) and Norovirus G2 (26 PDU/mL). Norovirus G1 was approximately 100 times less than Norovirus G2.

Enterovirus, hepatitis A virus, adenovirus and norovirus G2 were found in all samples taken from Ciliwung River whereas norovirus G1 was found in 4/7 samples. The most abundant virus in river water was adenovirus with a geometric mean of 14.0 PDU/mL, followed by hepatitis A virus (13.0 PDU/mL), norovirus G2 (5.3 PDU/mL), enterovirus (0.7 PDU/mL) and norovirus G1 (0.03 PDU/mL), respectively. The results revealed that enteric viruses, especially hepatitis A virus and adenovirus, were prevalent in Jakarta. The concentration of total coliforms and *E. coli* were in the range $1.1 \times 10^3 - 1.6 \times 10^4$ and $2.0 \times 10^2 - 1.1 \times 10^3$ CFU/mL, respectively, indicating that the Ciliwung River was heavily polluted. The concentrations of viruses, total coliforms and *E. coli* along the Ciliwung River are shown in Figure 2. Comparison of means (Mann–Whitney *U* test) of the $\log_{10}$ tramsformed values of microorganisms was performed between floodwater and river waters. The floodwater samples presented significantly higher concentration ($p < 0.05$) than river waters for all bacterial indicators and viruses except adenovirus.

The greater microbial contamination level in floodwater than in the Ciliwung River indicated a higher health risk during flood events than the normal seasons, especially to the people who walk through, and children playing in, floodwater. In addition, due to the lack of clean water, many people used the floodwaters for washing and other domestic purposes which increased the risk of infection to them. In this study, the floodwater samples were collected after 4 d of flooding when the floodwater started to recede. Such microbial contamination may further be exacerbated if the people suffer from

Table 1 Concentration of viruses and bacterial indicators in floodwater, river and groundwater

Parameters	Unit*	Geometric mean concentration (range)		Min and max concentration groundwater	
		Floodwater ($n = 2$)	Ciliwung River ($n = 7$)	Flooded area ($n = 3$)	Non-flooded area ($n = 5$)
Viruses					
Enterovirus	PDU/mL	30 (12–72)	0.7 (0.2–2.2)	ND–0.022	ND**
Hepatitis A virus	PDU/mL	79 (71–87)	13.0 (4.0–33)	ND–1.0	ND
Norovirus G1	PDU/mL	0.19 (0.18–0.19)	0.03 (0.01–0.04)	ND–0.013	ND
Norovirus G2	PDU/mL	26 (22–30)	5.3 (1.0–16)	ND–8.9	ND
Adenovirus	PDU/mL	55 (51–60)	14.0 (0.5–120)	ND–0.80	ND
Bacterial indicators					
Total coliforms	10^3 CFU/mL	440 (95–2000)	4.2 (1.1–16)	0.007–4	ND–0.014
E. coli	10^3 CFU/mL	24 (9–65)	0.6 (0.2–1.1)	ND–1.7	ND

*CFU/mL = colonies forming unit/mL, and PDU/mL = PCR detection unit/mL; **ND = not detected

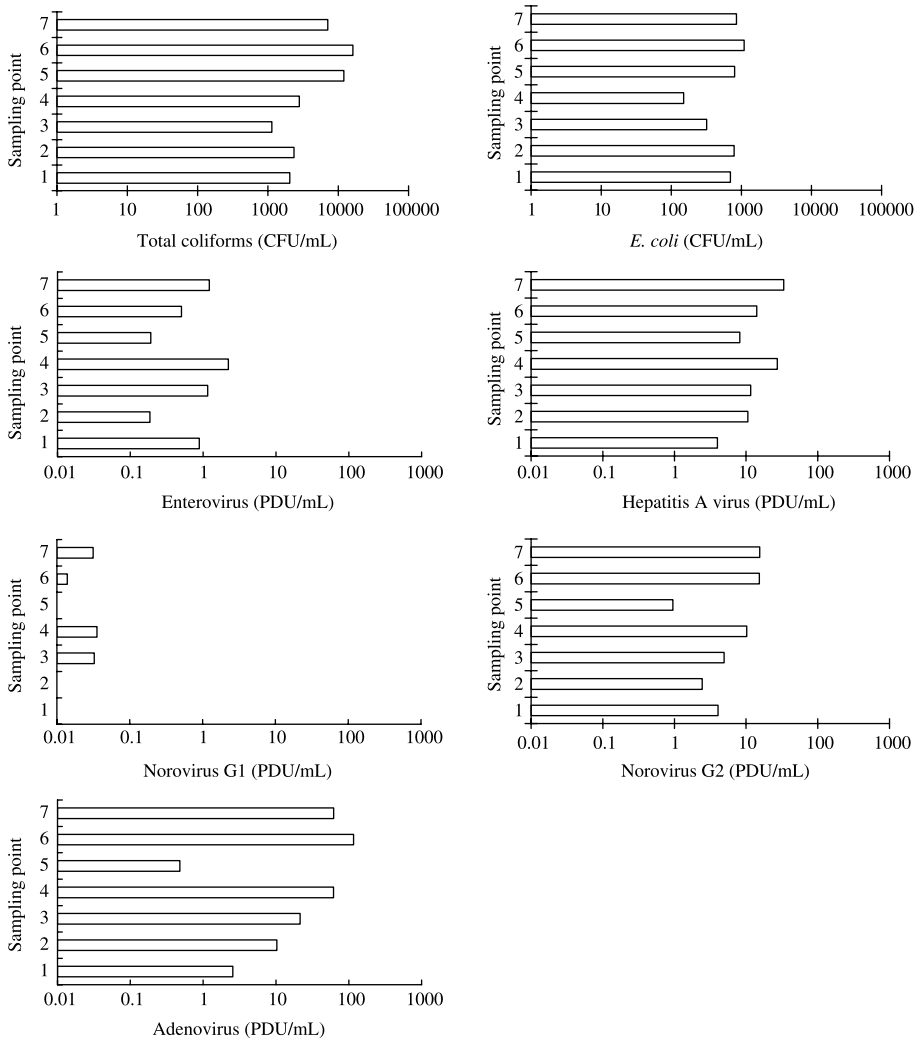

Figure 2 Concentration of viruses and bacterial indicators at each sampling point along Ciliwung River, Jakarta, Indonesia. (sampling point 1–7 referred to sampling point from upstream to downstream as shown in Figure 1)

elongated flooding in the future. Monitoring of viruses in floodwater and river water provides useful information on pathogenic microorganisms in the community.

Occurrence of enteric viruses and bacterial indicators in drinking water source

Total coliforms were the most common organism detected in six out of eight groundwater samples in flooded and non-flooded area, whereas *E. coli* was observed in two out of three groundwater samples in flooded area at concentrations of 1,700 and 16 CFU/mL. The sample with a higher concentration of *E. coli* (1,700 CFU/mL) was also contaminated with enterovirus, hepatitis A virus, norovirus (G1 and G2) and adenovirus at concentrations of 0.022, 1.0, 0.013, 8.9 and 0.80 PDU/mL, respectively. The sample with the lower number of *E. coli* (16 CFU/mL) was only positive for adenovirus (0.15 PDU/mL). PCR could not distinguish between infectious and non-infectious viruses but detection of the viral genomes by PCR raised the concern of viral infection to the people, which could be greater if people drank contaminated groundwater without boiling.

Total coliforms were detected in the range of 2.5–14 CFU/mL in three out of five groundwater samples but no *E. coli* and enteric viruses were detected in any groundwater samples in the non-flooded area. Two out of five wells in the non-flooding area were located near septic tanks (~5 m) and had high ammonia concentrations of 12 and 28 mg/L, but none were virus positive. Viruses and bacterial indicators were not detected in any tap water samples in both flooded and non-flooded areas. The average concentrations of total chlorine and free chlorine in tap water were 1.5 and 1.3 mg/L, respectively.

There were no *E. coli* or viruses detected in groundwater samples collected from a well located near a septic tank in the non-flooded area, whereas they were found in some groundwater samples in the flooded area, suggesting that flooding was more likely to be a source of microbial contamination in groundwater than septic tanks. The presence of viruses in *E. coli* positive samples implied that the presence of *E. coli* seemed to be an acceptable indicator to evaluate the presence of pathogenic virus in groundwater in this area.

Physicochemical parameters

Physicochemical parameters in floodwater, river and groundwater are shown in Table 2. Floodwater and river water had a significant difference in temperature and ORP values (Mann-Whitney U test, $p < 0.05$). Mean value of temperature and ORP determined in floodwater and river water was 26.3 °C (26.1–26.4 °C) and 24.7 °C (23.3–25.6 °C) and 110 mV (80–140 mV) and 250 mV (200–320 mV), respectively. No significant difference was found between physicochemical parameters measured in groundwater samples in flooded and non-flood area.

A comparison between virus positive and negative samples of groundwaters in the flooded area revealed that the viruses were detected in wells with higher turbidity but lower conductivity and ORP. High concentration of ammonia nitrogen (14 mg/L) was found in the virus positive groundwater sample in the flooded area. However, ammonia nitrogen at concentrations of 12 and 28 mg/L were also found in groundwater samples in non-flooded areas, while it was negative for virus. Therefore, ammonia nitrogen could not be used to predict the presence of virus in groundwater in this study.

Relationship among viruses, bacterial indicator and physicochemical parameters

Statistical non-parametric Spearman correlation was used to analyse the correlation between viruses and indicators (logarithmic basis), and physicochemical parameters with

Table 2 Physicochemical parameters in floodwater, Ciliwung River and ground water

Parameter*	Unit	Mean (range)			
		Floodwater ($n = 2$)	River ($n = 7$)	Groundwater	
				Flooded area ($n = 3$)	Non-flooded area ($n = 5$)
pH	–	7.2 (6.8–7.6)	7.4 (6.8–8.0)	6.9 (6.9–7.0)	6.7 (6.6–6.9)
Conductivity	mS/m	16 (14–17)	19 (10–29)	87 (31–120)	82 (59–110)
Turbidity	NTU	670 (410–920)	230 (40–660)	9 (0–26)	3.2 (0–13)
DO	mg/L	7.9 (7.8–8.0)	8.4 (7.8–8.8)	5.3 (5.0–5.5)	5.7 (4–6.4)
Temperature	°C	26.3 (26.1–26.4)	24.7 (23.3–25.6)	28.1 (27.5–28.7)	29.1 (28.0–29.8)
TDS	g/L	0.10 (0.09–0.11)	0.10 (0.07–0.16)	0.6 (0.2–0.8)	0.5 (0.4–0.7)
ORP	mV	110 (80–140)	250 (200–320)	250 (190–280)	320 (260–420)
Chloride	mg/L	22 (17–27)	17 (8–31)	63 (28–82)	81 (52–115)
Fe(II)	mg/L	18 (17–19)	0.09 (0.03–0.24)	0.04 (0.01–0.07)	0.06 (0.00–0.18)
NH_3-N	mg/L	2 (0–2)	0.14 (0.0–1.0)	4.7 (0–14)	8.6 (0–28)

*ADO: dissolved oxygen, TDS: total dissolved solids, ORP: oxidation reduction potential, Fe(II): ferrous iron, and NH_3-N: ammonia nitrogen

Table 3 Correlation half-matrix of viruses, bacterial indicators and physicochemical parameters

Parameter	Spearman's rank correlation coefficient (r), $n = 21$*											
	EV	**HAV**	**NG1**	**NG2**	**Ads**	**TC**	**EC**	**Cond**	**Turb**	**Temp**	**TDS**	**ORP**
Viruses												
EV	1.00											
HAV	0.99	1.00										
NG1	0.85	0.86	1.00									
NG2	0.96	0.97	0.89	1.00								
Ads	0.93	0.95	0.81	0.94	1.00							
Bacterial Indicators												
TC	0.86	0.88	0.72	0.90	0.89	1.00						
EC	0.87	0.89	0.74	0.93	0.89	0.94	1.00					
Physicochemical parameters												
Cond	−0.78	−0.76	−0.54†	−0.70	−0.67	−0.55†	−0.65	1.00				
Turb	0.90	0.90	0.75	0.87	0.84	0.80	0.81	−0.84	1.00			
Temp	−0.76	−0.75	−0.44†	−0.69	−0.76	−0.62	−0.67	0.85	−0.74	1.00		
TDS	−0.77	−0.77	−0.55	−0.74	−0.75	−0.62	−0.70	0.93	−0.86	0.87	1.00	
ORP	−0.69	−0.71	−0.62	−0.77	−0.70	−0.80	−0.83	n.s.‡	−0.60	n.s.‡	n.s.‡	1.00

*significant at $p < 0.01$; †significant at $p < 0.05$; ‡not significant; Cond = conductivity, Turb = turbidity, Temp = temperature, TDS = total dissolved solids, ORP = oxidation reduction potential

all water samples showing significant correlation are shown in Table 3. Total coliforms and *E. coli* were positively correlated with all viruses tested. However, as shown in Table 1, total coliforms were also detected in groundwater in non-flooded areas, whereas *E. coli* was not. *E. coli* seemed to be a better indicator than total coliforms to predict the presence of viruses in groundwater. The results showed a statistically significant correlation between the physicochemical parameters (including conductivity, turbidity, temperature, TDS and ORP) with all viruses and bacterial indicators tested. Turbidity showed positive correlation with all the microbes tested while conductivity, temperature, TDS and ORP showed negative correlation.

Conclusions

1. The enteric viruses, enterovirus, hepatitis A virus, norovirus (G1, G2) and adenovirus in contaminated water in Jakarta were successfully concentrated and detected by the methods used in this study.
2. The human enteric viruses, especially hepatitis A virus and adenovirus, were prevalent in Jakarta.
3. Higher contamination of viruses and bacterial indicators was found in floodwater than river water, indicating a higher health risk during flood events than the normal seasons.
4. The source of virus contamination in groundwater in the study area was likely to have been floodwater rather than other sources such as septic tanks.
5. The virus positive samples in groundwater were found in *E. coli* positive samples, the presence of which seemed to be an acceptable indicator to evaluate the occurrence of pathogenic virus in groundwater contaminated with floodwater in the study area.
6. Total coliforms and *E. coli* bacteria were positively correlated with all viruses tested. There was also significant correlation between some physicochemical parameters (conductivity, turbidity, temperature, TDS and ORP) with all viruses tested, total coliforms and *E. coli*.

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