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Prevalence of hepatitis A and E viruses in wastewater in Asian countries

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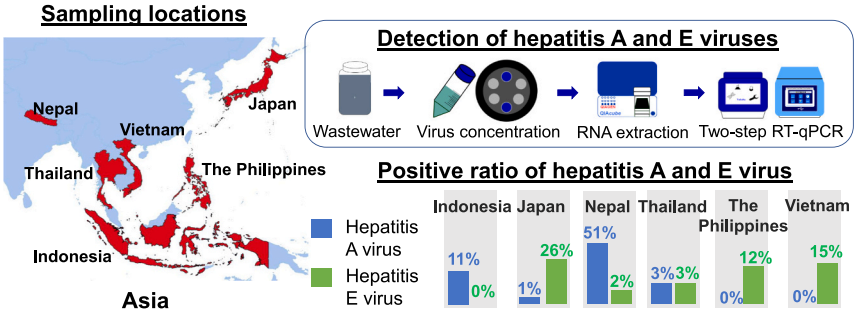
HIGHLIGHTS

- Hepatitis A and E viruses were monitored in six Asian countries using wastewater.
- The geographic variability of HAV and HEV among Asian countries was observed.
- Nepal exhibited the highest HAV prevalence (51 %), followed by Indonesia (11 %).
- Japan showed a high HEV prevalence (24 %), prompting the need for its subtyping.
- Continued monitoring and subtyping of HAV and HEV are recommended.

GRAPHICAL ABSTRACT



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ABSTRACT

Hepatitis A and E viruses (HAV and HEV, respectively) remain a significant global health concern despite advancements in healthcare and vaccination programs. Regular monitoring and vaccine efficacy of HAV are still lacking in different countries. This study aimed to investigate HAV and HEV prevalence in developed,

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developing, and least-developed Asian countries using wastewater as a surveillance tool. A total of 232 untreated wastewater samples were collected from six wastewater treatment plants, a sewage treatment plant, or an open drainage in six countries [Nepal (n = 51), Indonesia (n = 37), Thailand (n = 30), Vietnam (n = 27), the Philippines (n = 17), and Japan (n = 70)] between April and October 2022. Viruses in wastewater were concentrated by simple centrifugation or polyethylene glycol precipitation method, followed by viral RNA extraction and reverse transcription-quantitative polymerase chain reaction. HAV and HEV RNA were detected in the samples from Nepal (51 % for HAV and 2 % for HEV), Thailand (3 % for both viruses), and Japan (1 % for HAV and 24 % for HEV). Only HAV RNA was found in 11 % of the samples in Indonesia, whereas only HEV RNA was detected in Vietnam and the Philippines, with a positive ratio of 15 % and 12 %, respectively. These results highlighted the geographic variability in HAV and HEV prevalence, underscoring the need for localized public health strategies to address specific viral hepatitis challenges in each country.

1. Introduction

Hepatitis disease caused by hepatitis viruses is mainly characterized by the inflammation of the liver. Although viral hepatitis frequently results in jaundice, anorexia, and malaise, it can cause little or no symptoms (Gholizadeh et al., 2023). The five strains of hepatitis viruses (types A–E) cause viral disease in humans, but the disease severity and prevalence depend on the virus type and geographical distribution (Gholizadeh et al., 2023; Odenwald and Paul, 2022). Among the five strains, hepatitis B, C, and D viruses are transmitted via exposure to infected blood or bodily fluids, whereas hepatitis A and E viruses (HAV and HEV, respectively) are transmitted primarily by the fecal-oral route and are highly endemic in developing countries with poor sanitation (El-Mokhtar et al., 2023; Odenwald and Paul, 2022). HAV and HEV are the most common cause of acute hepatitis worldwide, and millions of HAV and HEV infection cases have been recorded (Guerra Veloz and Agarwal, 2023; Odenwald and Paul, 2022; Rau et al., 2024).

HAV causes mild and self-limiting disease with lifelong immunity after getting infected (Van Damme et al., 2023); however, it can result in hospitalization and, in rare instances, death in immune-compromised patients (Hernandez-Suarez et al., 2021; Hofmeister et al., 2023; Lemon et al., 2018). In the past few decades, HAV outbreaks were reported in many countries, including Thailand (2002−2003) (Poovorawan et al., 2005), India (2004), the Philippines and Vietnam (2011–2017) (Kwon et al., 2018), Egypt (2013–2014) (Hamza et al., 2017), Malaysia (2012) (Yusoff et al., 2015), Taiwan (2015–2017) (Chen et al., 2019), Indonesia (2015–2016) (Juniastuti et al., 2019), and the United States (2017–2018) (Snyder et al., 2019). In the current situation, due to immunization with human globulin for HAV and proper sanitation practices, the HAV trend has been greatly reduced in developed countries (Naoumov, 2007); however, HAV is still reported in developed and developing countries (Chatziprodromidou et al., 2022; Palewar et al., 2022). Genetically, HAV is a nonenveloped positive-sense RNA belonging to the member of the genus *Hepatovirus* in the *Picornaviridae* family (Wang et al., 2015). HAV is highly stable and resistant to diverse environmental and substrate conditions (De Paula et al., 2007; Sattar et al., 2000; Wang et al., 2015) and can survive on surfaces for months, depending on the temperature and relative humidity (Cook et al., 2018).

HAV endemicity is generally high in low- and middle-income countries, where children are typically exposed to the virus, unlike high-income countries with very low endemicity (Hernandez-Suarez et al., 2021; Jacobsen and Koopman, 2005). However, recent urbanization and increasing income in low- and middle-income countries have improved access to clean water, leading to a shift from high to low HAV endemicity as childhood exposure decreases (Jacobsen and Koopman, 2005). Paradoxically, this increases the risk of HAV outbreaks (Hernandez-Suarez et al., 2021; Jacobsen and Koopman, 2005; Kyrka et al., 2009). In less developed countries with highly endemic HAV infection, infection occurs early when the disease is mostly asymptomatic. However, in developed countries with proper sanitation, children avoid infection during the early stage, and transmission occurs in older age groups, leading to higher rates of severe disease and death in adults, particularly in the unvaccinated elderly population (Franco, 2012; Gurav et al., 2022; Posuwan et al., 2019).

The World Health Organization estimated that ~20 million new HEV infections arise annually, although this figure could be underestimated due to the absence of widespread screening programs to enhance surveillance efforts (Rau et al., 2024). Sporadic HEV cases have been reported in most developed countries, including Japan, the United States, and Europe (Sato et al., 2011; Yazaki et al., 2003). HEV is a non-enveloped RNA virus and is the sole member of the genus *Hepevirus* in the family *Hepeviridae*, with eight known genotypes (Takahashi et al., 2007). Swine HEV was first isolated and genetically characterized from pigs in 1997, and avian HEV was identified in chickens with hepatitis-splenomegaly syndrome in 2001 (Haqshenas et al., 2001; Meng et al., 1997). Genotypes 1 to 4 are the most studied genotypes of HEV, which are transmitted via the fecal-oral route (genotypes 1 and 2) or zoonotic transmission by ingestion of raw or undercooked meat (genotypes 3 and 4) (Odenwald and Paul, 2022). HEV strains have also been genetically identified in wild boars, deer, mongooses, rabbits, and rats, expanding their host range and genomic diversity (Meng, 2010; Sato et al., 2011). Sporadic hepatitis cases, likely of zoonotic origin and not related to travel to endemic areas, have been increasingly recognized in many industrialized countries, including the United States, European countries, and Japan (Sato et al., 2011). The infection caused by HEV is usually acute and self-limiting, yet it can escalate to severe levels in pregnant individuals and those with compromised immune systems (Odenwald and Paul, 2022).

Wastewater surveillance has been extensively documented as a valuable tool for the early detection of human pathogens and emphasizes the critical role of effective sanitation in preventing waterborne outbreaks, aligning with the Sustainable Development Goals of the United Nations. The presence of HAV and HEV RNA in wastewater reported in previous studies indicates shedding from infected individuals in the community (Botes et al., 2013; Cancela et al., 2023; Ouardani et al., 2016), highlighting the applicability of wastewater for HAV and HEV surveillance in the community level (Adefisoye et al., 2016; McCall et al., 2021; Palombieri et al., 2023). However, most studies focused on countries during the outbreak and/or based on the sentinelled clinical surveillance; thus, the actual situation of HAV and HEV at the community level remains unknown in recent years. This study addresses significant global health concerns posed by HAV and HEV. Although advances have been made in water, sanitation, and hygiene (WASH) programs, healthcare, and vaccination efforts, numerous waterborne outbreaks of HAV and HEV continue to occur in different countries. Many cases of these diseases often go unreported due to limitations in current clinical detection methods, highlighting the need for alternative monitoring strategies. Although the vaccine is available, it is not mandatory and is not included in the national immunization programs of most countries (World Health Organization, 2023). This lack of universal vaccination coverage makes it challenging to identify and protect at-risk populations in different regions. Without a standardized nationwide approach to vaccination, there are significant gaps in coverage, which can lead to varying levels of susceptibility and potentially higher rates of disease transmission within unvaccinated groups. This study

aims to fill this gap by investigating the prevalence of HAV and HEV in developed, developing, and least-developed Asian countries using wastewater surveillance so that the concerned authority can focus on high-risk countries and endemic areas to reduce the burden of HAV and HEV worldwide.

2. Materials and methods

2.1. Water samples

A total of 232 grab samples were collected from inlets of six wastewater treatment plants (WWTPs), an inlet of a sewage treatment plant (STP), or an open drainage in six countries [Nepal (WWTP A, $n = 25$; WWTP B, $n = 26$), Indonesia (WWTP C, $n = 17$; WWTP D, $n = 20$), Thailand (WWTP E, $n = 30$), Vietnam (open drainage, $n = 27$), the Philippines (STP, n = 17), and Japan (WWTP F, $n = 70$)] between April and October 2022. Among these samples, 51 samples collected from Nepal, 74 samples collected from Indonesia, Thailand, and Japan, and 17 samples collected from the Philippines were previously tested for the presence of respiratory viruses (Raya et al., 2024a), hand, foot, and mouth disease-causing enteroviruses (Shrestha et al., 2024), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Inson et al., 2024). The average flow of wastewater in WWTPs A, B, C, D, E, and F was 32,400, 7,000, 243,000, 28.8, 350,000, and 97,000 m^3/day, respectively. In Vietnam and the Philippines, wastewater samples from an open drainage and an STP, respectively, were collected in an autoclaved bottle and transferred to the laboratory on ice packs. The samples were stored at −20 °C until further processed.

2.2. Virus concentration

The water samples from WWTPs A to E, STP, and open drainage were concentrated by simple centrifugation method as described previously (Inson et al., 2024; Raya et al., 2024a; Shrestha et al., 2024), whereas those from WWTP F were concentrated by polyethylene glycol (PEG) precipitation method (Shrestha et al., 2023; Torii et al., 2021). In the simple centrifugation method, 40 to 45 mL wastewater sample was centrifuged at 4000 to 5000 ×*g* for 10 min at room temperature. The supernatant was discarded, and the pellet was recovered as a viral concentrate using polymerase chain reaction (PCR)-grade water (Sigma-Aldrich, St. Louis, MO, USA) or autoclaved MilliQ water (Merck, Rahway, NJ, USA). The final volume of the concentrate was recorded. An equal volume of DNA/RNA Shield (Zymo Research, Irvine, CA, USA) was added to the final concentrate of the samples from Nepal and the Philippines. For the PEG concentrate method, 4.0 g PEG 8000 (Sigma-Aldrich) and 0.94 g NaCl (Kanto Chemical, Tokyo, Japan) were added to 40 mL wastewater and continuously vortexed for 10 min. The mixture was centrifuged at 12,000 ×*g* for 99 min at 4 °C. The supernatant was discarded, and the pellet was recovered as a viral concentrate using 800 μL PCR-grade water. The concentrated samples were stored at −20 °C until processed.

2.3. Sample process control and RNA extraction

One microliter each of 20× diluted mixtures of coliphage MS2 [ATCC 15597-B1; ~10^{11} plaque-forming units (PFU)/mL; American Type Culture Collection, Manassas, VA, USA] and *Pseudomonas* phage Φ6 (NBRC 105899; ~10^{10} PFU/mL; National Institute of Technology and Evaluation, Tokyo, Japan) was added to 140 μL concentrated sample as a process control (Haramoto et al., 2018) and extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) to obtain 60 μL viral RNA, according to the manufacturer's instructions.

2.4. Reverse transcription-quantitative PCR (RT-qPCR)

RT was performed using a High-Capacity cDNA RT Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's guidelines. Briefly, 20.0 μL viral RNA with 4.0 μL RT buffer, 1.6 μL deoxynucleotide triphosphate, 4.0 μL RT random primers, 2.0 μL MultiScribe reverse transcriptase, 2.0 μL RNase inhibitor, and 6.4 μL PCR-grade water were used to obtain 40.0 μL cDNA. The thermal conditions were 25 °C for 10 min, followed by 37 °C for 120 min and 85 °C for 5 min.

The obtained cDNA was amplified in the Thermal Cycler Dice Real Time System III (Takara Bio, Kusatsu, Japan) to detect coliphage MS2, HAV, and HEV. Briefly, 2.5 μL cDNA was mixed with a 22.5 μL qPCR mixture containing 12.5 μL Probe qPCR Mix with UNG (Takara Bio), 0.1 μL each of forward and reverse primers (100 μM), 0.05 μL probe (100 μM), and 9.75 μL PCR-grade water. The sequences of the primers and probes are shown in Table 1 (Chou and Williams-Hill, 2018; Jothikumar et al., 2006). The thermal conditions were 25 °C for 10 min, followed by 95 °C for 30 s, and 45 cycles of 95 °C for 5 s and 60 °C for 30 s.

Pepper mild mottle virus (PMMoV) and Φ6 were also detected using the SARS-CoV-2 Detection RT-qPCR Kit for Wastewater (Takara Bio), in which reagents for one-step duplex RT-qPCR for PMMoV and Φ6 were included as previously mentioned by Raya et al. (2024a, 2024b). In brief, 5.0 μL viral RNA extract was mixed with 20.0 μL RT-qPCR mixture containing 12.5 μL one-step RT-qPCR mix, 2.5 μL of the mixture of primers and probes [Primer/Probe (PMMoV&Φ6)], and 5.0 μL RNase-free water. The thermal conditions were 25 °C for 10 min, 52 °C for 5 min, 95 °C for 10 s, and 45 cycles of 95 °C for 5 s and 60 °C for 30 s.

Six 10-fold serial dilutions (5.0×10^0–5.0×10^5 copies/reaction) of gBlocks Gene Fragments (Integrated DNA Technologies, Coralville, LA, USA; for HAV and HEV), artificially synthesized plasmid DNA (Takara Bio; for MS2), and Positive Control DNA (PMMoV/Φ6) included in the SARS-CoV-2 Detection RT-qPCR Kit for Wastewater (for PMMoV and Φ6) were used to obtain a standard curve. Negative control was included in each run to ensure no contamination. All samples, including positive and negative controls, were tested in duplicate. A threshold cycle (Ct) value of 40 was considered a cutoff point. Only two-well positive samples were used to calculate the copy number. One-well positive samples were considered positive but were not included in the calculation of the copy number and were considered below the limit of quantification (2.6 copies/reaction; 4.6 $\log_{10}$ copies/L). Negative samples were assigned the value of the limit of detection (1 copy/reaction; 4.2 $\log_{10}$ copies/L).

2.5. Extraction and RT-qPCR efficiency

PMMoV RNA was evaluated as an indicator of fecal contamination in this study. Extraction-RT-qPCR efficiency was determined by calculating the RNA concentration of recovered MS2. Although Φ6 was also tested as a molecular process control, recovery data were not considered in this

Table 1
Oligonucleotide sequences of primers and probes used for HAV and HEV detection.

Target	Function	Sequence (5′-3′)	Product length (bp)	Reference
HAV	Forward primer	GTAACAGCGGCGGATATTGG	113	Chou and Williams-Hill, 2018
	Reverse primer	CCTAGAGACAGCCCTGACA		
	Probe	CAACGCCGG/ZEN/ AGGACTGGCTCTCATCC-IBFQ		
HEV	Forward primer	GGTGGTTTCTGGGGTGAC	70	Jothikumar et al., 2006
	Reverse primer	AGGGGTTGGTTGGATGAA		
	Probe	FAM-TGATTCTCA/ZEN/ GCCCTTCGC-IBFQ		

FAM, 6-carboxyfluorescein; IBFQ, Iowa Black fluorescent quencher; ZEN, ZEN internal quencher.

study, as it was added as a control for enveloped viruses. The extraction-RT-qPCR efficiency was 49 ± 40 % ($n = 120$), ensuring no substantial viral loss and/or inhibition in the samples during RNA extraction and RT-qPCR. Since the samples were concentrated and stored at −20 °C until processing, viruses in some samples may have been degraded during storage, which could have affected the accuracy of data. A strong linear relationship was observed between the Ct value and the $\log_{10}$ concentration of the gBlocks gene fragments for HAV ($r = -0.993$) and HEV ($r = -0.997$), suggesting that qPCR assay is precise and reliable in quantifying HAV and HEV. Amplification efficiency was 98.0 % and 102.0 % for HAV and HEV, respectively.

2.6. Statistical analysis

Mean and standard deviation (SD) were calculated using Microsoft Excel 2019 (Microsoft Corp., Redmond, WA, USA) for only two-well positive samples. Statistical analysis was performed using R version 4.3.1 (R Core Team, 2020). A paired *t*-test was performed to assess whether there were significant differences in the PMMoV concentration across the sampling locations. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Detection of PMMoV RNA in water samples

Fig. 1 shows the PMMoV RNA concentrations in the water samples across Asian countries. Among the 232 samples, PMMoV RNA was detected in 230 samples (99 %). Two samples from WWTP C in Indonesia were negative for PMMoV RNA, which were excluded from the study. This exclusion was done to ensure the accuracy and reliability of the results, as the absence of PMMoV RNA in these samples could indicate potential issues with sampling and/or sample quality. All sampling locations showed high and stable PMMoV RNA concentrations, suggesting the applicability of these samples for wastewater-based epidemiology. However, a significant difference in PMMoV RNA concentrations was observed between the sampling locations ($p < 0.05$), except between Indonesia and Thailand ($p > 0.05$). The PMMoV RNA concentration was highest in Japan (8.6 ± 0.4 $\log_{10}$ copies/L) and showed the lowest variability among the tested countries, suggesting that the PMMoV RNA concentration was consistently high in Japan. In contrast, Thailand and Indonesia had comparatively lower PMMoV RNA concentrations, with 6.2 ± 0.4 and 6.3 ± 0.5 $\log_{10}$ copies/L, respectively.

3.2. Detection of HAV RNA in water samples

Table 2 shows the positive ratio of HAV and HEV RNA in the water samples. HAV RNA was detected in the samples of WWTPs from Nepal (51 %), Indonesia (11 %), Thailand (3 %), and Japan (1 %). However, it was not detected in any of the samples from Vietnam and the Philippines. The detection ratio was higher in Nepal compared to other countries. HAV RNA was detected in 72 % (18/25) and 31 % (8/26) of the samples from WWTPs A and B in Nepal, respectively. It was detected in only one sample from Thailand and Japan. Similarly, it was detected only in 13 % (2/15) and 10 % (2/20) of the samples from WWTPs C and D, respectively, in Indonesia, but it was in the quantifiable ratio in only WWTP C, indicating a higher HAV prevalence in Nepal compared to Indonesia, Thailand, and Japan and no or little prevalence in Vietnam and the Philippines.

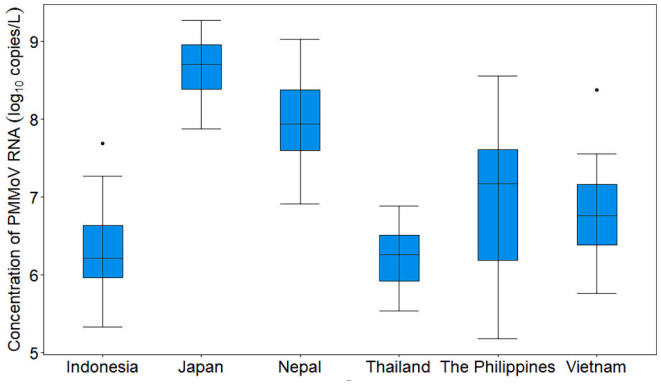

Fig. 1. PMMoV concentrations in water samples across sampling locations in Asia.

Fig. 2 shows the HAV RNA concentrations in two WWTPs in Nepal. HAV RNA was more frequently detected in WWTP B than WWTP A, with 6.5 ± 0.8 and 5.5 ± 0.6 $\log_{10}$ copies/L, respectively. HAV RNA was detected in all samples tested during the initial month of sampling in WWTP A. Then, it was detected in only one sample from June to October 2022. Alternately, HAV RNA was detected throughout the study period in WWTP B.

3.3. Detection of HEV RNA in water samples

HEV RNA was not detected in any of the samples from Indonesia, indicating low HEV prevalence in Indonesia, whereas it was detected in 2 % (1/51) of the samples from Nepal, with only one-well testing positive. Similar to Nepal, HEV RNA was detected in 3 % (1/30), 15 % (4/27), and 12 % (2/17) of the samples from Thailand, Vietnam, and the Philippines, respectively, with only one-well testing positive. In Japan, HEV RNA was detected in 24 % (17/70) of the samples. In total, 2 % (4/230) of the tested samples had two wells positive, with concentrations of 4.2 ± 0.6 $\log_{10}$ copies/L, and 9 % (21/230) had one-well positive, indicating a low HEV prevalence in Nepal and Thailand and notable presence of HEV RNA in wastewater in Vietnam, the Philippines, and Japan.

4. Discussion

This study described a large-scale environmental surveillance of HAV and HEV in developed, developing, and least-developed Asian countries. This study reported the presence of HAV and/or HEV RNA in wastewater collected from Nepal, Indonesia, Thailand, Vietnam, the Philippines, and Japan. HAV and/or HEV RNA in wastewater indicated the circulation of these viruses at the community level in the study regions. In contrast, the absence of HAV RNA in wastewater collected from Vietnam and the Philippines and the absence of HEV in Indonesia indicated a very low HAV/HEV prevalence at the community level in these study areas. However, a low concentration of a pathogen in wastewater does not necessarily indicate a low virus prevalence; it could result from low shedding rates by infected individuals or infrequent detection in wastewater samples (Tiwari et al., 2024). Given that the detection ratio of HAV/HEV in wastewater can be as low as 23 % (Tiwari et al., 2024), it is possible that the prevalence of these viruses might have been underestimated in this study. The water samples were collected from six WWTPs in Nepal, Indonesia, Thailand, and Japan. In Vietnam and the Philippines, where only a limited population has access to sewage systems (Inson et al., 2024), the samples were collected from open drainage systems and STP to better represent the local population. The PMMoV prevalence as a fecal indicator is well established due to its consistently higher presence throughout the year than pathogenic bacteria without substantial seasonal fluctuations (Kitajima et al., 2018). PMMoV was detected in high concentrations in all sampling locations, consistent with previous studies (Kuroda et al., 2015; Raya et al., 2024a; Tandukar et al., 2022). However, a significant difference was observed in PMMoV concentrations between the sampling locations across Asia. The difference in the variability in PMMoV concentrations among the sampling locations might be attributed to various physiochemical parameters and

Table 2
Positive ratio of HAV and HEV RNA in the water samples from Asian countries.

Country	Sampling points	No. tested samples	HAV RNA			HEV RNA		
			No. samples (%)		Concentration (mean ± SD) (log_{10} copies/L)	No. samples (%)		Concentration (mean ± SD) (log_{10} copies/L)
			Two-well positive	One-well positive		Two-well positive	One-well positive	
Nepal	WWTP A	25	11 (44 %)	7 (28 %)	5.5 ± 0.6	0	1 (4 %)	NA
	WWTP B	26	7 (27 %)	1 (4 %)	6.5 ± 0.8	0	0	NA
Indonesia	WWTP C	15	1 (7 %)	1 (7 %)	5.6	0	0	NA
	WWTP D	20	0	2 (10 %)	NA	0	0	NA
Thailand	WWTP E	30	0	1 (3 %)	NA	0	1 (3 %)	NA
Vietnam	Open drainage	27	0	0	NA	0	4 (15 %)	NA
The Philippines	STP	17	0	0	NA	0	2 (12 %)	NA
Japan	WWTP F	70	0	1 (1 %)	NA	4 (6%)	13 (19 %)	4.2 ± 0.6
Total		230	19 (8 %)	13 (6 %)	5.8 ± 0.8	4 (2 %)	21 (9 %)	4.2 ± 0.6

NA, not applicable.

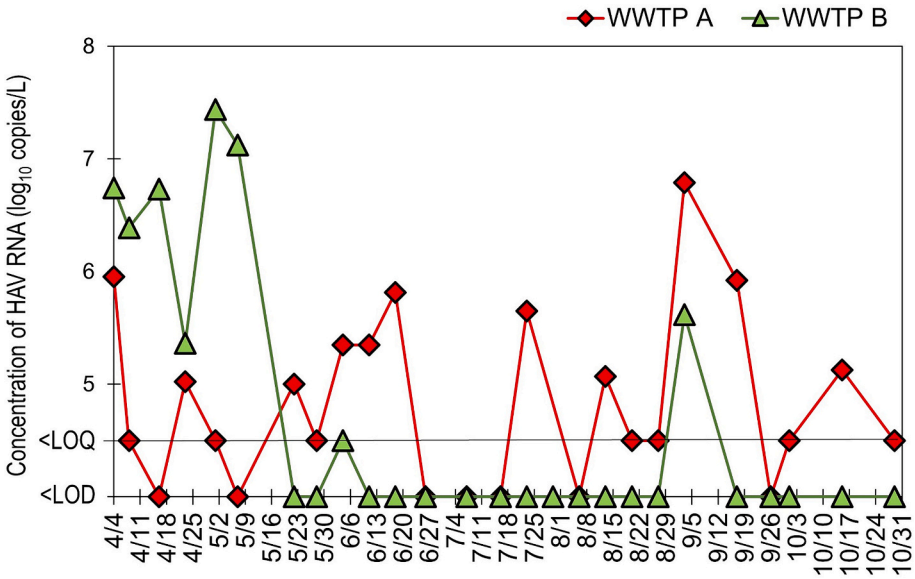

Fig. 2. HAV RNA concentrations in WWTP samples in Nepal.

environmental conditions across the sampling locations (Goitom et al., 2024). The overall lower levels of PMMoV RNA detected in Indonesia might be consistent with dietary or environmental factors. For example, the local population might consume fewer PMMoV-containing foods and/or have different waste management practices, which could affect the concentrations of PMMoV in wastewater. For Thailand, where one might expect higher levels of PMMoV based on dietary habits involving PMMoV-containing foods, the observed data might still be lower than anticipated due to several factors. One possible reason could be food preparation and handling practices in Thailand, which might reduce the presence of PMMoV RNA in wastewater. Thorough cooking or other food safety measures could decrease the concentration of PMMoV RNA that ultimately enters the wastewater system. Regional and cultural differences within Thailand might also have contributed to variability in PMMoV RNA levels. Differences in local diets and food consumption patterns could have resulted in varying levels of PMMoV in wastewater. This variability is further complicated by the fact that the quantification of multiple pathogens in wastewater is complex due to differences in their shedding rates, persistence, and decay (Tiwari et al., 2024). Additionally, the lack of standardized methods for wastewater sample concentration, nucleic acid extraction, purification, and enumeration can lead to inconsistent results. Variability in sample representativeness, transportation conditions, and pathogen stability further complicates the accuracy of the data (Tiwari et al., 2024). Despite these variations, the presence of PMMoV underscores the suitability of these samples for wastewater-based epidemiology.

Despite the introduction of vaccination, HAV outbreaks were reported in many countries (Hamza et al., 2017; Juniastuti et al., 2019; Snyder et al., 2019). The geographical distribution of HAV is linked to hygiene, sanitary conditions, and socioeconomic development, as highlighted in previous studies (Cao et al., 2021; Franco, 2012). In developing countries, higher incidence rates are closely related to lower socioeconomic indicators and limited access to safe drinking water. Conversely, in developed countries, HAV incidence decreases as incomes rise and access to clean water improves (Franco, 2012). A similar trend was observed in this study, providing valuable insights into HAV prevalence across multiple countries. In Nepal, a high HAV RNA detection ratio (51 %) was observed, indicating significant infection or exposure among the population in the study area. Considering that HAV can survive on surfaces for several days (Sattar et al., 2000) with longer incubation period in infected individuals (Feinstone, 2019) and the viruses are shed not only in feces but also in saliva (Shin and Jeong, 2018), it is likely that HAV can remain in high concentration in wastewater for an extended period. Research on HAV in Nepal has been limited, highlighting the need for further investigation into the shedding patterns of HAV and its impact on wastewater. However, high detection of HAV in wastewater is alarming and warrant immediate attention. A comprehensive serological survey from 1987 to 1996 revealed that 99.3 % of

the tested individuals had anti-HAV antibodies (Sawayama et al., 1999). In a similar study, the seroprevalence of anti-HAV antibodies was found to be >90 % among school children in India (Acharya et al., 2003). Recently, clinical surveys and case reports have documented HAV cases and associated complications among symptomatic patients (Pokhrel et al., 2020; Shrestha, 2006; Shrestha, 2016). However, there is a lack of surveillance data to accurately assess the prevalence and situation of HAV in Nepal. The HAV prevalence throughout the study period suggests substantial deficiencies in sanitation, hygiene, and access to safe drinking water, aligning with patterns observed in other developing countries where socioeconomic indicators are low (Franco, 2012), demanding targeted interventions against HAV in Nepal.

Indonesia exhibited an HAV RNA detection ratio of 11 %, indicating a low but still notable presence of the virus at the community level. This prevalence reflects ongoing public health challenges, although less severe than in Nepal. Although good sanitation, personal hygiene, and widespread HAV vaccination helped to reduce transmission of HAV infection, specific regions in Indonesia continue to face challenges due to poor sanitation and inadequate personal hygiene practices (Martini and Suryadi Rahman, 2022). The potential impact of HAV is significant in Indonesia, as HAV can persist for several hours on the hands of infected individuals with poor hygiene (Cook et al., 2018). A single HAV-infected food handler with poor hygiene can transmit HAV to dozens or even hundreds of people (Fiore, 2004), leading to widespread outbreaks in communities and schools. There is still a lack of routine hepatitis surveillance and low confidence in epidemiological data, underscoring the necessity for nationwide surveillance to obtain an accurate assessment (Hernandez-Suarez et al., 2021). In this study, the HAV prevalence in Indonesia also highlighted the need for continuous monitoring and public health interventions to mitigate HAV transmission, similar to trends observed in other countries.

In Thailand and Japan, the detection ratio for HAV RNA was very low. The HAV incidence in developed countries is mostly attributed to travelers and immigrants (Campagna et al., 2012). The latest updated information on HAV in Japan was reported in 2019 based on clinical surveillance with 425 cases. About 16 % of the cases were reported from overseas travelers, mainly those who visited South Korea, Pakistan, and India (Kiyohara et al., 2023). Recent updates have reported HAV outbreaks linked to frozen fruits, particularly berries and pomegranate arils, with most outbreaks occurring in industrialized countries (Nasheri et al., 2019). Interestingly, no HAV RNA was detected in the samples from Vietnam and the Philippines. Since HAV vaccination was not included in the national immunization program in both countries (Gloriani et al., 2024), the absence of detection could be attributed to successful public health interventions and improved sanitation. However, it may also reflect limitations in detection methods or sample sizes, suggesting the need for further research to confirm these findings and understand the dynamics of HAV transmission in these countries.

Unlike HAV, HEV is a zoonotic foodborne transmission due to ingestion of infected animal meat, especially in developed countries; however, transmission by the fecal-oral route, solid-organ transplantation, and blood transfusion routes have also been described (Mendoza et al., 2022). HEV has been linked to large waterborne epidemics and smaller outbreaks in developing regions. However, over the past decade, there has been an increase in sporadic, locally acquired cases in high-income countries, where determining the exact source of infection can be challenging (Treagus et al., 2021). This study reported the presence of HEV RNA in the samples from Japan. HEV RNA was detected at a relatively high ratio in Japan compared to other countries, suggesting that HEV is a more significant issue in Japan, with a much higher prevalence compared to HAV. In developed and non-endemic countries, HEV infections were previously linked mainly to travelers returning from endemic regions. However, recent evidence shows that sporadic, locally acquired cases of genotype 3 and 4 HEV now occur among individuals with no travel history to endemic areas (Mirazo et al., 2014). Over the past decade, reported autochthonous cases of these genotypes have surged, highlighting the role of animal reservoirs and zoonotic transmission. A national survey in Japan detected HEV RNA or anti-HEV-IgG prevalence in wild boars across different prefectures (Sato et al., 2011). Hunting is popular in Japan, which could facilitate the virus spread within the community. Foodborne transmission of HEV is associated with ingestion of contaminated animal meat and derivatives (Di Cola et al., 2021). HEV subtyping is crucial to identify transmission routes, a detail this study lacks.

In Vietnam, only HEV RNA was detected, with a prevalence ratio of 15 %, indicating that HEV is present in the population, and there were no detected HAV cases in the provided data, highlighting HEV as the primary concern. Similar to Vietnam, only HEV RNA was detected in the Philippines, suggesting that HEV is a notable concern, whereas HAV does not appear in the data, indicating a lack of detected HAV cases. The detection ratios for HAV and HEV RNA in Thailand were 3 % each, showing that both viruses are present at low levels within the population. Although HEV outbreaks have been reported in Indonesia in past years, no HEV RNA was detected throughout the study period, indicating a low HEV prevalence within the community.

In Nepal, unlike HAV, the HEV RNA detection ratio was much lower (2 %), suggesting that HEV is less prevalent in Nepal compared to HAV. The periodicity and fluctuation of HEV cases have been reported in the past, with five major outbreaks in 1973, 1981 to 1982, 1987, 2006, and 2007 in Nepal (Raji et al., 2021; Shrestha, 2006; Shrestha, 2016). A hospital-based study of hepatitis viruses in the Kathmandu Valley in 2014 reported HEV as the major cause of acute hepatitis, followed by HAV, in patients with the symptoms of jaundice (Gupta et al., 2018). In the same year, an HEV outbreak was reported in the eastern part of Nepal, with 14 deaths. The epidemic was assumed to be caused by contaminated drinking water, as water and sewage pipelines were damaged during road construction and repair (Shrestha et al., 2015). Genomic analysis of the HEV outbreak in Kathmandu and neighboring countries identified various HEV subtypes exhibiting increased pathogenicity and in vitro resistance to ribavirin (Shrestha et al., 2015; Shrestha, 2016), underscoring the importance of ongoing HEV surveillance despite low clinical case numbers, due to the potential of asymptomatic carriers as dynamic reservoirs and their possible role in HEV mutation leading to epidemics.

This study showed the presence of HAV at high concentrations in a less developed country, Nepal, whereas HEV was detected at high concentrations in a developed country, Japan. However, it was hard to compare the results of each country, as wastewater samples were collected from different conditions, and different protocols were used for sample processing. Viruses present in the sample were concentrated by two methods: simple centrifugation and PEG precipitation. Although the study found comparable results in SARS-CoV-2 detection between these methods (Raya et al., 2024a), the partition coefficient of respiratory viruses and hepatitis viruses differs (Roldan-Hernandez et al., 2024). This variation in partition behavior affects how these viruses are distributed between the liquid and solid phases of wastewater, influencing the efficiency of each concentration method (Roldan-Hernandez et al., 2024). As a result, the effectiveness of centrifugation and PEG precipitation may vary for different viruses, highlighting the need for further research to optimize detection methods for hepatitis viruses based on their unique partition characteristics. A DNA/RNA Shield was added to only several samples as pretreatment to minimize DNA or RNA degradation during the transportation time. The study was done between April and October 2022, which was generally considered to be wet season for most of the study countries except for Indonesia. HAV and HEV often exhibit peak prevalence during the summer and spring seasons (Fares, 2015), which can influence detection and surveillance efforts. Overall, data from this study underscored the need for tailored public health strategies to address the varying levels of HAV prevalence. In countries such as Nepal, improving sanitation, access to safe drinking water, and implementing widespread vaccination programs are critical. For countries with moderate prevalence, such as Indonesia, maintaining

and enhancing existing public health measures are essential. In regions with low detection ratios, continuous surveillance and targeted interventions are necessary to sustain and improve public health outcomes. These efforts are vital for the global objective of controlling and ultimately eliminating viral hepatitis. It is important to note that this study did not include clinical data to corroborate the environmental findings, no clinical data were available for comparison. Future research should integrate clinical case data to better understand the correlation between wastewater detections and actual infection rates. Additionally, expanding surveillance to include more regions and incorporating data on seasonal and demographic factors could provide a more comprehensive picture of virus prevalence. Future studies to enhance wastewater monitoring techniques, improve reporting systems, and implement targeted public health interventions based on wastewater data to effectively manage and mitigate the spread of HAV and HEV are recommended.

5. Conclusions

Despite the global goal of eliminating viral hepatitis by 2030, monitoring efforts for HAV and HEV are currently inadequate. HAV RNA was detected in WWTPs from Nepal, Indonesia, Thailand, and Japan, whereas HEV RNA was detected in Japan, Vietnam, the Philippines, Thailand, and Nepal. The presence of HAV in wastewater from Nepal, Indonesia, Thailand, and Japan, as opposed to its absence in Vietnam and the Philippines, emphasizes the role of sanitation, vaccination efforts, and public health interventions. In developed countries like Japan, where sanitation and public health intervention are generally robust, HAV detection may be linked to the presence of migrants from endemic countries or foodborne transmission from frozen fruits imported from endemic countries. HAV RNA was detected at a high ratio in wastewater in Nepal compared to Indonesia, Thailand, and Japan, which might indicate fecal-oral transmission due to poor sanitation practices, suggesting the higher HAV prevalence in the community. Although developed countries have low endemicity, they face contamination risks from migration from high-endemic regions. Therefore, efforts should also focus on eradicating HAV in high-endemic countries. Conversely, the high HEV prevalence in Japan compared to other countries underscores the need for continuous surveillance and genetic characterization of the virus. This is crucial for better understanding the transmission routes and implementing effective remedial measures. Understanding these differences is important to design targeted strategies to mitigate the risk of waterborne transmission and enhance overall public health in these diverse regions.

CRediT authorship contribution statement

Sunayana Raya: Writing – original draft, Investigation, Formal analysis. **Sarmila Tandukar:** Resources. **Hari Prasad Kattel:** Resources. **Sangita Sharma:** Resources. **Jatuwat Sangsanont:** Resources. **Kwanrawee Sirikanchana:** Writing – review & editing, Resources. **Huong Thi Thuy Ngo:** Resources. **Jessamine Gail M. Inson:** Resources. **Ma. Luisa D. Enriquez:** Resources. **Zeba F. Alam:** Resources. **Ahmad Soleh Setiyawan:** Resources. **Tjandra Setiadi:** Resources, Funding acquisition. **Eiji Haramoto:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

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

Data will be made available on request.

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