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Original Article

Detection and Characterization of Enteric Viruses in Flood Water from the 2011 Thai Flood

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SUMMARY: Severe flooding, which is associated with numerous outbreaks of a wide range of infectious diseases, particularly those caused by enteric viruses, occurred in all areas of Thailand in 2011. To determine the prevalence of five human enteric viruses, namely enterovirus, rotavirus (RV), norovirus (NV), hepatitis A virus (HAV), and hepatitis E virus, in the flood water, 100 water samples were collected from flood-damaged areas in central Thailand. Viral RNA was extracted from concentrated samples and analyzed by RT-PCR and sequencing. NV was the most commonly detected pathogen in the tested samples (14%). RV and HAV were detected in 9% and 7% of samples, respectively. This study is the first to detect enteric viral genes in flood water in Thailand. Furthermore, it is the first to detect an NV gene in any type of environmental water in Thailand. These results provide useful information for estimating the risk of flood waterborne viral infection.

INTRODUCTION

Severe floods occurred during the 2011 monsoon season in Thailand. These floods began in July 2011 and devastated large parts of central Thailand. Flood water reached the capital city of Bangkok in October. The floods persisted in some areas until mid-January 2012 and resulted in 815 deaths. A total of 13.6 million individuals were affected.

Floods are associated with numerous outbreaks of a wide range of infectious diseases (1). The pattern of prevalence of waterborne diseases such as typhoid fever, cholera, leptospirosis, diarrheal diseases, and hepatitis appears to have changed after the flood (2–4). The prevalence of not only waterborne diseases but also vectorborne diseases such as malaria, West Nile fever, and dengue fever has increased after the flood (5,6).

The incidence of gastrointestinal symptoms increased both during and after the flood (7). In Thailand, during 2007–2009, 1.9 acute diarrheal cases per 100,000 residents/province/week were reported. According to the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand (http://www.boe.moph.go.th/urgent.php?cat=1), this number increased to 27.3 acute diarrheal cases per 100,000 residents/province/week in areas affected by the flood in central Thailand during the 2011 flooding season. Direct contact with flood water was found to increase the relative risk of gastrointestinal symptoms (7) and enteric hepatitis A virus (HAV) and hepatitis E virus (HEV) infections (8–10). Moreover, an outbreak of gastrointestinal illness related to norovirus (NV) was reported during the flood (7). Other enteric viruses, including rotavirus (RV) and enterovirus (EV), also cause gastroenteritis and can be transmitted through flood waters (11). These viruses are primarily transmitted by the fecal–oral route, by person to person (or animal) contact, or by the ingestion of contaminated water (12).

The aim of the present study was to assess the risk of waterborne viral disease transmission during the 2011 Thai Flood. The possible presence of enteric viral genomic sequences (HAV, HEV, EV, RV, and NV) in flood water samples collected in central areas of Thailand was investigated.

MATERIALS AND METHODS

Sample collection: From November to December 2011, 100 flood water samples were collected in Ayutthaya, Bangkok, Nakhon Pathom, and Nonthaburi provinces of central Thailand (Fig. 1). Most of the water samples (70 samples) were collected from the Salaya area of Nakhon Pathom province. The other 30 samples were collected from Ayutthaya, Bangkok, Nakhon Pathom (Nakhon Chai Si area), and Nonthaburi provinces. All the water samples were transported to the laboratory on ice and stored at −80°C until use.

Virus concentration and RNA extraction: All the flood water samples were concentrated from 400 ml to 1 ml using polyethylene glycol (PEG). In brief, the water

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samples were sonicated for 15 min at 4°C and then centrifuged at 1,000 × *g* for 10 min. The supernatants were collected, and PEG (PEG 6000; Sigma, St. Louis, Mo., USA) and NaCl were added to a final concentration of 10% (w/w) and 1 M, respectively, followed by gentle stirring at 4°C overnight. After centrifugation at 10,000 × *g* for 60 min, the pellet was resuspended in 1 ml phosphate-buffered saline (PBS) (−) and sonicated for 15 min at 4°C. After centrifugation at 10,000 × *g* for 10 min at 4°C, RNA was extracted from the supernatant (200 μl) using an RNA extraction kit (PureLink™, Viral RNA/DNA mini kit; Invitrogen, Carlsbad, Calif., USA) according to the manufacturer's protocol.

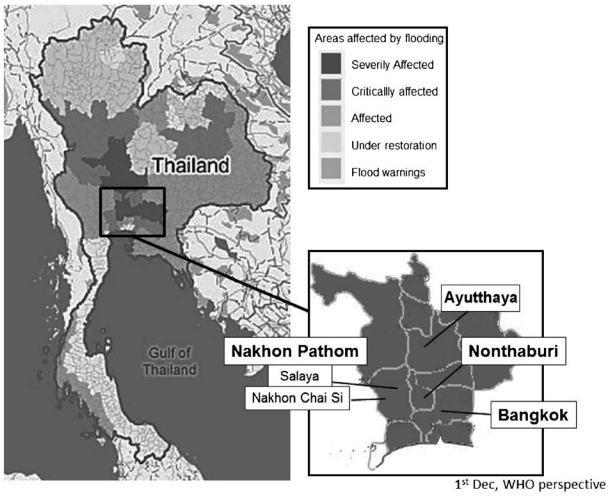

Fig. 1. Geographical distribution of the sampling sites in central Thailand. The areas affected by flooding are highlighted (gray).

Detection of viral RNA: Reverse transcription (RT)-PCR was used to detect viral RNA in the flood water samples using a SuperScript III One-Step RT-PCR with Platinum *Taq* kit (Invitrogen). The primer sequences and references (13–16) are listed in Table 1. One-step RT-PCR was performed for the detection of HEV, HAV, and EV, whereas nested RT-PCR was performed for the detection of NV and RV. Previously reported RT-PCR conditions were followed (13–16). Nested PCR conditions used for NV and RV are the same as those used for first-step RT-PCR, without the reverse transcription step. All PCR products were analyzed by 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized under ultraviolet (UV) light. A positive control for each virus was amplified in parallel with the samples. The standard positive sample for EV was kindly provided by Prof. Pilaipan Puthavathana; HAV and RV were provided by Assoc. Prof. Suda Louisirirotchanakul, Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; HEV was provided by Dr. Khin Saw Aye Myint; and NV (genotype I [GI] and genotype II [GII]) was provided by the National Institute of Infectious Diseases, Tokyo, Japan.

Sequence and phylogenetic analyses: PCR products

Table 1. Sequences of the oligonucleotide primers used for RT-PCR

Virus	Polarity	Sequence (5′–3′)	Size (bp)	Target gene	Reference
Hepatitis A virus	+	GAAATGTCTCAGGTACTTTCTTTG	247	VP1/VP3	13
	−	GTTTTGCTCCTCTTTATCATGCTATG			
Hepatitis E virus	+	GMYTGGTCDCGCCAAGHGGA	137	ORF2/3	13
	−	GYTGATTCTCAGCCCTTCGC			
Enterovirus	+	CTACTTTGGGTGTCCGTGTT	653	VP4	14
	−	GGTAAYTTCCACCACCANCC			
Rotavirus	1st PCR				
	+	GGCTTTAAAACGAAGTCTTC	1128	VP6	15
	−	CAGTCCAATTCATGCCTGGTGGA			
	2nd PCR				
	+	GGATCAGAAATTCAGGTCGCTGGAT		VP6	
	−	CGCATTTGGAAATAATGCTGC	381		
	−	ACATTCGCCAATAGAGTCTCAT	485		
Norovirus GI	1st PCR				16
	+	CGYTGGATGCGNTTYCATGA	381	Capsid	
	−	CCAACCCARCCATTRTACA			
	2nd PCR				
	+	CTGCCCGAATTYGTAAATGA	330	Capsid	
	−	CCAACCCARCCATTRTACA			
Norovirus GII	1st PCR				
	+	CARGARBCNATGTTYAGRTGGATGAG	387	Capsid	
	−	CCRCCNGCATRHCCRTTRTACAT			
	2nd PCR				
	+	CNTGGGAGGGCGATCGCAA	344	Capsid	
	−	CCRCCNGCATRHCCRTTRTACAT			

M, A/C; Y, C/T; D, A/G/T; H, A/C/T; N, A/G/C/T; R, A/G; B, C/G/T.

were purified using a gel extraction kit (QIAGEN, Tokyo, Japan), and purified DNA was directly sequenced (15,16) by a commercial DNA sequencing company (MACROGEN, Seoul, Korea). Nucleotide sequence similarities were evaluated using the BLAST search program and the NCBI GenBank database. To investigate the phylogeny of the samples, phylogenetic trees were constructed as follows: BLAST searches of viral nucleotide sequences in GenBank were performed using the sample nucleotide sequences as queries, and the corresponding regions of the viral sequences were extracted from the database. The extracted sequences were aligned using the MAFFT (v6.864b) program using default parameters after removing redundant sequences (17). Phylogenetic trees were then constructed using the maximum likelihood method based on the Kimura-2 parameter model by MEGA 5.05 (18). The GenBank accession numbers of the reference strains are shown in each figure.

RESULTS

Detection of viral genes in flood water samples: A total of 100 flood water samples were collected in Thailand during the 2011 flood. Total positive rates for RT-PCR are shown in Table 2. Thirty samples (30%) contained at least one of the viral pathogens. Mixed contamination by 2 viruses, HAV and RV, was found in 1 sample (1%). NV was the most commonly detected pathogen in the tested samples (14%). RV and HAV were detected in 9% and 7% of the samples, respectively. EV and HEV genes were not detected in any of these samples.

Most water samples were collected in the Salaya area of Nakhon Pathom province (70 samples). The temperature of the samples at the time of collection was in the range of 19.8–39.1°C and the pH was 6.38–7.64. Of these 70 samples, NV was detected in 12 (17.1%), RV was detected in 8 (11.4%), and HAV was detected in 5 (7.1%). Of the 30 samples collected from other areas, NV was detected in 2 (6.7%), HAV was detected in 2 (6.7%), and RV was detected in 1 (3.3%).

Characterization of viral genes detected in flood water: In total, 14, 9, and 7 samples were positive for NV, RV, and HAV by RT-PCR, respectively. Of these positive samples, partial sequences of the capsid gene of NV, the VP6 gene of RV, and the VP1/VP3 gene of HAV were used for future characterization of strains/genotypes through sequencing and phylogenetic analysis (Fig. 2 and Table 3).

NV-specific sequences were obtained from the PCR products amplified from the 14 NV-positive samples. Specific primer pairs for the GI and GII groups were separately used to amplify the NV gene from the samples. Twelve samples belonged to the GI genotype. Of these, five fell within the GI/3 cluster (87–99% identity), one fell within the GI/4 cluster (97% identity), and the other six were most closely related to GI/9 cluster (87–99% identity), according to the phylogenetic tree (Fig. 2A). One of the GII samples was positioned within the GII/3 cluster, and the other was positioned within the GII/4 cluster (Fig. 2B). In case of RV, the first round of RT-PCR was performed to identify the RV-positive samples. Subsequently, nested PCR was used for subgrouping. The RV sequences (9 samples) were almost identical, with high similarity to the sequence of the RV A G2P[4] MMC84 strain (99% identity, GenBank accession no. HQ641367.1). No other RV group was detected in this study. Furthermore, the detected RVs were grouped within the same cluster as the RVs isolated in 2007 in Thailand (Fig. 2C). The HAV sequences (5 samples) clustered near the genotype IA cluster and were highly similar (99% identity) to the sequence of the HAV strain IVA, genotype IA (GenBank accession no. DQ646426.1; Fig. 2D).

DISCUSSION

The present study showed that the NV, RV, and HAV genes were present in the flood water samples collected in Thailand in 2011. This is the first surveillance of viral genes in flood water samples in Thailand. NV was the major pathogen identified in these samples. In addition, this study was the first to detect an NV gene in environmental water samples in Thailand.

Data from other studies conducted in Thailand showed that some enteric viruses (or their genes and/or antigens) were present in non-flood environmental water from canals, swamps, or sewage (19–21). However, to the best of our knowledge, there are no previous reports of NV or HEV in any type of environmental water in Thailand. In a study of sewage samples, 8% samples were positive for the RV antigen (19). In another study, the RV gene was detected in 20% (river), 26% (canal), and 25% (sewage) of samples (20). The HAV gene was detected in 15% (sewage, swamp) and 10% (canal) of samples, and the genotypes of these HAVs were GIA (19,21). Kittigul et al. performed a surveillance of EV in environmental water but did not detect EV (19). Their report suggested that enteric viral contamination is impacted by sewage from households (21). Enteric viruses are transmitted by the fecal–oral route; the spread of these viruses has been attributed to the consumption of food and drinking water contami-

Table 2. Prevalence of enteric viral genes in flood water samples collected in central Thailand

Area (tested no.)	No. of positive samples (%)					
	HAV	HEV	Enterovirus	Rotavirus	Norovirus	Total
Salaya (70)	5[1)] (7.1)	0 (0)	0 (0)	8[1)] (11.4)	12 (17.1)	25 (35.7)
Others (30)	2 (6.6)	0 (0)	0 (0)	1 (3.3)	2 (6.6)	5 (16.6)
Total (100)	7 (7)	0 (0)	0 (0)	9 (9)	14 (14)	30 (30)

[1)]: One sample was a mixed contamination by both HAV and RV.
HAV, hepatitis A virus; HEV, hepatitis E virus.

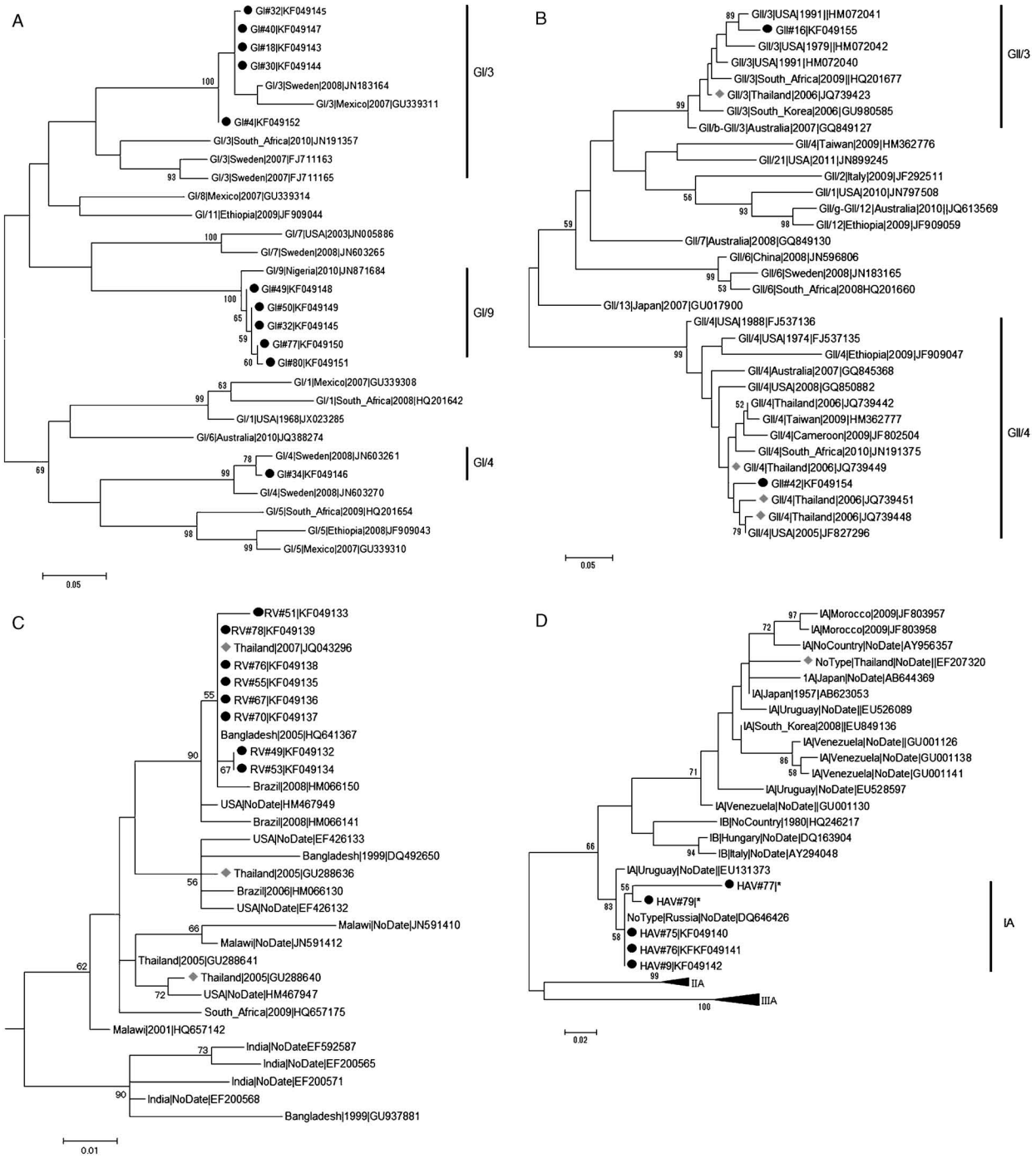

Fig. 2. Phylogenetic analysis of NV GI (A), NV GII (B), RV (C), and HAV (D) sequences from flood water samples from Thailand. The country in which the viruses were detected, the year of detection, and GenBank accession numbers subjected to analysis are described beside the strain information (*, nucleic acid less than 200 bp, which cannot be accepted by Genbank). The genotype clusters are described on the right. The tree was generated using the maximum likelihood method, based on the Kimura-2 parameter model. Bootstrap values are indicated on each branch. Black circle, detected sequence in this study; gray diamond shape, reference sequence detected in Thailand.

Table 3. Prevalence of the NV, RV, and HAV genotypes in flood water in central Thailand in 2011

Area	Norovirus							Rotavirus	HAV
genotype	GI	(GI/3	GI/4	GI/9)	GII	(GII/3	GII/4)	G2	IA
Salaya	11	(5	1	5)	1	(0	1)	8	5
Others	1	(0	0	1)	1	(1	0)	1	2

HAV, hepatitis A virus; RV, rotavirus; NV, norovirus.

nated with fecal matter (22,23). Our water samples were also collected from residential areas (Fig. 1). Sewage from households may have been present in the tested water samples. However, the detection rates of viral genes in our study were lower than those in reports examining other environmental water sources in Thailand (19–21). The differences in these detection rates may also be explained by differences in the targeting samples (flood water versus normal environmental water).

Children are potentially at a greater risk of infection with enteric viruses (24). In a study of children with diarrhea admitted to a hospital in Thailand, some enteric viruses (or their genes) were found in fecal samples. The prevalence of NV in fecal samples from children hospitalized with acute gastroenteritis in Thailand was 0.65–60% (25–27). In the present study, the circulation of GI/3, 4, and 9 and GII/3 and 4 genotypes was observed in flood water samples. These genotypes had already been reported in infected cases in Thailand. The dominant genotype of the NV cases in Thailand was found to be GII/4, although several other genotypes were also present, namely GI/3, 4, 6, and 9 and GII/1, 3, and 6 (25–27).

The prevalence of RV in infants and young children with diarrhea admitted to hospitals in Thailand was reported to be 27.5–28.4%, and all these viruses belonged to RV group A (25,28). These studies identified the globally common RV genotypes G1P[8], G2P[4], and G9P[8] (28). In the present study, the circulation of RV G2P[4] was also observed in the 2011 flood water. This genotype is highly prevalent in patients in Bangkok, Thailand (28).

Serosurveillance of acute viral hepatitis cases in Thailand showed that 39.6% of cases were positive for HAV (29). The dominant genotype of HAV in Thailand was genotype IA. However, a previous report detected genotype IB for the first time (30). In the present study, we observed the circulation of genotype IA in the 2011 flood water. Thus, we detected indigenous viral genotypes in the flood water from central Thailand. The viral genes detected in the flood water samples were highly similar to those detected in patients in Thailand. These data also suggest that contamination of flood water with enteric viruses is impacted by sewage from households.

Neither EV nor HEV genes were detected in any of the water samples. However, these viruses were detected in Thailand in previous studies (22,25,31–34). The rate of EV gene detection ranges from 2.5–58.3% in children (25,31), and EV 71 is the dominant group (31). HEV seroprevalence in young adults ranges from 9–22% (22). Pigs are suspected to be a source of HEV transmission to humans in Thailand (32). In other countries, water samples collected near pig farms have tested positive for HEV genes (33). Flooding conditions have also been linked to increased incidences of EV and HEV infections (34). Vigilance regarding EV and HEV infections is therefore still warranted, although we did not detect these viruses in the flood water in Thailand.

There is some evidence that the increase in gastrointestinal symptoms during the flood may have been due to direct exposure to flood water. During the flood, individuals who were in direct contact with the flood water were at an increased risk of gastrointestinal symptoms (7). The present study confirmed the presence of enteric viral genes in flood water in Thailand. NV was the major pathogen in these samples. The presence of viral genes does not directly correlate with viral infectivity but suggests a potential risk for the spread of enteric viruses by flood water. Contact with flood water or items contaminated with flood water is a risk factor for enteric viral infections.

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Conflict of interest None to declare.

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