การประชุมเสนอผลงานวิจัยระดับชาติ มหาวิทยาลัยสุโขทัยธรรมาธิราช ครั้งที่ 11
The 11th STOU National Research Conference

Surveillance of Human Bocavirus in Recycled Water, Sewage Sludge, and Bivalve Shellfish Samples

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Abstract

Human bocavirus (HBoV) infections are related to respiratory and gastrointestinal diseases. The genetic diversity of HBoVs in the environment is limited. This study aimed to assess the presence and molecular characterization of HBoV species in recycled water and sewage sludge samples collected from a wastewater treatment plant and bivalve shellfish collected from fresh markets using nested PCR with broad-range primer pairs targeting the capsid proteins VP1 and VP2. HBoV DNA was detected in recycled water of 9/106 (8.49%) samples and sewage sludge of 27/86 (31.39%) samples, whereas HBoV DNA was not detected in bivalve shellfish (0/106 samples). Thirty-five HBoV-positive samples were identified to species as the predominant HBoV2; 26 samples followed by HBoV3; 8 samples and the rare HBoV4; 1 sample. Based on DNA sequencing and alignment with nucleotide sequences from HBoV strains reported globally, the sequences of HBoV found in this study showed nucleotide identity of 96.84—100.00% similar to the sequences from HBoV infected patients in South America, Africa, Europe, and Asia. This is the first study determining the contamination of HBoV species in recycled water and sewage sludge samples in Thailand. HBoVs detected in recycled water may cause harmful effects to exposed consumers. The data of HBoVs in the recycled water and sewage sludge emphasizes the circulation of virus in environment and the potential vehicle of its transmission.

Keywords: Bocavirus, Nested PCR, Gastroenteritis, Environmental sample

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Introduction

Human bocavirus (HBoV) is an emerging virus first discovered in pediatric respiratory samples in 2005 (Allander et al., 2005). The virus is associated with respiratory tract and gastrointestinal infections mainly in children less than two years (Kapoor et al., 2009). HBoV is a non-enveloped virus belonging to the family *Parvoviridae*, subfamily Parvovirinae, and genus Bocavirus. The virus contains 5300 nucleotides single-stranded DNA genome bearing different regions: ORF1 encodes the nonstructural protein NS1; ORF2 encodes the nuclear phosphoprotein NP1; and ORF3 encodes the viral capsid proteins called VP1 and VP2. HBoVs are classified into 4 species: HBoV1 is often detected in the upper and lower respiratory tracts, whereas HBoV2, HBoV3, and HBoV4 are more frequently found in fecal specimens (Guido et al., 2016).

HBoV transmits through contact with respiratory droplets from infected patients and contaminated food or water (Schildgen et al., 2008). Since HBoVs are excreted in the feces of infected patients and dispersed in aquatic environments, the viruses probably cause a potential risk to humans via environmental exposure of viral contamination. In addition, filter-feeding bivalve shellfish tend to accumulate water circulating viruses in their tissues (La Rosa et al., 2018; Purpari et al., 2019). There are reports of HBoV found in sewage in Egypt (Hamza et al., 2016) and Uruguay (Salvo et al., 2018), sewage sludge in Egypt (Shaheen et al., 2020), treated water in Norway (Myrmel et al., 2015), river water in Germany (Hamza et al., 2009) and Italy (La Rosa et al., 2017) and bivalve shellfish in Italy (La Rosa et al., 2018; Purpari et al., 2019) and South Africa (Onosi et al., 2020). Previous studies found that HBoVs detected in environmental samples were predominant HBoV2 and HBoV3, whereas HBoV4 was rarely detected in environmental samples (Hamza et al., 2017; Iaconelli et al., 2016; La Rosa et al., 2018; Myrmel et al., 2015; Onosi et al., 2020). The presence of HBoV in the environmental water and bivalve shellfish can result in a risk to consumers and consequently cause respiratory or gastroenteritis illness in humans.

In Thailand, the presence of several enteric viruses in environmental water has been reported such as norovirus (Asami et al., 2016; Ngaosuwankul et al., 2013), rotavirus (Ngaosuwankul et al., 2013), hepatitis A virus (Kittigul et al., 2006; Ngaosuwankul et al., 2013), salivirus (Badru et al., 2018) and JC polyomavirus (Asami et al., 2016) exhibiting the possible presence of other enteric viruses including HBoV. These enteric viruses which are excreted in feces from infected individuals circulate and distribute in the environment. HBoV was found in oysters (Kumthip et al., 2021). Although HBoVs have been frequently detected in patients admitted to hospitals with the respiratory syndrome (Chieochansin et al., 2008) and acute gastroenteritis (Khamrin et al., 2012), the data of HBoV in environmental water samples is limited.

Purpose

The objective of this study was to determine the presence and molecular characterization of HBoV species in recycled water, sewage sludge, and bivalve shellfish samples. The amplicons were subjected to DNA sequencing and aligned with reference strains in the GenBank using BLAST analysis for HBoV species identification.

Research Methodology

Recycled water and sewage sludge samples: From June 2007 to March 2021, 106 recycled water samples and 86 sewage sludge samples were obtained from a wastewater treatment plant in Samut Prakan Province, Thailand. The capacity of the wastewater treatment plant is 16,000 cm^3/day and serves 60,000 inhabitants. Recycled water samples were collected from the outflow after chemical and biological treatment processes. The sewage sludge samples were obtained from semi-solid wastes produced as a by-product of wastewater treatment after a mechanical sludge dewatering process with lime stabilization. This recycled water was reused for irrigation and agricultural fields and even discharged into a surrounding canal. The sewage sludge was disposed on land application.

Bivalve shellfish samples: From 2011 to 2016, 106 bivalve shellfish samples including oysters (*Crassostrea belcheri*), mussels (*Perna viridis*), and cockles (*Anadara nodifera*) were obtained from fresh local markets in Bangkok.

The recycled water and sewage sludge, and bivalve shellfish samples were collected and processed for virus concentration in previous studies (Kittigul et al., 2016; Kittigul et al., 2019; Tunyakittaveeward et al., 2019). In this study, nucleic acid extraction and detection for HBoV DNA using nested PCR were performed.

Virus processing and DNA extraction: The recycled water (5 l per sample) was processed using an adsorption-elution technique with membrane filtration as described previously (Kittigul et al., 2019). Briefly, the samples were adjusted to pH 3.5 and added with aluminum chloride at a final concentration of 0.0015 N. For filtration and viral adsorption, a 0.45 m pore size mixed cellulose ester membrane was utilized. The virus was eluted from the membrane using 2.9% tryptose phosphate broth (TPB) containing 6% glycine, pH 9.0. The sewage sludge (5 g per sample) was processed using an adsorption-elution method as described previously (Kittigul et al., 2019). Briefly, the specimens were treated with deionized water (20 mL) and adjusted to a pH of 5.0. The virus was eluted by adding 2.9% TPB containing 6% glycine, pH 9.0 followed by 0.5 M arginine-0.15 M NaCl, pH 7.5. The digestive tissues (4 g) of bivalve shellfish samples were processed using an adsorption-twice elution-extraction method, as previously described (Kittigul et al. 2016; Tunyakittaveeward et al. 2019). The volumes of concentrates were reduced to 1.3—2.2 ml for recycled water, 0.7—1.4 ml for sewage sludge, and 0.8 ml for bivalve shellfish using a speed vacuum centrifuge (UNI-EQUIP Laborgeratebau und-vertriebs GmbH, Munich, Germany). The concentrates of recycled water,

sewage sludge and bivalve shellfish were stored at -80 ºC until used for nucleic acid extraction. Viral DNA was extracted from each 200 µl of recycled water and sewage sludge concentrates using QIAamp® DNA Mini Kit or QIAamp® RNA Extraction Kit (QIAGEN GmbH, Hilden, Germany) in accordance with the manufacturer's instruction. For bivalve shellfish, viral DNA was extracted from each 200 µl of concentrates using QIAamp® DNA Mini Kit or RNeasy® Mini Kit (QIAGEN GmbH, Hilden, Germany) in accordance with the manufacturer's instruction. The final volume of 60 µl DNA or RNA extract was used for HBoV detection. Total nucleic acid concentrations of DNA and RNA in the extracts were determined by measuring absorbance at 260 nm using BioDropTM spectrophotometer (NanoDropTM 2000 Spectrophotometry; Thermo Fisher Scientific, Wilmington, DE).

Nested PCR: Detection of HBoV in extracted DNA or RNA was investigated using nested PCR as described previously (La Rosa et al., 2017). Primers 2028F [5'-GAAATGCTTTCTGCTGYTGAAA-3'] and 2029R [5'-GTGGATATACCCACAYCAGAA-3'] were used for amplification of first-round PCR and 2030F [5'-GGTGGGTGCTTCCTGGTTA-3'] and 2031R [5'-TCTTGRATTTCATTTTCA GACAT-3'] for nested PCR. The primer pairs are specific to the sequence of the capsid proteins VP1 and VP2 of HBoV. Briefly, a 25 µl reaction mixture consisted of 5 µl DNA and 20 µl of reaction mixture containing 10 pmol of each primer 2028F and 2029R, 1X PCR buffer, 0.2 mM dNTPs, 2 U *Taq* polymerase (Invitrogen, Carlsbad, CA), 2 mM $MgCl_2$ and nuclease-free water. The reaction tube was inserted into a BIO-RAD T100TM Thermal cycler (Applied Biosystems, Foster City, CA). The cycling conditions were an initial denaturation at 94℃ for 10 min followed by 35 cycles of 94℃ for 30 sec, 51℃ for 30 sec, 72℃ for 1 min with a final extension at 72℃ for 5 min. HBoV DNA amplicon in the length of 543 bp was amplified. Nested PCR was carried out in a 23 µl reaction mixture contained 2 µl PCR product, 10 pmol of each primer 2030F and 2031R, 1X PCR buffer, 0.2 mM dNTPs, 2 U *Taq* polymerase (Invitrogen, Carlsbad, CA), 2 mM $MgCl_2$, and nuclease-free water. The condition of PCR was the same as the first PCR, except for an annealing temperature of 50℃. The nested PCR product of HBoV generated a DNA amplicon in the length of 382 bp. The HBoV DNA product was purified using a QIAquick gel extraction kit (QIAGEN, Valencia, CA) and subjected to DNA sequencing. The obtained nucleotide sequences were aligned and assembled into a consensus sequence using BLAST analysis for HBoV species identification.

Data analysis: HBoV detection rates in recycled water, sewage sludge, and bivalve shellfish samples were calculated and described in percentages. All calculations were performed using SPSS for Windows (version 18.0; SPSS Inc., Chicago. IL, USA) for statistical analyses.

Results

DNA and RNA concentrations in environmental samples

Although HBoV is a DNA virus, a previous study using an RNA extraction kit could detect HBoV (Onosi et al., 2020). This study used both DNA and RNA extraction kits to assess the presence of HBoVs in the recycled water, sewage sludge, and bivalve shellfish samples to facilitate the detection. The results showed that total DNA and RNA concentrations of DNA extracts from the DNA extraction kit ranged from 7.00—246.00 µg/ml (Geometric mean, II = 46.68) and 4.80—186.40 µg/ml (Geometric mean, II = 35.89). Total DNA and RNA concentrations of RNA extracts from the RNA extraction kit were in a range of 10.00—348.00 µg/ml (Geometric mean, II = 109.65) and 6.40—276.00 µg/ml (Geometric mean, II = 91.91), respectively. Using the DNA extraction kit, the geometric means of total DNA (57.08 µg/ml) and RNA (43.91 µg/ml) concentrations were highest in the DNA extracts from bivalve shellfish. Using the RNA extraction kit, the geometric mean of total DNA (165.67 µg/ml) and RNA (126.64 µg/ml) concentrations were highest in the RNA extracts from sewage sludge (Table 1).

Table 1 The total concentrations of DNA and RNA extracted from recycled water, sewage sludge, and bivalve shellfish samples presented as geometric mean

Sample type	Total no.	DNA extraction kit		RNA extraction kit	
		DNA[a]	RNA[a]	DNA[a]	RNA[a]
Recycled water	10	45.85	35.69	121.87	99.91
Sewage sludge	10	42.98	32.62	165.67	126.64
Bivalve shellfish	5	57.08	43.91	31.02	21.88

[a]Total DNA and RNA concentrations were determined by measuring absorbance at 260 nm and calculated as geometric mean of µg/ml.

The presence of human bocavirus in recycled water and sewage sludge

A total of 298 environmental samples were tested for HBoV and gave positive HBoV results of 36 samples (12.08%). Of 106 recycled water samples, 9 (8.49%) were detected positive for HBoV (Table 2). The DNA extracted samples yielded single and sharp bands of HBoV. Some non-specific bands were observed but they did not pose any problems for the results interpretation, as shown in Figure 1. Of 86 sewage sludge samples, 27 (31.39%) were detected positive for HBoV (Table 2). The DNA extracted samples yielded single and

sharp bands of HBoV. There were some non-specific bands, as shown in Figure 2. Of 106 bivalve shellfish, none of them (0.0%) was detected for HBoV (Table 2), the specific band of HBoV has not appeared at 382 bp.

Table 2 The presence of HBoV in recycled water, sewage sludge, and bivalve shellfish samples tested by nested PCR

Sample type	Total no.	No. of HBoV-positive samples (%)
Recycled water	106	9 (8.49)
Sewage sludge	86	27 (31.39)
Bivalve shellfish	106	0 (0.00)
Total	298	36 (12.08)

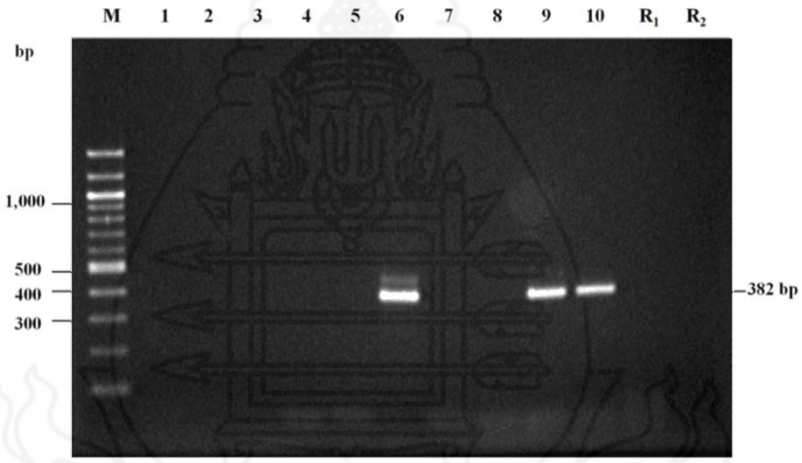

Figure 1 Detection of HBoV in recycled water using nested PCR. Lane: M, DNA marker (100 bp DNA ladder); 1—10, concentrates from recycled water samples determined for HBoV; R_1 and R_2, negative controls for first PCR and nested PCR, respectively. Gel electrophoresis of nested PCR products of HBoV showed 382-bp bands.

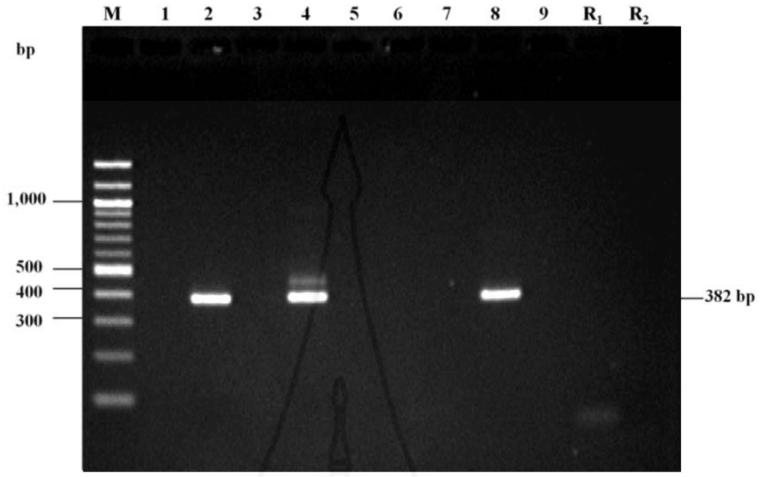

Figure 2 Detection of HBoV in sewage sludge using nested PCR. Lane: M, DNA marker (100 bp DNA ladder); 1—9, concentrates from sewage sludge samples determined for HBoV; R_1 and R_2, negative controls for first PCR and nested PCR, respectively. Gel electrophoresis of nested PCR products of HBoV showed 382-bp bands.

Molecular characterization of human bocavirus in recycled water and sewage sludge

Thirty-six HBoV-positive PCR products were subjected to DNA sequencing and partial nucleotide sequences of capsid genes were analyzed by BLAST program. Thirty-five samples (97.22%) were characterized successfully including 8 recycled water samples (88.88%) and 27 sewage sludge samples (100%). In recycled water, 3 samples had a nucleotide sequence identity of 99.12—100.00% similar to the sequences from HBoV infected patients in Ethiopia, and the United Kingdom belonged to HBoV2 species. Five samples had a nucleotide sequence identity of 98.82—100.00% similar to the sequences from HBoV infected patients in China belonged to HBoV3 species (Table 3). In sewage sludge, 23 samples had a nucleotide sequence identity of 96.84—100.00% similar to the sequences from HBoV infected patients in Brazil, Ethiopia, China, South Korea, Tunisia, and the United Kingdom belonged to HBoV2 species. Three samples had a nucleotide sequence identity of 98.72—100.00% similar to the sequences from HBoV infected patients in China belonged to HBoV3 species. One sample had a nucleotide sequence identity of 98.53% similar to the sequence from HBoV infected patients in Ethiopia belonged to HBoV4 species (Table 4).

Table 3 Identities of the HBoV nucleotide sequences detected in recycled water samples to the sequences of HBoV strains deposited in the NCBI GenBank database

No.	Sample ID[a]	HBoV strain deposited in the GenBank[b]	Identity (%)	Species
1	RE11-11-17	Human bocavirus/ ETH_P4/2016/ Ethiopia	99.70	HBoV2
2	RE11-11-19	Human bocavirus/ ETH_P4/2016/ Ethiopia	99.12	HBoV2
3	RE04-04-19	Human bocavirus/ CU1557UK/2008/ United Kingdom	100.00	HBoV2
4	RE04-04-17	Human bocavirus/ 46-BJ07/2007/ China	100.00	HBoV3
5	RE05-05-17	Human bocavirus/ 46-BJ07/2007/ China	98.82	HBoV3
6	RE07-12-07	Human bocavirus/ 46-BJ07/2007/ China	100.00	HBoV3
7	RE12-12-17	Human bocavirus/ 46-BJ07/2007/ China	100.00	HBoV3
8	RE12-12-19	Human bocavirus/ LZFB199/ 2007/ China	100.00	HBoV3

[a], RE: recycled water. [b], Analyzed by the BLAST program.

Table 4 Identities of the HBoV nucleotide sequences detected in sewage sludge samples to the sequences of HBoV strains deposited in the NCBI GenBank database

No.	Sample ID[a]	HBoV strain deposited in the GenBank[b]	Identity (%)	Species
1	SS08-07-14	Human bocavirus/ BRA/ TO-237/ 2014/ Brazil	99.14	HBoV2
2	SS10-09-15	Human bocavirus/ BRA/ TO-237/ 2014/ Brazil	98.86	HBoV2
3	SS12-11-15	Human bocavirus/ BRA/ TO-237/ 2014/ Brazil	96.84	HBoV2
4	SS02-01-16	Human bocavirus/ BRA/ TO-237/ 2014/ Brazil	99.69	HBoV2
5	SS04-03-16	Human bocavirus/ BRA/ TO-237/ 2014/ Brazil	99.12	HBoV2
6	SS06-05-16	Human bocavirus/ BRA/ TO-237/ 2014/ Brazil	97.66	HBoV2
7	SS08-07-16	Human bocavirus/ BRA/ TO-237/ 2014/ Brazil	99.41	HBoV2
8	SS03-05-17	Human bocavirus/ BRA/ TO-237/ 2014/ Brazil	99.70	HBoV2
9	SS06-09-17	Human bocavirus/ BRA/ TO-237/ 2014/ Brazil	99.41	HBoV2
10	SS02-01-18	Human bocavirus/ BRA/ TO-237/ 2014/ Brazil	98.00	HBoV2
11	SS04-05-18	Human bocavirus/ BRA/ TO-237/ 2014/ Brazil	99.12	HBoV2
12	SS06-09-18	Human bocavirus/ BRA/ TO-237/ 2014/ Brazil	99.41	HBoV2
13	SS04-05-19	Human bocavirus/ BRA/ TO-237/ 2014/ Brazil	99.12	HBoV2
14	SS01-01-20	Human bocavirus/ BRA/ TO-237/ 2014/ Brazil	98.86	HBoV2

No.	Sample ID[a]	HBoV strain deposited in the GenBank[b]	Identity (%)	Species
15	SS10-09-13	Human bocavirus/ UK-648/ 2008/ United Kingdom	100.00	HBoV2
16	SS04-03-14	Human bocavirus/ UK-648/ 2008/ United Kingdom	99.73	HBoV2
17	SS02-01-19	Human bocavirus/ LZ55602/ 2007/ China	98.82	HBoV2
18	SSD01-01-20	Human bocavirus/ LZ55602/ 2007/ China	99.12	HBoV2
19	SS06-09-19	Human bocavirus/ BJQ435/ 2011/ China	98.82	HBoV2
20	SS10-09-16	Human bocavirus/ ETH_P4/ 2016/ Ethiopia	97.95	HBoV2
21	SS12-11-16	Human bocavirus/ ETH_P4/ 2016/ Ethiopia	99.41	HBoV2
22	SS02-01-15	Human bocavirus/ HBoV2A-TU-A-114-06/ 2006/ Tunesia	99.73	HBoV2
23	SS02-01-17	Human bocavirus/ CUK18/ 2013/ South Korea	97.94	HBoV2
24	SS04-03-15	Human bocavirus/ LZFB199/ 2013/ China	98.72	HBoV3
25	SS06-05-15	Human bocavirus/ LZFB199/ 2013/ China	98.99	HBoV3
26	SS02-01-13	Human bocavirus/ 46-BJ07/ 2007/ China	100.00	HBoV3
27	SS04-05-17	Human bocavirus/ ETH_P3/ 2016/ Ethiopia	98.53	HBoV4

[a], SS: wet sewage sludge, SSD: dry sewage sludge. [b], Analyzed by the BLAST program.

Discussion

The detection of HBoVs in clinical and environmental samples has been widely reported in many countries worldwide. In Thailand, HBoV infection was found in sporadic cases admitted to hospitals (Chieochansin et al., 2008; Khamrin et al., 2012). However, HBoV prevalence in the environment remains largely unknown. Environmental samples contain several organic and inorganic compounds such as heavy metals, humic acids, and polyphenols. These are toxic and might form complexes with nucleic acid extracts to inhibit the enzyme in DNA amplification (Schlindwein et al., 2010). In this study, the nested PCR method was chosen for the detection of environmental samples in order to increase the specificity of detection and eliminate any false-positive results. In addition, DNA and RNA extraction kits were employed to facilitate HBoV detection. Since HBoV is a DNA virus, DNA extraction kits have been used in various studies (Shaheen et al., 2020; Hamza et al., 2017). In previous studies on HBoV detection, an RNA extraction kit without DNase digestion was also used (Onosi et al., 2020). We found that the RNA extraction kit gave a higher yield of both RNA and DNA concentrations than the DNA extraction kit suggesting the higher efficiency of extraction. Notably, the higher amount of DNA than RNA extracted by QIAamp® RNA Extraction Kit might be explained by some RNA degradation that occurred during extraction steps or by an ineffective binding of RNA to the membrane of the spin column (Klenner et al., 2017). Low virus concentration present in the environmental samples is a possible

factor that could result in insufficient nucleic acids for molecular detection. This study also used the QIAamp® DNA Mini Kit for HBoV DNA extraction due to the fact that HBoV is a DNA virus similar to previous studies of HBoVs (Shaheen et al., 2020; Hamza et al., 2017).

The present study showed HBoVs in recycled water (8.49%) at lower frequencies than the previous reports of HBoVs in secondarily treated sewage in Norway (42.00%) (Myrmel et al., 2015), and treated raw sewage in Egypt (25.00%) (Shaheen et al., 2020). On the contrary, HBoVs in sewage sludge (31.39%), were approximately two times more prevalent than that in a previous study in Egypt (16.60%) (Shaheen et al., 2020). These differences in the frequency of HBoV detection might be the differences in the sample types, the geographical area, concentration methods, and detection methods. HBoV from infected individuals can be discharged in untreated wastewaters through virus shedding and consequently disperse throughout water environments. High detection rates of HBoVs in sewage in Uruguay (Salvo et al., 2018), Norway (Myrmel et al., 2015), and in Egypt (Shaheen et al., 2020), which is usually directly discharged into surface waters suggest a waterborne transmission is likely to occur if people enter in contact with polluted surface waters for recreational activities such as fishing or swimming. HBoV was not detected in all tested bivalve shellfish samples. This is probably due to the low frequency of HBoV detection in bivalve shellfish samples as reported in Italy (8.50% and 3.70%) (La Rosa et al., 2018; Purpari et al., 2019) and Thailand (7.60%) (Kumthip et al., 2021). The different findings might be the differences in the species of bivalve shellfish, the geographical area, sample collection, and detection method. Additional monitoring is necessary to assess the potential risk of HBoV transmission to humans through raw-consumption food.

Nearly all HBoV DNA-positive samples could be successfully sequenced and aligned with the deposited nucleotide sequences in the GenBank. Only one recycled water sample could not be aligned with HBoV sequences in the NCBI GenBank database due to the extremely low concentration of PCR product. All sewage sludge samples with positive HBoVs provided a relatively high yield of PCR products that could be characterized for HBoV species. Both HBoV2 and HBoV3 were detected in recycled water and sewage sludge samples with HBoV2 predominance. This finding is consistent with the HBoV study in urban sewage in Italy (Iaconelli et al., 2016). HBoV3 was found in raw sewage in South Africa (Onosi et al., 2020). Of interest, the rare HBoV4 was detected in one sewage sludge sample. This result was in accordance with previous studies confirming that HBoV4 is rarely detected both in environmental (Iaconelli et al. 2016; Salvo et al. 2018) and clinical samples (Khamrin et al. 2012). In the present study, no HBoV1 was detected in all tested samples. HBoV1—3 was reported in Egypt (Shaheen et al., 2020), whereas the presence of the four HBoV species in the aquatic environment, mainly in sewage was registered in Uruguay (Salvo et al., 2018). Since HBoV2, HBoV3, and HBoV4 are associated with gastrointestinal infections, they replicate in the epithelium of the gastrointestinal tract, while HBoV1 infects epithelial cells of the respiratory tract. It has been suggested

that HBoV1 once replicated in the respiratory tract can be swallowed together with nasopharyngeal secretion and can be detected in feces or sewage with lower titer than that observed in nasopharyngeal swabs (Schildgen et al., 2008). In Thailand, HBoV1—4 were reported in pediatric patients admitted to hospital (Khamrin et al., 2012) together with the results of the present findings of HBoV2—4 indicate the importance of genetic diversity of HBoVs in humans and the environment.

The detection of HBoV in treated recycled water revealed the difficulty in removing the virus thoroughly by the procedure applied in the current wastewater treatment. Our previous reports demonstrated that processes of wastewater treatment are not completely effective in removing norovirus (Kittigul et al., 2019) and rotavirus (Kittigul & Pombubpa, 2021) and therefore human viruses are constantly discharged into the environmental waters. In this study, the detection rate of HBoV in sewage sludge was higher than in recycled water exhibiting that sewage sludge used for land application can serve as a potential reservoir of human enteric viruses which can be released again into the water environment as a result of agitation by storm action, heavy rain, etc. The data highlight the important role of environmental contamination in the dissemination of viral infection in the community.

Suggestion

This is the first investigation on HBoV in environmental samples in Thailand. HBoV contamination in recycled water and sewage sludge samples indicates the presence of human feces and the circulation of three HBoV species including the predominant HBoV2 followed by HBoV3 and HBoV4. The wastewater treatment system can only reduce but not inactivate the virus completely. Therefore, the potential role of treated water used for irrigation and sewage sludge disposed on a land application in the transmission of HBoV should not be neglected. The limitations of the present study are the small number of samples and the nested PCR used to test both viable and non-viable viruses. Thus, the presence of samples positive for viral genome does not necessarily correspond to a threat to human health. Further study on a large number of samples and viable PCR would give a clearer picture of HBoV prevalence in the environment. Phylogenetic analysis of HBoV should be further analysed to extend our understanding of the molecular epidemiology of HBoV.

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