MOLECULAR DETECTION OF HUMAN BOCAVIRUS 1 AND 2 IN CHILDREN WITH ACUTE GASTROENTERITIS IN TAIWAN

Meng-Bin Tang[1,2], Chi-Ming Chu[3], Yu-Ching Chou[3], Jen-Chun Kuan[3] and Chia-Peng Yu[4]

[1]Department of Family Medicine and Department of Hospice Palliative Medicine, Wei-Gong Memorial Hospital, Miaoli County; [2]Department of Public Health and Department of Health Services Administration, China Medical University, Taichung City; [3]School of Public Health, National Defense Medical Center, Taipei; [4]Department of Bioengineering, Tatung University, Taipei, Taiwan

Abstract. Human bocaviruses (HBoVs) have been detected in human gastrointestinal infections worldwide. Although HBoV global prevalence and strains diversity have been reported, but epidemiological data from Taiwan is largely unavailable to date. A total of 110 fecal samples from stools of diarrheic children at a general hospital, Taiwan, obtained from August 2012 to July 2013, were analyzed by nested PCR targeting a partial fragment (576 bp) of HBoV VP1/VP2 gene, which revealed 4 positive fecal samples. Clinical symptoms of HBoV-associated acute gastroenteritis (AGE) were not different from those without HBoV. HBoV infection was seen only during the fall and winter seasons. This is the first description of HBoV infection in children with AGE in Taiwan. Systematic surveillance and evidence-based studies are required to determine the transmission pathways and spread of HBoV in Taiwan.

Keywords: acute gastroenteritis, human bocavirus, phylogenetic analysis, Taiwan

INTRODUCTION

Human bocavirus (HBoV) was originally isolated in the respiratory secretions of Swedish patients with symptoms of acute respiratory infection in 2005 (Allander *et al*, 2005). Since its discovery, HBoV also has been shown to circulate globally (Kesebir *et al*, 2006; Allander *et al*, 2007; Monavari *et al*, 2013; Xiang *et al*, 2014), predominantly in urine, serum, and stool specimens of children with respiratory infections (Lindner and Modrow, 2008).

HBoVs are classified into the *Bocaparvovirus* genus (family Parvoviridae, subfamily Parvovirinae), which includes minute, non-envoloped, icosahedral viruses that package a linear negative- and positive-sense single-stranded DNA genome of ~5,000 bases. It is assumed that HBoV is an autonomously replicating virus (Manteufel and Truyen, 2008; Schildgen *et al*, 2012). HBoV genome contains three open reading frames (ORFs), the first two sequential ORFs (ORF1 and

Correspondence: Chia-Peng Yu, Department of Bioengineering, Tatung University, Number 40, Sec. 3, Zhongshan N. Road, Taipei 10452, Taiwan.
Tel: +886 2 21822918; Fax: +886 2 21822918
E-mail: yu6641@gmail.com

ORF2) encoding nonstructural protein NS1 and NP1 (nuclear phosphoprotein 1), respectively, and the third downstream ORF3 encoding two structural capsid viral proteins, VP1 and VP2 (Chen *et al*, 2010; Babkin *et al*, 2015). ORF1 and ORF3 are present in all parvovirus genomes (Berns and Parrish, 2007), but the specific feature that distinguishes bocaviruses from the remaining parvoviruses is the presence of an additional ORF2 in their genome. HBoV NS1 is essential for replication of the viral single-stranded DNA genome, DNA packaging and may play a number of versatile roles in virus-host interaction (Tewary *et al*, 2013). NP1 displays no similarity to other parvovirus proteins and is known to play a key role in virus replication, viral RNA processing, arrest of host cell cycle, and inducing apoptosis of infected (HeLa) cells (Sun *et al*, 2009; Sukhu *et al*, 2013; Sun *et al*, 2013). HBoV VP1 has an amino acid sequence identical to that of the VP2, except for an additional 129 amino acids at its N-terminus, commonly referred to as the VP1 unique region (Chow and Esper, 2009; Chen *et al*, 2010).

Four genotypes of HBoV have been reported, namely, HBoV1, HBoV2, HBoV3, and HBoV4 (Allander *et al*, 2005; Arthur *et al*, 2009; Kapoor *et al*, 2009, 2010). HBoV1 and HBoV3 belong to sp. *Primate bocaparvovirus 1*, while HBoV2 and HBoV4 to sp. *Primate bocaparvovirus 2* (Cotmore *et al*, 2014). HBoVs have been associated with upper and lower respiratory tract infections and gastroenteritis, with HBoV1 being an important pathogen in low respiratory tract infections, but also found in fecal samples (Jartti *et al*, 2012). A recent study has revealed that there is a direct correlation of high viral load with increasing disease severity in patients co-infected with HBoV1 and at least one other respiratory virus (Zhao *et al*, 2013). In 2009-2010, HBoV2-4 also were detected in stool specimens (Arthur *et al*, 2009; Kapoor *et al*, 2010) as well as in sewage samples (Blinkova *et al*, 2009) and that there is an association between HBoV2 infection and gastroenteritis (Kantola *et al*, 2010).

In Taiwan, epidemiological surveillances of HBoV infection in children with respiratory tract infection have been reported since 2009, 4 years after its discovery. Currently, HBoV-associated respiratory tract infection is not uncommon and is responsible for 2%-22.6% of such infections in Taiwan (Lin *et al*, 2009; Chuang *et al*, 2011; Sung *et al*, 2011; Chen *et al*, 2014; Lee *et al*, 2015).

Although there have been scattered reports describing the epidemiology of HBoV in specific areas of Taiwan, the epidemiological features of HBoV infection in patients with acute gastroenteritis (AGE) have previously not been described. In this study, we report the molecular epidemiology of HBoV detected in stool specimens from children with AGE in Taiwan, which could provide a greater understanding of the genetic evolutionary relationship among the viruses circulating in Taiwan.

MATERIALS AND METHODS

Specimen collection

AGE patients are defined as those with clinical diarrhea (≥3 loose stools within a 24-hour period), which may be accompanied by abdominal pain, fever, nausea, and vomiting. The study was conducted from August 2012 to July 2013 at Wei-Gong Memorial Hospital, Miaoli County, Taiwan. Stools of 110 children with AGE were collected and stored at -20°C before being transferred on ice to the Department of Bioengineering, Tatung

University, Taipei, Taiwan, where they were stored at -70°C. Samples were examined for the presence of HBoV using PCR before storage in a balanced salt solution at 10% suspensions at -70°C until used.

The study was approved by the Human Subject Research Ethics Committee, Wei-Gong Memorial Hospital (approval no. 101003). Prior informed written consent was obtained from adult participants and parents of minors.

Questionnaire

Patients were sent a questionnaire the week after enrollment to indicate their epidemiological features and clinical symptoms and to ascertain that they had AGE.

Nested PCR detection of HBoV

Nucleic acid was extracted from 200 μl of 10% fecal suspension in a final volume of 50 μl of RNase-free water using a viral nucleic acid extraction kit (Geneaid; New Taipei City, Taiwan) according to the manufacturer's instructions. Extracted DNA was stored at -20°C until used. Nested PCR targeting VP1/VP2 region of both HBoV1 and HBoV2 (nucleotide positions 3233-3808 according to HBoV2 prototype sequence deposited in GenBank, accession no. FJ170278). First round PCR was performed in a 25-μl reaction volume containing 10 μl of RNase-free water, 5 μl of template DNA, 0.5 μl (of 10 μM stock solution) of primers AK-VP-F1 (5'- CGCCGTGGCTCCTGCTCT -3') and AK-VP-R1 (5'- TGTTCGCCATCACAAAAGATGTG -3') (Kapoor *et al*, 2010), 0.5 μl of dNTPs (of 10 mM stock), 12.5 U *Taq* DNA polymerase (IT'S Science, Taipei, Taiwan), and 2.5 μl of 10X buffer (500 mM Tris-HCl pH 9.2, 160 mM ammonium sulfate, 25 mM $MgCl_2$ and 1% Tween 20). Thermocycling (conducted in Thermo Electron, West Palm Beach, FL) conditions were as follows: 95°C for 10 minutes; 35 cycles of 94°C for 30 seconds, 54°C for 45 seconds, and 72°C for 45 seconds; with a final step at 72°C for 10 minutes. Amplicons (611 bp) were separated by electrophoresis in 2% agarose gels and visualized after staining by ethidium bromide. DNA 100 bp size markers (Geneaid, New Taipei City, Taiwan) were used for calibration. Second round PCR was performed as in the first round PCR except that primers AK-VP-F2 (5'-GGCTCCTGCTCTAGGAAATA-AAGAG-3') and AK-VP-R2 (5'-CCTGCT-GTTAGGTCGTTGTTGTATGT-3') and 3 μl from the first round reaction solution were used; and the annealing temperature was 58°C. The amplicons (576 bp) were analyzed as described.

RESULTS

Study population

During the study period, 110 children with AGE comprising 58 (53%) males were enrolled. Fecal samples (including 11 obtained from out-patients, 62 from the emergency unit and 37 from in-patients) were collected and screened for HBoV.

HBoV positive cases and clinical features

HBoV was detected in 4 (3.5%) of all samples (Table 1). All occurred in children with AGE during the fall-winter season. Clinical symptoms of HBoV-positive AGE patients included fever (50%), vomiting (75%), watery stool (75%), and abdominal pain (75%).

DISCUSSION

This study demonstrates the presence of HBoV genomic DNA in stool samples from 3.5% of children with AGE at a hospital in Taiwan. This is in line with previous studies in Iran and Italy, higher than that reported in China, Mexico, Thailand, and

Table 1
Epidemiological, clinical features and HBoV genotype of patients at Wei-Gong Memorial Hospital, Taiwan.

Parameter	Patient (N = 110)	
	HBoV positive (n = 4)	HBoV negative (n = 106)
Gender		
Male	2	56
Female	2	50
Setting		
Out-patient	0	11
Emergency unit	2	60
In-patient	2	35
Age (years)		
< 2	2	35
2-10	2	51
10-18	0	20
Season		
Spring	0	22
Summer	0	18
Fall (November)	3	16
Winter (February)	1	50
Fever > 38°C		
Yes	2	52
No	2	54
Vomiting		
Yes	3	72
No	1	34
Stool type		
Watery	3	83
Bloody	0	2
Non-watery, non-bloody	1	21
Dehydration		
Moderate	0	1
Mild	0	32
No	4	73
Abdominal pain		
Yes	3	101
No	1	5

USA, but lower than in Brazil, Finland, Japan, Pakistan, and Paraguay (Table 2).

In this study, HBoV-positive cases were detected during November to February. In some previous studies, HBoV1 was detected throughout the year, but higher incidences during winter months have been reported (Brieu *et al*, 2008; Yu *et al*, 2008; Risku *et al*, 2012). In China, HBoV2 also has been detected throughout the

Table 2
Prevalence of HBoV detected by PCR and features from different studies.

Location	Year	Outbreak	Stool sample		Patient	Prevalence	PCR detection of HBoV			Reference
			Source	no.		(%)	Genome region	Primer name	Genotype (no.)	
Taiwan	2015	Sporadic	AGE	110	C	4 (3.6)	VP1/VP2	AK-VP-F2 AK-VP-R2	HBoV (4)	Present study
Mexico	2015	Sporadic	AGE	76	C	1 (1.3)	-	-	HBoV (1)	Martinez *et al*, 2015
China	2015	Sporadic	AGE	1,128	C	17 (1.5)	-	-	HBoV 1 (14)	Zhang *et al*, 2015
Pakistan	2015	Sporadic	AGE	365	C	47 (12.9)	VP1	Boca F_2 Boca R_2^c	HBoV 1 (26) HBoV 2 (1) HBoV 2 (3)	Alam *et al*, 2015
Brazil	2015	Sporadic	AGE	105	C	44 (41.9)	VP1/VP2	AK-VP-F2 AK-VP-R2	HBoV 1 (3) HBoV 2 (7)	Campos *et al*, 2015
China	2014	Sporadic	AGE	1,121	C	25 (2.2)	NP1/VP1	HBoV-c1 HBoV-c2	HBoV 1 (9) HBoV 2 (15) HBoV 3 (1)	Zhao *et al*, 2014
China	2014	Sporadic	AGE	331	C	49 (14.8)	-	-	HBoV 1 (26) HBoV 2 (15) HBoV 3 (7) HBoV 4 (1)	Xiang *et al*, 2014
USA	2013	Sporadic	AGE	782	C	11 (1.4)	NS1	HBoVF2 HBoVR2	HBoV (11)	Chhabra *et al*, 2013
Italy	2013	Sporadic	AGE	246	C	5 (2)	VP1/VP2	AK-VP-F2 AK-VP-R2	HBoV (5)	Rovida *et al*, 2013
Iran	2013	Sporadic	AGE	294	C	11 (3.7)	NP1	188F 542R	HBoV (8)	Romani *et al*, 2013
Iran	2013	Sporadic	AGE	200	C	16 (8)	-	Boca-forward Boca-reverse	HBoV (16)	Monavari *et al*, 2013
Paraguay	2013	Sporadic	AGE	349	C	22 (6.3)	VP1/VP2	VP1-F, VP2-R	HBoV 1(37)	Proenca-Modena *et al*, 2013
Italy	2012	Sporadic	AGE	712	C	22 (3.1)	-	Adel1F Adel1R	HBoV (23)	Medici *et al*, 2012
Japan	2012	Sporadic	AGE	177	C	11 (6.2)	VP1/VP2	AK-VP-F2 AK-VP-R2	HBoV 1 (7) HBoV 2 (4)	Khamrin *et al*, 2012
Finland	2012	Sporadic	AGE	878	C	85 (9.7)	NS1	HBoV NS1 2^{nd} fwd HBoV NS1 2^{nd} rev	HBoV1 (49) HBoV2 (29) HBoV3 (8)	Risku *et al*, 2012
Thailand	2011	Sporadic	AGE	82	C	1 (1.2)	NP1	188F 542R	HBoV (1)	Pham *et al*, 2011
Japan	2011	Sporadic	AGE	247	C	5 (2)	NP1	188F 542R	HBoV (5)	Pham *et al*, 2011

C, children.

year, with the highest incidences from February to April (Xu *et al*, 2011). These epidemiologic features of HBoV infection have crucial implications for health authorities in Taiwan regarding detection and control measures in children with HBoV-associated AGE, in particular as their clinical symptoms do not appear to be different from nonhuman bocavirus-infected AGE patients. In addition, it is crucial to emphasize the existence of HBoV in Taiwan as implicated that HBoV sporadic gastroenteritis, seen worldwide, also has occurred in Taiwan.

In conclusion, this study is the first to examine HBoVs in children with AGE in Taiwan. This study investigated stool samples from children with AGE. Systematic surveillance and evidence-based studies are required to determine the transmission pathways and spread of HBoV.

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