Short Communication

Human Bocavirus Infection in Children with Acute Gastroenteritis and Healthy Controls

Thaweesak Chieochansin, Chitima Thongmee, Linda Vimolket, Apiradee Theamboonlers and Yong Poovorawan*

Center of Excellence in Clinical Virology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

(Received February 8, 2008. Accepted July 31, 2008)

SUMMARY: Human bocavirus (HBoV) is a novel virus which can cause respiratory tract disease in infants and children. Recently, the prevalence of this virus was studied worldwide not only in the respiratory tract, but also in the gastrointestinal tract. The results of this study focusing on the HBoV detection in stool samples showed that HBoV could only be found in the stool of children with acute gastroenteritis (0.9%), not in the healthy control samples. Moreover, the complete coding sequences of these HBoV also showed very conserved sequences.

Human bocavirus (HBoV) is a newly discovered member of the Parvoviridae family that has been identified as a cause of human respiratory tract infection (1). The prevalence of this virus has been reported to be from 1.5 to 19% in North America, Europe, South Africa and Asia indicating its global distribution (1-5). HBoV can be detected not only in the respiratory tract, but also in the gastrointestinal (GI) tract, as has been reported in Spain, Brazil, Korea and Hong Kong (6-9). However, all data presented thus far have exclusively originated from children with acute diarrhea. Comparative data on healthy children have not yet been provided. Therefore, the purpose of this study was to establish HBoV prevalence in children hospitalized with acute gastroenteritis in comparison with healthy children serving as controls.

The stool samples were collected from two population groups. The first was comprised of 225 children from 4 months to 4 years of age who had been admitted to the King Chulalongkorn Memorial Hospital, Bangkok and Buriram Provincial Hospital, Buriram and diagnosed with acute diarrhoea between November 2005 and September 2006. Some of these samples on rotavirus infection and characterization of the rotaviruses have been reported elsewhere (10,11). The control group consisted of 202 healthy children aged between 2 months and 5 years from the well baby clinic, King Chulalongkorn Memorial Hospital and from children attending a kindergarten in Bangkok, whose parents had volunteered their stool samples to be taken from May to September, 2007. All stool samples were diluted 1:10 in phosphate-buffered saline, thoroughly mixed on a vortex and centrifuged. Supernatants were collected and stored at -70°C until tested. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University for the stored specimens to be examined and normal stool samples to be collected. Prior to enrolling the healthy children, all participating parents were informed about the study objective and their written consent was obtained.

DNA and RNA were extracted from 150 μl of stool suspension using TRI REAGENT® LS (Molecular Research Center, Inc., Cincinnati, Ohio, USA) and solubilized in 20 μl of 8 mM NaOH or 12 μl of DePC treated water for DNA or RNA, respectively. For rotavirus detection, RNA was subjected to one-step reverse transcription-polymerase chain reaction (PCR) as described elsewhere (11). HBoV detection was performed by PCR with two sets of primers derived from the NS1 gene (118F and 542R) and the VP1/VP2 gene (VPF2 and VPR2), as previously described (1,12). Moreover, the HBoV positive samples were also subjected to sequence analysis as previously described (12). Positive and negative controls were included in both the PCR and extraction steps to avoid contamination and false negative results.

Of all 225 stool specimens collected from children with acute diarrhea, HBoV was detected in 2 samples (0.9%, 95% confidence interval [CI] = 0.15 - 2.9%), in which CUS8N (female, 3 years old) and CU74W (female, 8 months old) had been collected in September and May 2006, respectively. Strain CUS8N was isolated from a patient admitted to King Chulalongkorn Memorial Hospital, Bangkok, whereas CU74W was isolated from a patient admitted to Buriram Provincial Hospital, both of whom showed acute diarrhea as a symptom without any respiratory diseases. In contrast, HBoV could not be detected in any of the 202 healthy controls (0%, 95% CI = 0 - 1.5%). There was no statistically significant difference between the HBoV cases and the control group (P = 0.17). The HBoV prevalence in the GI tract of children with acute gastroenteritis determined in this study was lower than that established in Spain; 9.1% (6), but similar and almost identical to the results obtained in South Korea; 0.8% (7), Brazil; 2% (8) and Hong Kong; 2.1% (9) (Table 1). The complete coding sequences were determined as previously described (12), with nine PCR products amplified by using overlapping primer sets and subjected to sequencing. Upon analysis, two HBoV positive samples (CUS8N and CU74W) sequences were submitted to the GenBank database under accession nos. EU262978 and EU262979, respectively. Both sequences were more than 97% identical to the HBoV sequences previously published in the GenBank database. Human rotavirus was detected in 81 of 225 acute diarrhea stool samples (36%) with one sample coinfected with HBoV (CU74W), and in one of 202 healthy children (0.5%). Phylogenetic analysis indicated that HBoV is a rather conserved
virus (Figure 1). Based on amino acid alignment, variations almost exclusively appear in the capsid proteins (VP1 and VP2) while NS1 and NP1 represent the most conserved regions (data not shown). Comparison based on amino acid sequence identity showed a minimum to maximum identity range of 99.1 - 100%, 99.8 - 100% and 99.1 - 99.9% for the NP1, NS1 and VP1/VP2 gene, respectively. Based on previous results, the complete coding sequences of the HBoV isolates obtained from stool samples could be classified into Group 3 with 98 as the bootstrap value (Figure 1B).

HBoV could only be found in the stool of two children with acute gastroenteritis symptoms but could not be detected in the healthy control samples. Unfortunately, in this study HBoV analysis was not performed on isolates obtained from respiratory secretions and, thus, the possibility cannot be excluded that HBoV detected in patients originally infected the respiratory tract and subsequently passed through the GI tract. However, previous studies have demonstrated HBoV isolation from patients diagnosed with gastroenteritis only (6-9). The study presented here has limitations due to the small population size and insufficient amounts of specimens to test for other pathogens except HBoV and rotavirus. Our group’s previous study performed on children with respiratory tract symptoms did not show any seasonal distribution of HBoV infection (13). Therefore, the children recruited as healthy controls during this short period of time could serve as controls. We were not able to detect HBoV in normal pediatric stool samples, which may be due to the small sample sizes and very low prevalence of HBoV infection among these children. Hence, a large number of cases and controls ought to be evaluated. These preliminary data may prove crucial to advancing our understanding of HBoV infection of the GI tract. However, the pathogenicity of this virus in the respiratory and GI tract remains to be elucidated.

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Table 1. Summary of human bocavirus (HBoV) detection in children with gastroenteritis

<table>
<thead>
<tr>
<th>Country</th>
<th>Duration of sample collection</th>
<th>Sample size</th>
<th>HBoV Positive (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>Dec 2005-Mar 2006</td>
<td>ND</td>
<td>527</td>
<td>9.1 ND</td>
</tr>
<tr>
<td>South Korea</td>
<td>Jan 2005-Dec 2006</td>
<td>ND</td>
<td>926</td>
<td>0.8 ND</td>
</tr>
<tr>
<td>Brazil</td>
<td>Jan 2003-Dec 2005</td>
<td>ND</td>
<td>705</td>
<td>2.0 ND</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>Nov 2004-Oct 2005</td>
<td>ND</td>
<td>1,435</td>
<td>2.1 ND</td>
</tr>
<tr>
<td>Thailand</td>
<td>Nov 2005-Sep 2006 May 2007 - Sep 2007</td>
<td>225 202</td>
<td>0.9 0</td>
<td>This study</td>
</tr>
</tbody>
</table>

1) Samples from children with gastroenteritis.
2) Samples from healthy children.
ND, not determine.

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Fig. 1. Phylogenetic analysis of the complete coding sequences of human bocavirus (HBoV) with bovine parvovirus (BPV), canine minute virus (CMV) and Parvovirus B19 (B19) serving as the out group sequences (A) and without outer group that described previously (11) (B). The tree was created by Neighbor-Joining method and bootstrapped with 1,000 replicates. The bootstrap numbers are given for node. Numbers above the nodes correspond to bootstrap values derived from 1,000 pseudoreplicates. The two isolates from this study are represented by arrows.
ACKNOWLEDGMENTS
This study was supported by the Thailand Research Fund (Senior Research Scholar), Royal Golden Jubilee Ph.D. Program, the Thailand Research Fund, the Center of Excellence in Clinical Virology, Chulalongkorn University, Biomedical Science, Graduate School, Chulalongkorn University and the Commission on Higher Education, Ministry of Education.

REFERENCES