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Newly recognized bocaviruses (HBoV, HBoV2) in children and adults with gastrointestinal illness in the United States

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ABSTRACT

Background: The human bocavirus (HBoV) is a newly recognized parvovirus associated with respiratory and gastrointestinal disease. Recently, two new members of the parvovirus family have been recognized, HBoV2 and HBoV3.
Objectives: Here we investigate stool and respiratory samples for the presence of HBoV, HBoV2 and HBoV3.
Study design: Stool samples collected from 12/1/2007 to 3/31/2008 were screened by PCR for the presence of HBoV, HBoV2, and HBoV3. Extracted DNA from respiratory specimens archived between 10/17/2005 and 3/29/2006 were screened by PCR for HBoV2 and HBoV3. Medical records for all bocavirus positive patients were reviewed.
Results: Of 479 stool samples screened, 328 (68.5%) were from adults, and 151 (31.5%) were from children. Sixteen (3.4%) patients were positive for the presence of a bocavirus, including 10 (2.1%) HBoV and 6 (1.3%) HBoV2. No HBoV3 was detected in stool samples. Frequency of HBoV and HBoV2 in stool samples from children was 3.3% and 0.7%, and from adults was 1.5% and 1.5% respectively. Clinical findings in patients with HBoV and HBoV2 in stool include diarrhea (50% and 83.3%), abdominal pain (40%, 33.3%), and cough (10%, 50%). Of 868 respiratory samples screened, none were positive for either HBoV2 or HBoV3.
Conclusions: The newly recognized parvovirus HBoV2 circulates in the United States. Patients with bocaviruses in stool have evidence of gastrointestinal illness. HBoV2 was not detected in respiratory samples. HBoV3 was not detected in either stool or respiratory samples.

1. Background

Gastroenteritis is a major cause of morbidity and mortality, with acute gastroenteritis causing 3 million deaths annually worldwide.[1] Many viruses are associated with acute gastroenteritis, including rotaviruses, astroviruses, adenoviruses, and calciviruses.[2–5] Despite sensitive molecular techniques, most cases of gastroenteritis remain without an identifiable cause, suggesting the presence of unrecognized pathogens.[6–8]

Parvoviruses have been recognized as a cause of gastrointestinal illness in numerous animal species. Diarrheal illness has been associated with parvovirus species in dogs and cats, and hepatitis in geese and hamsters.[9] Prior to the description of human bocaviruses, the only recognized human parvoviruses were parvovirus B19,[9] PARV4[10] and PARV5.[11]

Since its discovery in 2005, the human bocavirus (HBoV) has been associated with upper and lower respiratory tract illness.[12–18] HBoV is now implicated in gastrointestinal illness with 0.8–9.1% of stool samples screening positive for HBoV DNA.[19–25] Recently, two viruses related to HBoV have been recognized, provisionally named human bocavirus 2 (HBoV2) and human bocavirus 3 (HBoV3). Human bocavirus 2 was discovered in stool samples from patients with acute flaccid paralysis in Pakistan.[26] Soon thereafter, researchers in Australia identified HBoV2 as well as HBoV3.[27]

In genomic analysis, HBoV2 has between 67% and 80% nucleotide homology compared to HBoV.[26] Analysis of the HBoV3 genome shows close homology to HBoV in the nonstructural protein encoding regions NS1 and NP-1 (87% nucleotide similarity), but is more similar to HBoV2 in the structural protein encoding regions VP1/VP2 (77% nucleotide similarity). This suggests that HBoV3 may have arisen from a recombination event between HBoV and HBoV2.[26] The epidemiology and clinical man-

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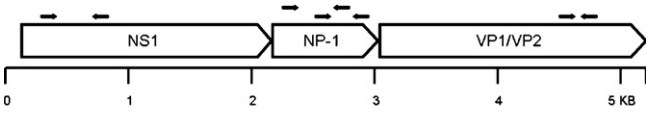

Primer Set	Gene target	Primer	Location	Amplicon size (bp)
HBoV	NP-1	Forward: GACCTCTGTAAGTACTATTAC Reverse: CTCTGTGTTGACTGAATACAG	2351-2704	353
HBoV2	VP1/VP2	Forward: TCCTTCAAACAACAACGCAG Reverse: TGCGGTGTGTCTTCTTTCTG	4706-4832	126
HBoV3	NS1	Forward: TAGAAGATCGCCATTTTGGG Reverse: AGCGAAGAGCATCCACTTGT	429-842	413
Bocavirus Consensus	NP-1	Forward: TCGTCTTACACTGCTTCGA Reverse: GCGCTTTCTGTATTTTTGATCTA	2593-2900	307

Fig. 1. Human bocavirus primer locations. Diagrammatic representation of a typical bocavirus genome and primer sets used in screening and analysis. Locations based on the primer locations of the following isolates: HBoV (GenBank accession number NC_007455), HBoV2 (NC_012042), HBoV3 (NC_012564) and Bocavirus Consensus (Displayed primer position based on NC_007455; GC clamp underlined). Approximate primer locations are marked with black arrows on genomic map.

ifestations associated with these viruses are only now being realized.[28]

2. Objectives

To investigate the presence of HBoV, HBoV2, and HBoV3 in patients with gastrointestinal illness and to determine if HBoV2 and HBoV3 have involvement with respiratory tract disease.

3. Study design

3.1.1. Gastrointestinal sample collection

From December 1, 2007 to March 31, 2008, stool samples were collected from the core laboratory at University Hospitals – Case Medical Center of Cleveland, OH. We obtained all clinical specimens from children and adults that screened negative for *Clostridium difficile* A and B toxin by enzyme immunoassay (Meridian Bioscience, Cincinnati, OH). For each month within the study period, random samplings of stool specimens were selected for bocavirus screening. Other than age, no selection criteria were used.

3.1.2. Respiratory sample collection

Archived DNA originating from pediatric and adult respiratory samples previously analyzed for HBoV[15] were selected for HBoV2 and HBoV3 screening. DNA originated from patients presenting from October 17, 2005 to March 29, 2006 and have been stored at −20 °C. All clinical specimens had negative results for RSV, parainfluenza viruses (1–3), influenza A and B, and adenovirus by direct immunofluorescence assay (DFA).

3.1.3. DNA extraction and polymerase chain reaction (PCR)

Nucleic acid from each stool specimen was extracted with the MagMAX™-96 Total Nucleic Acid Isolation Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol using 0.3–0.5 g of stool. After disruption and centrifugation, 175 μl of supernatant were used for nucleic acid extraction.

Primers used for HBoV screening originated from published reports[15] (Fig. 1). Primers used for screening of HBoV2 and HBoV3 were constructed using GenBank sequences provided by the original reports.[26,27] The sensitivity and specificity of bocavirus primer sets are unknown. Recent analysis suggest detection rates of HBoV by PCR is similar despite genes targeted or primer sets used.[29] Bocavirus consensus primers were constructed targeting a conserved region of the NP-1 gene identified through ClustalW multiple sequence alignment of human and animal parvovirus sequences (Bioedit, Version 7.0.5.3[30]). A GC clamp was applied to the consensus reverse primer to ensure compatibility of annealing temperature.

Each sample was separately screened for the presence of HBoV, HBoV2 or HBoV3 by PCR using Platinum Taq Polymerase (Invitrogen, Carlsbad, CA) according to the manufacturer's specification. Amplification conditions for all reactions were as follows: 95 °C for 3 min; followed by 40 cycles of 94 °C for 1 min, 55 °C for 1 min (for bocavirus consensus primer reaction, annealing was set at 50 °C), and 72 °C for 30 s; and completed with a final extension cycle of 72 °C for 10 min. Each set of polymerase chain reactions contained appropriate positive and negative controls. An amplicon of the HBoV3 NS1 region was generously provided by Jane Arthur and Rodney Ratcliff for use in this study. Sequence confirmation was performed on ABI Prism 3730 DNA Analyzer automated sequencers at the Genomics Core Facility, Case Western Reserve University School of Medicine.

All isolates positive for bocaviruses were screened for the presence of common gastrointestinal viruses including adenovirus, rotavirus, and noroviruses by RT-PCR using primer sets and reaction conditions as described.[31–33]

3.1.4. Clinical data

Medical records of all bocavirus positive patients were reviewed. Demographic data, history of illness, physical exam and laboratory studies were recorded on a standard collection form. Collection of specimens and clinical data was approved by the University Hospitals – Case Medical Center Human Investigation Committee and is compliant with the Health Insurance Portability and Accountability Act regulations.

3.1.5. Phylogenetic analysis

Cleveland isolates of HBoV and HBoV2 were amplified using a bocavirus consensus primer set and sequenced. The region of analysis corresponded to a 307 base pair region of the NP-1 gene spanning nucleotides 2593–2900 of the HBoV genome (Fig. 1). The phylogenetic analysis included representative samples of Cleveland isolates in addition to GenBank sequence data from original bocavirus prototype strains [HBoV, NC_007455; HBoV2, NC_012042; HBoV3, NC_012564] and animal parvoviruses [Bovine Parvovirus, DQ335247; Canine Minute Virus, NC_004442]. ClustalW alignment was generated using Bioedit 7.0.5.3 alignment software.[30] One hundred bootstrap data sets were created using the PHYLIP program SEQBOOT. Phylogenetic analyses were constructed using the PHYLIP program DNAML, with the default transition to transversion ratio of 2.0 and 1 jumble.[34]

4. Results

Four hundred seventy-nine stool samples were collected between December 2007 and March 2008 corresponding to the peak prevalence of HBoV in several North American studies.[15,35] Of these, 151 were from children (<18 years of age), and 328 from adults (≥18 years of age). Sixteen samples (3.4%) contained one of the bocavirus species; 6 from children, and 10 from adults (4.0% and 3.0% respectively) (Table 1). Ten (2.1%) stool samples screened positive for HBoV; 5 from children and 5 from adults (3.3% and 1.5% respectively). Six (1.3%) stool samples contained HBoV2; 1 from a child, and 5 from adults (0.7% and 1.5% respectively). No stool sample screened positive for multiple bocaviruses. HBoV3 was not detected in any stool samples.