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Short communication

Human bocavirus species 2 and 3 in Brazil

Norma Santos[a,*], Teresa C.T. Peret[b], Charles D. Humphrey[a,b], Maria Carolina M. Albuquerque[a], Raquel Cirlene Silva[a], Fabrício José Benati[a], Xiaoyan Lu[b], Dean D. Erdman[b]

[a] Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-590, Brazil
[b] Center for Diseases Control and Prevention, Atlanta, GA 30333, United States

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ABSTRACT

Background: The newly described human bocavirus (HBoV) species 2 and 3 have been repeatedly detected in stool strengthening the possibility that these viruses might present a tropism for the gastrointestinal tract and may be etiological agents of diarrhea.
Objective: In this study we assessed the presence of HBoV2 and HBoV3 in stool specimens from Brazilians with acute gastroenteritis.
Study design: Stool samples from Brazilian patients with acute diarrhea were analyzed for HBoV2 and HBoV3 by PCR assay. Full or partial genome sequences were obtained for selected isolates. Electron microscopy analysis was used to investigate virus morphology.
Results: Electron microscopy confirmed the presence of virus-like particles in HBoV PCR-positive specimens, with morphology similar to other members of the Parvoviridae family. Five samples out of 807 (0.6%) were positive for HBoV3. Three of the HBoV3-positive patients were HIV/AIDS positive. A selected group of 144 samples was also tested for HBoV2 and 30 samples (20.8%) were positive, 11 of which were HIV/AIDS positive.
Conclusion: This study reports the detection and genetic characterization of HBoV3 and HBoV2 in the stool of Brazilian patients with acute diarrhea. This is the first description of HBoV3 outside Australia, suggesting a wide global distribution of this virus. Further studies are needed to better understand the role of HBoV in gastrointestinal infections, particularly among patients with HIV/AIDS.

1. Background

Human bocavirus (HBoV), a member of the family Parvoviridae, was proposed to be an etiologic agent of respiratory disease.[1] HBoV has also been detected in the blood of some patients with respiratory illness[2,3] and has become recognized as a possible agent of acute gastroenteritis.[4–11]

The HBoV genome possesses two major ORFs encoding a nonstructural protein (NS1) and at least two capsid proteins (VP1 and VP2). There is also a third ORF that encodes a nonstructural protein (NP1) with unknown function.[1] Based on the sequences of the NS1 gene, there are three HBoVs species: HBoV1, HBoV2 and HBoV3.[12,13]

2. Objective

We report the full genomic sequences of novel strains of HBoV3 and the detection of HBoV2 and HBoV3 in stool specimens from Brazilians with acute gastroenteritis.

3. Study design

3.1. Stool specimens

Eight hundred and seven stool samples from Brazilian patients with acute diarrhea were analyzed for HBoV. These samples were randomly selected based solely on the stool availability. The study protocol was approved by the Ethics Committees of the Instituto de Puericultura e Pediatria Martagão Gesteira and the Hospital Universitário Clementino Fraga Filho of the Federal University of Rio de Janeiro.

3.2. Virus strains

The MC8 strain was detected in the stool sample from an 18-month-old boy, and was the only pathogen detected in the stool.[5]

* Corresponding author at: Departamento de Virologia, Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Cidade Universitária, CCS – Bl. I. Ilha do Fundão, Rio de Janeiro 21941-590, RJ, Brazil. Tel.: +55 21 2560 8344x165; fax: +55 21 2560 8028.
E-mail address: nsantos@micro.ufrj.br (N. Santos).

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The IM10 strain was detected in the stool from a HIV-positive, 9-year-old boy, which was also positive for norovirus.[5]

3.3. DNA sequencing

DNA extraction was performed using NucliSens Magnetic Extraction Reagent (Biomerieux, Durham, NC). Sequencing was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit and the 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequence editing and assembling were performed using Sequencher v4.7 (Gene Codes Corporation, Ann Arbor, MI). Sequence alignments were performed using Clustal W and phylogenetic trees were constructed using neighbor joining, maximum parsimony and maximum likelihood methods implemented in PAUP* 4.0.[14] Full-genome sequences of HBoV3 strains MC8 and IM10 were deposited into GenBank (GQ867666 and GQ867667).

3.4. Negative stain EM

One HBoV3-positive stool specimen was concentrated by sucrose cushion ultracentrifugation prior to negative stain electron microscopy. Specimens were viewed in an FEI Technai BioTwin^R transmission electron microscope (FEI Company, Hillsboro, OR).

3.5. HBoV1/3 PCR assay

A PCR assay for HBoV1 and HBoV3 was developed that targets the NS1 gene. Primer set P1 (CAT ATT ATA GTT GGG GGA GAA GG) and P2 (GGT AGT TTT TGA AGA AGC GAA GAG) amplifies a 286 bp fragment of both HBoV species; primer set P5 (TCA GAA GCA TCG GAA GTG GGT GTT) and P6 (ATG TGA GGC TTT ATG CTG GCT GAA) amplifies a 440 bp fragment of only HBoV3. The PCR reaction was performed using $MgCl_2$ at a final concentration of 3 mM and each primer at a concentration of 0.4 μM. Amplification consisted of 1 step of 95 °C/15 min; 45 cycles of 94 °C/20 s, 52 °C/20 s, 72 °C/40 s, followed by extension at 72 °C/10 min. The PCR products were detected by agarose gel electrophoresis and ethidium bromide staining.

3.6. HBoV2 PCR assay

HBoV2 was tested by using nested-PCR reactions previously described.[12] The amplified DNAs of positive samples were sequenced. The nucleotide sequences obtained in this study were deposited in GenBank (GU256650–GU256655).

4. Results

4.1. Genome analysis

The complete coding sequences of HBoV3 strains MC8 and IM10 were determined. Both strains were closely related, showing greater than 99% sequence identity. The predicted proteins for the NS1, NP1, VP1/VP2 open reading frames are similar to that described for HBoV3.[13]

Phylogenetic analyses confirmed that MC8 and IM10 form distinct clade with published HBoV3 strain W471 (Fig. 1). The genomes of these new viruses showed 79.7–80.6% identity with HBoV1; 79.2–80.7% identity with HBoV2; and 97.8–99.2% with published HBoV3. Pairwise analysis demonstrated that the nucleotide divergence of NS1 and NP1 was higher between the Brazilian strains and HBoV2 while divergence of the structural gene (VP1/VP2) was higher between the Brazilian strains and HBoV1.

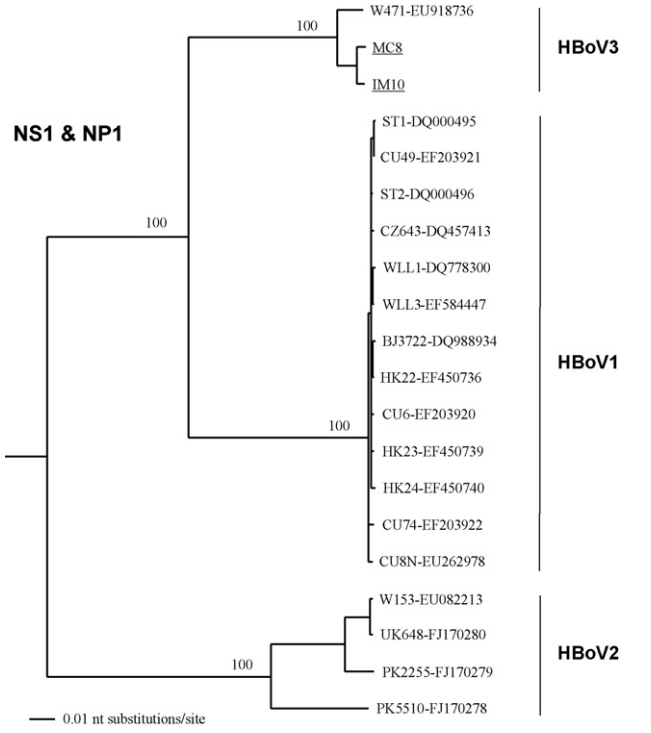

Fig. 1. Neighbor-joining trees of HBoV NS1 and NP1 (combined) nucleotide sequences. Bootstrap values (1000 replicates) are indicated at nodes of HBoV1, HBoV2 and HBoV3 clades. Trees were outgrouped with MVC sequence NC_00442 (not shown). GenBank accession numbers are shown with published HBoV strain names.

4.2. Electron microscopy

HBoV3 PCR-positive specimens contained 22–24 nm parvovirus-like particles (Fig. 2).

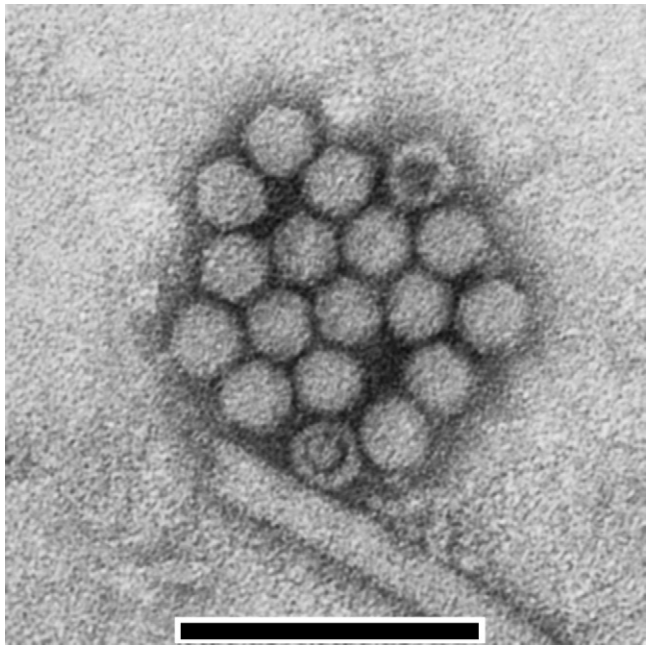

Fig. 2. HBoV cluster as shown by negative stain electron microscopy. The stain used was 5% ammonium molybdate–1% trehalose in water, pH 6.9. The bar marker represents 100 nm.