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Human bocavirus in hospitalized children with acute gastroenteritis in Russia from 2010 to 2012

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ABSTRACT

Human bocavirus (HBoV) can cause respiratory diseases and is detectable in the stool samples of patients with gastroenteritis. To assess the prevalence of HBoV in children hospitalized with acute gastroenteritis in Novosibirsk, Russia, as well as its genetic diversity and the potential role in the etiology of gastroenteritis in this region, a total of 5502 stool samples from children hospitalized with gastroenteritis from 2010 to 2012, $n = 5250$, and healthy children, $n = 252$, were assayed for the presence of HBoV DNA by semi-nested PCR. The HBoV DNA was found in 1.2% of stool samples from children, with gastroenteritis varying from 0.5% in 2012 to 1.7% in 2011. The prevalence of HBoV in healthy children was 0.3%. HBoV strains were detected throughout the year with an increase in the fall–winter season. In 87% of cases, HBoV was detected in children before 1 year of age. All known HBoV genetic variants have been detected in Novosibirsk, although with different prevalences: HBoV2 > HBoV1 > HBoV4 > HBoV3. At the beginning of 2011, HBoV2 replaced HBoV1 as the most prevalent variant. The median age of children with detected HBoV1 was 8.3 months, and that with HBoV2 was 8.0 months. All HBoV-positive samples were assayed for the presence of the rotaviruses A and C, norovirus GII, astrovirus, enterovirus, adenovirus F, *Salmonella* spp., *Campylobacter* spp., *Shigella* spp., and EIEC. HBoV1 and HBoV2 as single agents were found in 45.8% and 60% samples, respectively, although this difference was not statistically significant. In the case of co-infections, HBoV was most frequently recorded with rotavirus A and norovirus GII. This study demonstrated that the detection rate of HBoV in stool samples from children with gastroenteritis was low, although both HBoV1 and HBoV2 could be found as the sole agents in children with gastroenteritis in Novosibirsk.

1. Introduction

Human bocavirus (HBoV), which belongs to the family *Parvoviridae*, was first discovered in the nasopharyngeal aspirate samples from children with upper and lower respiratory diseases in Sweden in 2005 (Allander et al., 2005). Since 2007, HBoV has been detected in stool samples from children with gastroenteritis (GE) (Albuquerque et al., 2007; Chieochansin et al., 2008; Lau et al., 2007; Lee et al., 2007; Vicente et al., 2007). From 2009 to 2010, genetically different HBoV variants were found in stool samples and designated as HBoV2–HBoV4 (Arthur et al., 2009; Kapoor et al., 2009, 2010), whereas the previously discovered virus was referred to as HBoV1 (Chow and Esper, 2009). Currently, a new taxonomy for the family *Parvoviridae* is proposed; according to this new system, HBoV1–HBoV4 along with *gorilla bocavirus* belongs to the genus *Bocaparvovirus*, in which HBoV1 and HBoV3 are members of the species *Primate bocaparvovirus 1*, whereas HBoV2 and HBoV4 belong to the species *Primate bocaparvovirus 2* (Cotmore et al., 2014).

HBoVs have a small (18–26 nm) nonenveloped icosahedral virion (Gurda et al., 2010) with a linear single-stranded DNA. The genome is approximately 3.5-kb long (Schildgen et al., 2012) and contains three open reading frames (ORFs). ORF1 encodes two variants of the nonstructural multifunctional protein NS1, ORF2 (a unique feature of HBoV) encodes an additional nonstructural protein, a nuclear phosphoprotein NP1 (Gurda et al., 2010; Schildgen et al., 2012), ORF3 contains two overlapping genes, which encode two major structural proteins, VP1 and VP2 (Allander et al., 2005). The degree of identity of the HBoV amino acid sequences belonging to different genotypes is rather small, amounting to 70% to 80% (Kapoor et al., 2009, 2010). In particular, the degree of homology between the NS1, NP1, and VP1/VP2 amino acid sequences of HBoV1 and HBoV2 genotypes is 78%, 67%, and 80%, respectively. Due to high genetic divergence, HBoV2 has been subdivided into three variants: HBoV2a, HBoV2b, and HBoV2c (Kapoor et al., 2009, 2010). By this time, HBoV has been detected in stool samples from

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children with GE worldwide, including Europe (Albuquerque et al., 2007; Kapoor et al., 2009; Risku et al., 2012), Asia (Ahn et al., 2014; Alam et al., 2015; Cheng et al., 2011; Jin et al., 2011; Kapoor et al., 2009; Khamrin et al., 2012; Lau et al., 2007; Monavari et al., 2013; Nakanishi et al., 2009; Wang et al., 2011; Zhang et al., 2014), Americas (Campos et al., 2015; Chhabra et al., 2013; Kapoor et al., 2011; Levican et al., 2013; Proenca-Modena et al., 2013; Santos et al., 2010), Africa (Kapoor et al., 2010), and Australia (Arthur et al., 2009) and the prevalence of HBoV varies from 0.5% to 20% depending on the region. However, studies of HBoV in GE in Russia are limited and do not allow to assess its role in the GE etiology in this region (Babkin et al., 2013; Zhirakovskaia et al., 2013). The aim of this study was to assess the prevalence of HBoV in children hospitalized with acute GE and healthy children (controls) in Novosibirsk, Russia and to characterize the genetic diversity of the detected HBoV strains.

2. Materials and methods

2.1. Patients and sample collections

A total of 5,250 stool samples were collected from children ≤5 years of age hospitalized with diarrhea in the Novosibirsk Municipal Children's Hospital No. 3 from February 2010 to December 2012. Most cases of GE from Novosibirsk city and adjacent rural areas are referred to this hospital. The ages of children with diarrhea ranged from 3 days to 60 months (mean age ± SD, 7.44 ± 7.63 months). The majority of patients (85.2%) were 1 to 12 months old. The ratio of boys to girls was 1.2:1. Stool samples were collected on the day of hospital admission. A comparison group included stool samples from 252 healthy children aged ≤5 years, which underwent a regular outpatient examination in Novosibirsk Regional Hospital No. 1 in 2012. These children displayed no fever, diarrhea, or vomiting during the previous three weeks. The ages of healthy children ranged from 1 to 60 months (mean age ± SD, 27.65 ± 14.45 months). The majority of these children (64.6%) were 1–36 months of age. The ratio of boys to girls was 1.1:1. All stool samples were stored at −20 °C. Stool suspensions 10% (w/v) in phosphate-buffered saline (PBS) containing glycerol (15%) were stored at −80 °C before use.

This study was approved by the Ethical Committee with the State Research Center of Virology and Biotechnology Vector (FWA00000621).

2.2. HBoV detection

Viral nucleic acid was isolated from stool suspensions by affinity sorption using AmpliSens® RIBO-sorb extraction kit (Central Research Institute of Epidemiology, Russia) according to the manufacturer's protocols. The samples were assayed for the presence of HBoV DNA by semi-nested polymerase chain reaction (PCR) using DreamTaq Green PCR Master Mix (Thermo Scientific™, Lithuania) and the primers HBoV2-sf2 (5′-TGCTTCAACAGGCAAAACAA-3′) and HBoV2-sr1 (5′-AGGACAAAGGTCTCCAAGAGG-3′) for the first round and HBoV2-sf2 and HBoV2-sr2 (5′-TCCAAGAGGAAATGAGTTTGG-3′) for the second round of PCR (Kapoor et al., 2009).

2.3. Detection of other enteric viruses and bacteria

All HBoV positive samples were screened for the presence of nucleic acids of enteric viruses and bacteria. The AmpliSens® RIBO-sorb extraction kit and AmpliSens® Reverta-L reverse-transcription kit (Central Research Institute of Epidemiology, Russia) were used according to the manufacturer's protocols (Podkolzin et al., 2009). AmpliSens® Rotavirus/Norovirus/Astrovirus-FL and AmpliSens® Enterovirus-FL PCR kits (Central Research Institute of Epidemiology, Russia) were used for detection of group A human rotavirus (HRVA), human norovirus genogroup II (HNoV GII), human astrovirus (HAstV) and enterovirus RNAs, respectively, according to the manufacturer's protocols. Group C human rotavirus (HRVC) was identified using previously described primers specific for VP6 gene, RCVP6-F and RCVP6-R (Nilsson et al., 2000). DNA from *Salmonella* spp., *Campylobacter* spp., *Shigella* spp. and enteroinvasive *Escherichia coli* (EIEC) as well as adenovirus F (HAdV-F) DNA were extracted using the AmpliSens® RIBO-sorb Extraction Kit and detected by PCR using the AmpliSens® *Shigella* spp. and EIEC/*Salmonella* spp./*Campylobacter* spp.-FL and AmpliSens® AGE-screen-FL PCR kits (Central Research Institute of Epidemiology, Russia) according to the manufacturer's protocols. All extracted nucleic acids were stored at −80 °C..

2.4. HBoV genotyping and phylogenetic analysis

The PCR products were purified by electrophoresis in 0.6% "SeaKem® GTG-agarose" (Lonza, ME). The Sanger sequencing reaction was conducted using "BigDye™ Terminator v. 3.1 Cycle Sequencing kit" (Applied Biosystems Inc., CA) in both directions. The corresponding products were analyzed using an ABI 3500 Genetic Analyzer (Applied Biosystems Inc., CA). All obtained sequences were compared with those of reference strains available in the NCBI website using the BLASTN 2.2.31 +. Molecular phylogenetic analyses were conducted using Maximum Likelihood (ML) method based on Hasegawa–Kishino–Yano (HKY) nucleotide substitution model in MEGA6.06 (Tamura et al., 2013). The statistical significance of the branch was assessed by bootstrap resampling analysis (1,000 replicates).

2.5. Statistical analysis

Statistical analysis of the data was performed using R-language 3.0.1 and Excel based on earlier published recommendations (Armitage et al., 2002). A comparison of non-parametric variables and a definition of the Odds Ratio with 95% confidence intervals were performed by Fisher's exact test. p-Values < 0.05 were considered to be statistically significant.

2.6. Nucleotide sequence accession numbers

The partial nucleotide sequences determined in this study were deposited in GenBank under the following accession numbers: JX046072-JX046105 and KJ492895-KJ492923.

3. Results

3.1. HBoV detection

To assess the HBoV molecular epidemiology in Novosibirsk, Russia, a total of 5502 stool samples from young children hospitalized with acute GE ($n = 5{,}250$) and healthy children ($n = 252$) were assayed for the presence of HBoV DNA by semi-nested PCR using only three primers from a primer set described by Kapoor et al. (Kapoor et al., 2009). According the PrimerBlast the selected primers were specific to a conservative region of the NS1 gene of HBoV1–HBoV4 and gorilla bocavirus. The size of the PCR product including a fragment of the NS1 gene (ORF1) was 496 bp.

The HBoV DNA was found in 1.2% (62/5250) of the stool samples from children with acute GE. The HBoV prevalence in GE cases varied from year to year, amounting to 1.3% in 2010 (19/1467), 1.7% in 2011 (34/1980), and 0.5% in 2012 (9/1817). The HBoV prevalence in 2012 differed in a statistically significant manner from those in 2010 and 2012 ($\chi^2 = 6.14$ $p = 0.025$ and $\chi^2 = 12.63$, $p = 0.005$, respectively). Only one HBoV-positive sample (0.3%) was found in the collection of samples from healthy children. No statistically significant differences were observed between the HBoV prevalence in the sample collections of healthy children and those hospitalized for GE ($\chi^2 = 0.25$, $p = 0.05$). Moreover, the HBoV prevalence in 2012 in specimens from children