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Human Bocavirus in Acute Gastroenteritis in Children in Brazil

Gubio Soares Campos, Madina Lyve Silva Sampaio, Aline Dorea Luz Menezes, Dellane Martins Tigre, Lilia Ferreira Moura Costa, Fabio Alexandre Chinalia, and Silvia Ines Sardi*

Laboratory of Virology-Health Science Institute, Federal University of Bahia, Salvador, Bahia, Brazil

Epidemiological surveillance for Human Bocavirus (HBoV) was conducted on 105 fecal specimens from children with acute gastroenteritis in Bahia, Brazil. Among of a total 105 stool samples, 44 samples were positive for HBoV as detected by nested-PCR. Of the 44 positive samples, co-infections with other enteric viruses (Norovirus, Adenovirus, and Rotavirus) were found in 12 pediatric patients. Mixed infections among HBoV with Norovirus were frequently observed in this population. The phylogenetic analysis identified the presence of HBoV-1, and HBoV 2A species. This study shows that HBoV is another viral pathogen in the etiology of acute gastroenteritis in children in Bahia, Brazil. ***J. Med. Virol. 88:166–170, 2016***.

KEY WORDS: bocavirus; children; gastroenteritis; Brazil

INTRODUCTION

Infectious gastroenteritis, a pathological condition frequently observed in both developed and underdeveloped countries, is considered to be a major cause of mortality and morbidity in children, especially during the first few years of life. The etiological agents of this disease can be parasitic, bacterial, or viral. Among the viral agents, the primary agents are Norovirus (NoV), Rotavirus (RV), Adenovirus (AdV), or Human astrovirus (HAstV) [Rimoldi et al., 2011]. Additionally, the Human Bocavirus (HBoV) has been recently discovered to cause gastroenteritis [Allander et al., 2005].

HBoV, a human parvovirus recently discovered to be an etiologic agent of gastroenteritis belongs to the *Parvoviridae* family, and it is classified into the subfamily *Parvovirinae*, and genus Bocavirus. Owing to its close relation to the Bovine parvovirus (BPV) and the Canine minute virus (CnMV), the new virus was named as Human Bocavirus [Kapoor et al., 2009]. The HBoV family is composed of four distinct species: HBoV-1 [Cashman and O'Shea, 2012]; HBoV-2 [Albuquerque et al., 2007], HBoV-3 [Allander et al., 2005], and HBoV-4 [Kapoor et al., 2009]. HBoV-2 is further classified into two variants: 2A and 2B [Arthur et al., 2009]. The viral genome consists of a linear single-stranded DNA, it is a non-enveloped virus, with an icosahedral capsid consisting of approximately 60 capsomeres. HBoV has three open reading frames (ORFs) encoding four proteins: VP1 and VP2, which are the structural proteins (capsid) of the virion; NS1, a non-structural protein; and the nucleoprotein NP1, with unknown function [Cashmann and O'Shea, 2012].

HBoV, was initially identified in Sweden by Allander et al., 2007, from the respiratory secretions in patients with pneumonia. Subsequent reports have highlighted the presence of HBoV in stool samples collected from patients with gastroenteritis, which suggested a tropism for the digestive tract. The role of HBoV in pneumonia and gastroenteritis remains to be elucidated. However, it has been increasingly detected in patients with gastroenteritis, particularly in children aged between 6 and 24 months [Cashmann and O'Shea, 2012]. HBoV has also been found to co-infect humans with other enteric viruses, at very high frequencies. The signs and symptoms of HBoV-induced acute gastroenteritis are similar to those observed in patients infected with another animal parvovirus, the Canine parvovirus. These symptoms include the presence of leukopenia and frequent diarrhea [Chieochansin et al., 2010].

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*Correspondence to: Silvia Ines Sardi, Instituto de Ciências da Saúde, Laboratório de Virologia, Universidade Federal da Bahia, Av. Reitor Miguel Calmon s/n 40110-100-Vale do Canela, Salvador, Bahia, Brazil.
E-mail: sissardi@yahoo.com.br

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In Brazil, patients younger than five years with clinical symptoms of gastroenteritis (with or without symptoms of respiratory infection) are known to acquire HBoV-1 or HBoV-3 infection, either as a single agent or in co-infection with other viral intestinal pathogens [Schildgen et al., 2008; Silva et al., 2010]. However, to date, HBoV 2 A has not been reported in children with gastroenteritis in Brazil. This study aims to demonstrate that HBoV 1 and HBoV 2 are the new etiological agents in pediatric patients with acute gastroenteritis in Bahia, Brazil, either alone, or in co-infection with other enteric viruses.

MATERIALS AND METHODS

Sample Collection

This research was conducted with 105 stool specimens obtained from children with acute gastroenteritis attending in the emergency pediatric hospital in Salvador, city of Bahia, Brazil. The ages of the patients enrolled in the study ranged from neonate to five years old. The study period was from January to July, 2012 with the approval of the Ethical Committee from University of Salvador, Bahia, Brazil, protocol number 04.11.12. FR: 345422.

Detection of Nov, RV, and AdV

All stool specimens were screened using the commercial immunoenzimatic tests RIDASCREEN® 3rd generation Norovirus (R-Biopharm, Germany), RIDASCREEN® Adenovirus (R-Biopharm, Germany) and RIDASCREEN® Rotavirus (R-Biopharm, Germany) for NoV, AdV, and RV, respectively.

Detection of HBoV: Nested-PCR

Total DNA extraction was carried out on stool samples from symptomatic and asymptomatic children using QIAmp Viral DNA Mini kit (Qiagen, Brazil). Stools of asymptomatic children were used as the control group. First, stools were dissolved on phosphate-buffered saline solution at a 1:2 ratio (w/v); centrifuged (6,000 xg 1 min) and the supernatant was then used for the total DNA extraction. The positive result of a nested-PCR targeting the partial region of the VP1/2 gene was considered as indicative of the presence of HBoV. The primers described by Kapoor et al. (2009) are considered universal for HBoV1 and HBoV2 strains. The first-round of the nested-PCR was carried out using the AK-VP-F1 and AK-VP-R1 primers set, and the second round using the AK-VP-F2 and AK-VP-R2, respectively. The cycling conditions are described by the former authors. The final PCR product of 576 bp was subjected to gel electrophoresis (2% agarose), stained with ethidium bromide, and visualized on UV-transilluminator (301 nm).

Sequencing and Phylogenetic Analysis

PCR products were treated using the QIAquick PCR purification kit (Quiagen, Brazil) and products were sequenced using ABI model XXX and sequences deposited on NCBI (accession numbers KM366083–KM366092). Phylogenetic analyses were carried out by comparing the obtained sequences with others of type-like category which were listed on NCBI GenBank. Sequences were treated and aligned using BioEdit and CLUSTRAL W software and phylogenetic tree using Mega 6.0 software. It was used the Hasegawa-Kishino-Yano algorithm with 1,000 bootstrap replication.

RESULTS

HBoV Virus Detection

The nested-PCR analysis of 105 stool samples obtained from children with symptoms of acute gastroenteritis showed that 42% of the samples (44/105) were positive for HBoV, while 58% (61/105) were negative. The 576 bp fragment was amplified in the positive samples. Stool samples from healthy children were negative in the nested-PCR for HBoV confirming the specificity of the reaction.

An analysis of the age of patients with acute gastroenteritis showed a greater number of HBoV-positive results among children aged between 7 and 23 months (47.72%), followed by children aged between 24 and 35 months (15.90%) and children aged from 36 to 47 months and 48 to 60 months (13.66%). Nine percent of the children aged between 0 and 6 months showed HBoV-positive results (Table I).

NoV, ADV, and RV Viral Detection

The presence of NoV, AdV, and RV in the 105 samples was also detected using immunoassays. Twenty samples were positive for NoV (19%), three each for AdV (3%) and RV (3%), and one sample showed a positive result for both AdV and RV (1%).

Table I lists the age groups of children with acute gastroenteritis, who tested positive for NoV, AdV, and RV. The viruses were detected at a higher scale (including co-infection) in children aged between 0 and 23 months, highlighting the presence of RV within this age group. NoV was detected in almost all age groups, except for the 48–60 months age group, where the presence of viral infection was not detected. In the case of AdV infection, of the four positive samples, three corresponded to the 7–23 month age group.

HBoV and Co-Infection

From the total number of samples found to be positive for HBoV (n = 44), 12 (27%) were also positive for another virus. Eleven of these 12 HBoV infected samples were also infected with NoV

TABLE I. Distribution of Positive Samples to HBoV, NoV, AdV, and RV in Children With Acute Gastroenteritis

Age (Months)	HBoV (n =)*	NoV (n =)	AdV (n =)	HRV (n =)	AdV+HRV (n =)
0–6	4[a]	0	0	0	0
7–23	21[b]	16	3	3	1
24–35	7[c]	3	0	0	0
36–47	6[d]	1	1	0	0
48–60	6[e]	0	0	0	0
Total	44/105	20/105	4/105	3/105	1/105

*HBoV strains isolated in each category.
[a]HBoV 2A (KM 366083 and KM366085).
[b]HBoV 1 (KM 366086; KM 366087 and KM 366088) HBoV 2A (KM 366090and KM366091).
[c]HBoV 2A (KM366089).
[d]HBoV 2A (KM 366084).
[e]HBoV 2A (KM 366092).

(91.6%). The remaining co-infected sample was associated with AdV (8.4%). The HBoV positive samples were not co-infected with more than one additional virus.

Phylogenetic Analysis

Genetic sequencing and construction of the maximum likelihood phylogenetic tree (-InL = -1780.609) (Fig. 1) was performed using 10 samples from patients of different age groups. Figure 1 shows two clades. The top clade shows subdivisions identified as HBoV-2 strains (A and B), HBoV-3, and HBoV-4, and the lower clade, which did not contain any subdivisions, identified only the HBoV-1 strains. Among the 10 sequenced samples, 7 were phylogenetically related to the HBoV-2A species (strains KM366092, KM366089, KM366085, KM366084, KM366090, KM366091, and KM366083) and three were correlated to the HBoV-1 species (strains KM366088, KM366086, and KM366087).

DISCUSSION

Acute gastroenteritis is considered to be one of the most important causes of morbidity and mortality in children around the world. Among the viral etiology agents, the HBoV has been recently discovered to cause gastroenteritis. Thus far, few studies have been conducted related to the occurrence of gastrointestinal HBoV infection in Brazilian children [Albuquerque et al., 2007; Souza et al., 2012]. In this study, HBoV detection rate (42%) in children with gastroenteritis was higher than that previously reported in Brazil [Souza et al., 2012]. Several countries have also reported a low frequency of HBoV in patients with gastroenteritis [Chow et al., 2010; Cashman and O'Shea, 2012; Kharim et al., 2012]. The different frequencies found in these studies could be related to the methodology used to detect the presence of HBoV; this could include the choice of primers for amplification of the target regions of viral DNA. Unlike the other studies conducted in Brazil, the identification of multiple species (HBoV-1, HBoV-2, HBoV-3, and HBoV-4) from a pan-bocavirus PCR primer in the screening test would be widely beneficial [Kapoor et al., 2009]. This study identified two species, HBoV-1 and HBoV-2A, and to date, HBoV-2A occurrence has not been reported in Brazil [Silva et al., 2010; Souza et al., 2012].

Although HBoV-1 was the first species to be detected, the pathogenesis of this virus in patients with acute gastroenteritis remains unclear. Originally discovered in secretions of patients with respiratory infection, studies have shown that, after primary infection, HBoV-1 persists asymptomatically in the body for several months; after being swallowed, HBoV-1 can also affect the enteric tract [Vicente et al., 2007; Silva et al., 2010]. On the other hand, HBoV-2, and HBoV-3, which are often detected in samples obtained from individuals with gastroenteric symptoms, are rarely found in patients with respiratory infection [Schildgen et al., 2008; Arthur et al., 2009; Chieochansin et al., 2010; Chow et al., 2010].

The age of infected patients is an important factor, as most studies show a high incidence of HBoV infection among children; this is similar to the results of this study , where a high frequency of HBoV infection was observed among children under 2 years of age [Chow et al., 2008; Cashman and O'Shea, 2012; Romani et al., 2013]. However, there are few reported cases in children aged between 0 and 6 months, which can be explained by the by the multiple immunologic factors acquired through breastfeeding [Turin and Ochoa, 2014].

Another finding of this study was the detection of co-infection of HBoV with other viral pathogens such as NoV and AdV. These results corroborated similar results detailed in literature, and in Brazil where the occurrence of co-infection in cases of acute gastroenteritis has been predominantly higher than that of mono-infection [Arthur et al., 2009; Cashman and O'Shea, 2012 ; Jartti et al., 2012]. In contrast, this study showed that the majority of HBoV-positive samples displayed mono-infection, while a small number demonstrated co-infection with NoV and AdV. In addition, NoV was observed to be more prevalent and was expressed in greater frequency

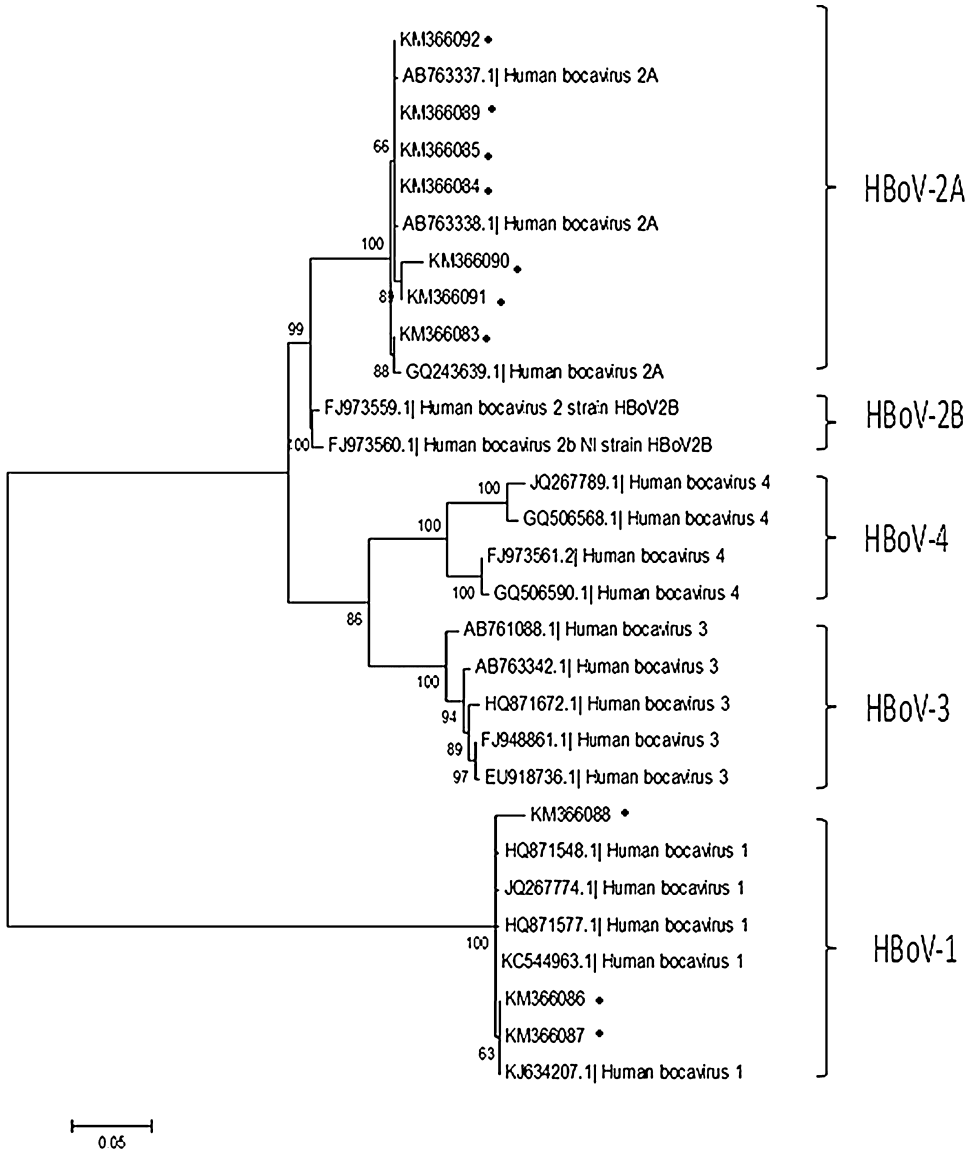

Fig. 1. Phylogenetic tree constructed from the HBoV polyprotein VP1-VP2 partial gene sequences. The nucleotide sequences were analyzed using Hasegawa-Kishino-Yano substitution model (HKY + G), and the reliability estimated by the bootstrap method using 1,000 pseudo-replicates. The virus names correspond to the country of origin/year collected/GenBank accession number and the strains in this study are labeled as follows: KM36683 to KM36692 (points). The scale bar represents a genetic distance of 0.05%, divergence in the nucleotide sequence.

compared to AdV, similar to previous studies, showing its relevance among viral agents in acute gastroenteritis [Campos et al., 2008].

In conclusion, this study show that HBoV is another viral pathogen that must be taken into account in the etiology of acute gastroenteritis in children. The high rate of HBoV detection observed in this study is of concern. This shows the importance of epidemiological monitoring of gastroenteritis, and the need for future inclusion of tests aimed at HBoV detection. In this sense, it is necessary to alert pediatric medical professionals to the presence of HBoV in the etiology of viral gastroenteritis.

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