

Human Bocavirus detected in stool samples from ≤ 5 years children with Acute Gastroenteritis

Neeraj Kumar^{1*}, S.K Mehra¹, Bharti Malhotra², M. Anjaneya Swamy² and Renuka Chauhan³¹Geetanjali University, Udaipur, Rajasthan, India²S M S Medical College, Jaipur, Rajasthan, India³R H G P G College, Kashipur, Uttarakhand, India

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*Corresponding author

Neeraj Kumar

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Abstract: Acute gastroenteritis (AGE) is a common infirmity intestinal disorder in children and adults, affecting all age groups but more common in children worldwide, causing an estimated three million deaths annually. Human bocavirus (HBoV), a newly identified member of the Parvoviridae family is associated with gastrointestinal tract diseases and respiratory infections, mostly of young children. In our study, 100 stool samples from children (≤ 5 years) with acute gastroenteritis were collected. Samples were screened for HBoV by real-time PCR. HBoV was detected in 3 (3%) out of 100 samples. The virus is commonly infected 1 to 5 years old children. The ratio of HBoV positive samples is similar to global results (2.1–5.5%). The positivity of HBoV is very less (3%). HBoV is the third most prevalent virus, after rotavirus and astrovirus, associated with pediatric AGE.

Keywords: AGE, Human Bocavirus (HBoV), Real Time PCR, Stool Sample.

INTRODUCTION

Acute gastroenteritis (AGE) is a common infirmity intestinal disorder in children and adults, affecting all age groups but more common in children worldwide. In developed countries, most of the children will average one episode of AGE viruses every year [1]. The rate of gastroenteritis infection is higher in children under five year age and which estimated 1.5 to 2.5 million deaths annually with AGE viruses [2, 3]. Globally, approximately 1.5 billion episodes and, the majority occurs in developing countries [4].

Human Bocavirus (HBoV) was first identified in 2005 by nonspecific genome amplification methods. Comprehensive studies on sequence and phylogenetic analysis led to classification of the virus to the Parvoviridae family. Due to its close relation to bovine parvovirus and minute virus of canines, the novel parvovirus was named 'Human Bocavirus' [5]. Two variants of the virus have been described [6] so far. However, differences in biological characteristics, disease association, epidemiology and geographical distributions of the genotypes are still poorly understood. HBoV infections show a seasonal distribution, with the peak in temperate areas being in the winter months. Infections are associated with acute gastroenteritis and respiratory diseases, mostly among young children [7-9].

The presence of HBoV in Rajasthan has not yet been investigated, so this lead study aimed to collect data on the presence of the virus in Rajasthan for the first time. The virus could be isolated in co-infection with other gastroenteritis viruses (rotavirus

and astrovirus, 4/5) [10]. HBoV has been detected worldwide, as reviewed by Lindner and Modrow [11]. To better understand the epidemiology of the HBoV infection, in conjunction of a viral surveillance program, we investigated the presence of HBoV in patients with acute gastroenteritis infection in Jaipur, a city located in India. Geographically, the city is characteristics of a tropical-subtropical climate, with the average annual temperature of 25-42°C and average relative humidity range of 22-83%.

These socio-natural factors make the region generally vulnerable to air-borne as well as food-borne viral infection. In our study, we screened fecal specimens from patients with acute gastroenteritis tract infection symptoms for HBoV and other common gastroenteritis viruses over a 1-60 month period using Real Time Reverse Transcriptase Polymerase Chain Reaction (real time RT-PCR) methods.

MATERIALS AND METHODS

Inclusion criteria

Children were presenting recent onset of vomiting and/or diarrhoea, with or without fever or abdominal pain.

Sample collection and transportation

A 100 stool samples were collected from clinically suspected gastrointestinal tract infections. The samples were properly labeled & transported in cold chain (4-8°C) at the earliest to the laboratory. The study was approved by institutional ethics committee.

Viral Nucleic Acid Extraction

Viral nucleic acid were extracted in a final volume 110µl from the stool samples by using NucliSENS Easy MAG automated nucleic acid extractor instrument (Biomeurix) as per the manufacturer's instruction. In brief, 400µl homogenized stool sample with 1600µl lysis buffer was added in columtube. 140µl of magnetic silica was added to the sample with internal control, mixed well by up & down followed by automated processing. The

nucleic acid was eluted in a volume of 110µl and processed for PCR.

Real Time PCR

Real-Time PCR assays were performed on ABI 7500 FDx RT PCR (Life Technologies, USA) using AgPath-IDTM One-Step RT-PCR kit (Ambion) thermocycler by using human Rotavirus specific primers and probe. The thermal profile followed was set at 50 °C for 15 minutes, 95 °C for 10 minutes, 40 cycles of 95 °C for 8 sec, 60 °C for 34 sec.

RESULTS

We observed that total positivity of HBoV is found 3%. Out of total suspected samples 65% were males and 35% were females whereas in positive samples 2 (66.67%) were male and 1 (33.33 %) was female. Positivity of HBoV is found to blow 2 year age group children. The ratio of HBoV positive samples is similar to international results (2.1–5.5%). The distribution of the samples as per age group is given in Table 1.

Table-1: Age wise distribution of samples

Age (Months)	Samples collected			Positive
	Male	Female	Total	
1-12	25	12	37	2
13-24	15	9	24	1
25-36	12	6	18	0
37-48	8	5	13	0
49-60	5	3	8	0
Total	65	35	100	3

CONCLUSIONS

In adolescent children, infectious gastroenteritis is one of the most frequent diseases. The etiology of diarrhoea of 'non-viral' gastroenteritis outbreaks remains unidentified in about 25–40% of cases, although the definition 'non-viral' could simply mean that virological studies produced negative results. As we get additional data about the wide range of viral pathogens which may play a role in acute gastroenteritis the percentage of outbreaks with clarified etiology will increase. The epidemiology and clinical aspects of the newly detected HBoV are inadequately understood, but gastroenteritis may be the possible consequence of infection primarily in young children.

We found one child with acute but mild gastroenteritis positive for HBoV in the winter season similarly 2 children with acute but mild gastroenteritis proved to be positive for HBoV in Hungary, of 2007/2008 [12]. The children were kindergarteners, where gastroenteritis outbreaks occurred a week before each the child's infirmity.

Acute HBoV infections are often observed to be accompanied by other infectious agents of both the respiratory and gastrointestinal band. Therefore, as only a limited number of prospective studies have been published, a final statement on HBoV as the causative agent of infectious disease in children cannot yet be given.

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