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A Detection of Human Bocavirus from Fecal Samples of Indian Children with Acute Gastroenteritis

KEYWORDS: AGE, Human Bocavirus (HBoV), Rotavirus, Astrovirus and RT-PCR.

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ABSTRACT

Objectives: Acute gastroenteritis (AGE) is a common illness affecting all age groups 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. To investigate the epidemiological and genetic variation of HBoV in Jaipur, India, we screened 239 stool samples from children with acute gastroenteritis infection symptoms for HBoV between March 2012 and December 2012.

Methods: In our study, 239 stool samples from children (≤5 years) with acute gastroenteritis were collected. Specimens were screened for HBoV by real-time PCR and other 6 common gastroenteritis viruses by RT-PCR or PCR. We have used specific primer and probes.

Results: HBoV was detected in 5(2.09%) out of 239 samples, mostly from inpatient children. The virus carrier children were 1to 5 years old. The ratio of HBoV positive samples is similar to global results (2.1–5.5%).

Conclusions: The positivity of HBoV is very less (2.09%). HBoV is the third most prevalent virus, after rotavirus and astrovirus, associated with pediatric AGE in this study. It is observed that HBoV is found in mix infection commonly.

INTRODUCTION

Acute gastroenteritis (AGE) is a common infirmity affecting all age groups but more common in children worldwide. Even in developed countries, most of the children will average one episode of AGE viruses per 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 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^{0}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.

Material and methods:

Patient Inclusion criteria:

Stool samples collected during the period March 2012 to December 2012 who attended the inpatient wards of the Department of General Pediatrics patients at J K Lone and SMS Hospitals, Jaipur were enrolled. All children were of ≤ 5 years of age, presenting with diarrhoea, vomiting, headache, signs and symptoms fever, chills and abdominal pain.

Sample collection and transportation:

A total 239 of stool samples were collected from suspected or clinically diagnosed cases of gastrointestinal tract infections in to a sterile transport container. The stool samples were properly labeled & transported in cold chain (4-8^0 C) at the earliest to the laboratory and stored the samples -80^0 C till further processing. The study was approved by institutional ethics committee.

Sample Size:

Sample size has been calculated using this formula

$$n = {}^{4pq}/_{l^2}$$

(where n= Total samples, 4 is the factor to achieve the confidence level of 96%, p= known Prevalence that is 10%, q= 100-p and l= permissible (absolute) error, set at 4%)

n=4×10×(100-10)/4×4
n~225

So we choose 239 samples for better resulting in present study.

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 (Biomeuriex) as par the manufacturer's instruction. In brief, 400µl homogenized stool sample with 1600µl lysis buffer was add 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 multiplex PCR.

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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 primer probe. Real-time RT-PCR 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:
Presently we studied 239 stool samples from March 2012 to December 2012(in figure1). Figure shows the variation of monthly diarrhoea sample collected and number of positive HBoV for less than five years aged hospitalized children. We observed that maximum children hospitalized from May to August and minimum in winter season. The total positivity of HBoV is found 2.09% in 239 samples.

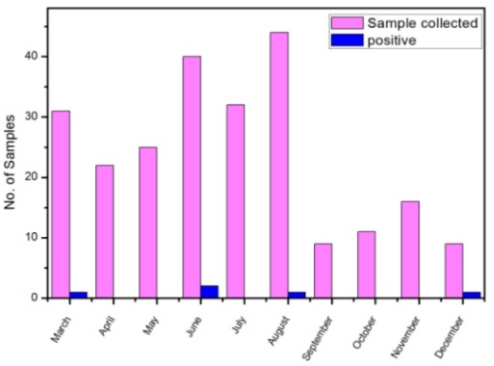

Figure 1: Monthly distribution of sample collection and positivity of Bocavirus.

The distribution of the samples collected as per age group is given in Table 1. Maximum number of samples was collected for the age group 1-12 months i.e. 158(66.11%), out of these 105(66.46%) were males and 53(33.54%) were females. Positivity of HBoV is also the highest for this age group. Gastroenteritis viral infection is very less in 49-60 months age group children according to present study. In total positive samples four belong to males and only one is to female child. 163(68.2%) samples were males in total 239 collected samples while only 76(31.8%) were females.

Age group	Sample collected			Positive
	Male	Female	Total	
1-12	105	53	158	3
13-24	36	10	46	0
25-36	10	4	14	1
37-48	10	7	17	1
49-60	2	2	4	0
Total	**163**	**76**	**239**	**5**

Table 1: Age wise distribution of samples

Out of five HBoV positive sample, only one is single infected and four are mixed infected with other gastroenteritis virus (two with rotavirus and two with Rotavirus + Astrovirus). The virus carrier children were 1- 4 years old. The ratio of HBoV positive samples is similar to international results (2.1–5.5%).

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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