Journal of Medical Virology 79:539–543 (2007)

Human Bocavirus in Iranian Children With Acute Respiratory Infections

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Human bocavirus (HBoV), a virus discovered in Sweden in 2005, has been associated with acute respiratory infections in young children and subsequent reports suggest that HBoV may have a worldwide distribution. This report describes the frequency and clinical presentation of HBoV in 261 Iranian children<5 years old with acute respiratory infections attending two regional hospitals in Rasht, Iran in the winter of 2003–2004. Polymerase chain reaction (PCR) and reverse transcription PCR (RT-PCR) were used for the detection of HBoV and other respiratory pathogens from nasopharyngeal specimens. HBoV was detected in 21 (8%) children. Fifteen (12%) of these children were identified among 122 children admitted to hospital and 6 (4%) from 139 outpatients ($P < 0.05$). Most children with HBoV were less than 2 years (17/21, 81%) and 7 (33%) were less than 1 year old. Although HBoV was identified in all ages it affected slightly older children than the respiratory syncytial virus (RSV). The frequency of the virus varied from 1 (3%) in 40 patients in November to 7 (12%) of 61 in February, suggesting a seasonal pattern during the autumn and early winter. Seven children had co-infections with RSV, adenovirus or influenza A. The relatively high frequency of HBoV suggests that the virus may contribute substantially to acute respiratory infections in children. ***J. Med. Virol. 79:539–543, 2007.***

KEY WORDS: human bocavirus; acute respiratory infections; children; clinical presentation; Iran

INTRODUCTION

Acute respiratory infections are among the most important causes of childhood morbidity and mortality and are responsible for one-fifth of all deaths in children under five, resulting mainly from pneumonia and bronchiolitis [Bryce et al., 2005]. Viruses play a significant role in these infections and the number of viruses associated with severe acute respiratory infections has increased in recent years with the detection of new pathogens such as human metapneumovirus (HMPV) [van den Hoogen et al., 2001] and severe acute respiratory syndrome associated with a coronavirus [Chan-Yeung and Yu, 2003].

Despite the advances in understanding the aetiology of acute respiratory infections, a significant proportion of episodes remain unclassified and it is likely that more "new" viruses will be discovered [Snell, 2004]. Human bocavirus (HBoV) was first described in 2005 [Allander et al., 2005] and it was suggested that the virus might be a cause of acute respiratory infections. A case series of 21 patients infected with HBoV who presented to two referral hospitals in Iran is described.

MATERIALS AND METHODS

The study was conducted from November 2003 to March 2004 based on 17-Shahrivar and Rasoul-e-Akram hospitals. 17-Shahrivar is a Paediatric Reference University Hospital with 200 beds and Rasoul-e-Akram is a general regional referral hospital in Rasht, Guilan in northern Iran. The original aim of the study was to describe the etiology of acute viral respiratory infections in children in Northern Iran. These included respiratory syncytial virus (RSV), HMPV, influenza A and B, parainfluenza, and adenovirus, plus *Chlamydia spp.* and *Mycoplasma pneumoniae*.

Children less than 5 years of age with acute respiratory infections of less than 7 days duration attending the outpatient department or being admitted to hospital from Saturday to Thursday were enrolled

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Accepted 11 January 2007
DOI 10.1002/jmv.20815
Published online in Wiley InterScience (www.interscience.wiley.com)

after informed parental consent. Acute respiratory infections were defined following the World Health Organization (WHO) protocol and were classified into upper and lower tract infections on the basis of the children's respiratory frequency and the presence of subcostal indrawing [Pio, 2003]. Oxygen saturations (pO_2) were measured in all patients admitted using a pulse oximeter (Nonin Medical, Inc. MPL, MN, model 8500) before initiation of oxygen therapy. Children were classified as having mild/moderate ($pO_2 \geq 94\%$) or severe hypoxia ($pO_2 < 94\%$) and a questionnaire containing socio-demographic, clinical, therapeutic, and outcome data was completed for each patient. Ethical approval for the study was obtained from the Research Ethics Committees of the Liverpool School of Tropical Medicine and Guilan University of Medical Sciences and informed consent was obtained from all parents.

Nasopharyngeal aspirates or swabs (Medical Wire & Equipment Co. Ltd., Corsham, Wilts, UK) were collected from all children using sterile mucus extractors for nasopharyngeal aspirates and stored at $-80^{\circ}C$ until processed. Samples were processed in the Department of Medical Microbiology, University of Liverpool, UK. DNA and RNA were extracted using QIAamp® DNA and RNeasy Mini Kits (Qiagen Ltd., Crawly, West Sussex, UK). Extracted RNA was processed using reverse transcriptionpolymerase chain reaction (RT-PCR) for detection of RSV and HMPV [Greensill et al., 2003], influenza A & B, and parainfluenza 1–4 viruses [Templeton et al., 2004], using primers and methods described previously. PCR assays were used to amplify the DNA of adenovirus, *Chlamydia spp*, and *Mycoplasma pneumoniae* [Couroucli et al., 2000]. The HBoV NP-1 gene was detected by PCR using the primers 188 F (GAGCTCTGTAAGTACTATTAC) and 542 R (CTCTGTGTTGACTGAATACAG) and the same reactions and thermal cycler programme as described and corrected subsequently by Allander et al. [Allander et al., 2005] to detect a 345-bp product. One-third of the HBoV amplicons were sequenced to confirm the identity of the virus (Lark Technologies, Essex, UK).

Epi Info 2002 (CDC, Atlanta) was used for the descriptive analysis of characteristics of the children included means, standard deviations (SD), median, inter quartile range (IQR), and percentages. Cross tabulation of the frequency of the virus in hospitalized and ambulatory children and their clinical presentations were compared using parametric tests. P values <0.05 were considered statistically significant.

RESULTS

Respiratory specimens were collected from 261 children. Of these, 139 were ambulatory and 122 were hospitalized. Their median (IQR) age was 14 (7–32) months and 167 (64%) were male. HBoV DNA was detected in 21 (8%) children; 15 (12%) were identified among the 122 hospitalized children and 6 (4%) among the 139 outpatients ($P < 0.05$). Fourteen (67%) cases were male and seven female. Five patients were known to have a history of asthma and six had been hospitalized previously with asthma or pneumonia.

The age distribution of the children with HBoV is shown in Figure 1. Most cases (17, 81%) were less than 2 years of age and 7 (33%) were less than 1 year old. The numbers of children enrolled and those with HBoV by month are shown in Figure 2. The proportion of children infected with HBoV each month ranged from 1 (3%) out of 40 patients in November to 7 (12%) out of 61 in February, suggesting a seasonal pattern (Chi square for trend, $P = 0.05$).

In total, 39 (15%) children with RSV, 37 (14%) with adenovirus, 11 (4%) with influenza A, 4 (2%) with *Chlamydia spp*, 2 (1%) with *M. pneumoniae*, and none with HMPV or parainfluenza were identified. Seven children with HBoV were co-infected with another respiratory pathogen (1 with Inf A and 3 each for RSV and adenovirus). None of the HBoV positive children were co-infected with HMPV, parainfluenza viruses, *Chlamydia spp*, or *M. pneumoniae* [Naghipour et al., in press].

The clinical presentation of the patients is described in Table I and are compared to children without HBoV in Table II. All children had a history of cough (100%) with a mean duration of 4 days (range 1–7), 17 (81%) had fever with a mean duration of 2 days (range 1–5), and 11 (52%) had tachypnoea. The pO_2 concentrations $<94\%$ were recorded in 3 of the 15 hospitalized children, and one was co-infected with influenza A. The clinical diagnoses on discharge were pneumonia in ten, upper respiratory infections in six, tracheobronchitis in three, and asthma in two cases. Fourteen of the children admitted had chest radiography performed and hyperinfiltration was the most frequent finding reported in 11 children (8 infected with HBoV alone), in addition to consolidation in 3. The mean (SD) duration of admission in the patients was 5 (2) days with a range from 3 to 9 days.

A total of six of the amplicons were subjected to DNA sequencing of both strands. Of these, four were identical to those of the Swedish reference strain. Two showed changes, one with a point mutation at codon 47 (R → K) and the other at codon 78 (S → N). These are available at www.ddbj.nig.ac.jp with accession numbers of AB257721 and AB257722, respectively.

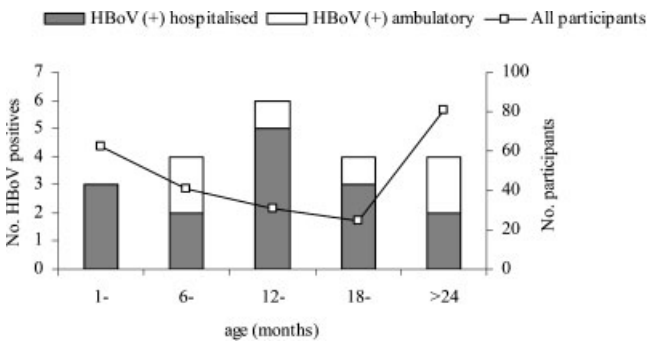

Fig. 1. Age distribution of all participants (right scale) and of those infected with human bocavirus (HBoV).

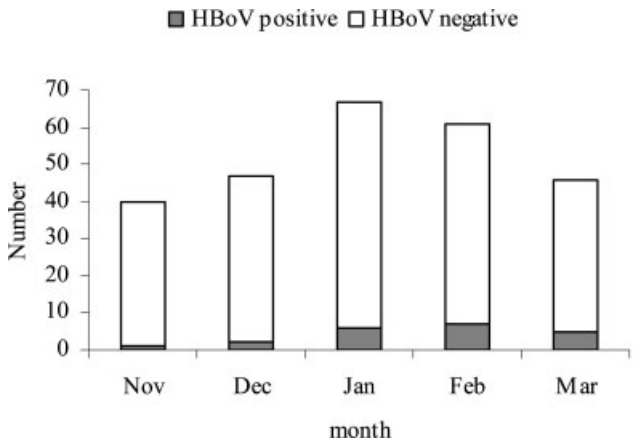

Fig. 2. Number of children infected with human bocavirus (HBoV) by month.

DISCUSSION

This case series describes the presence of HBoV outside industrialized countries. The frequency of HBoV in the study was 8%, which is slightly higher than in earlier reports (Canada 1.5%, Sweden 3.1%, Australia 5.6%, and Japan 5.7%) [Allander et al., 2005; Bastien et al., 2006; Ma et al., 2006; Sloots et al., 2006], but lower than reported recently from Germany (10.3%) [Weissbrich et al., 2006] and Korea (11.3%) [Choi et al., 2006] indicating that more studies are required to assess its relative importance. HBoV was observed more frequently in patients admitted to the hospitals, some of whom had severe hypoxia, than in ambulatory children. This higher frequency however could also reflect the sampling technique used, as samples for admitted patients were collected using Nasopharyngeal aspirates while ambulatory patients were tested with throat swabs, and further studies would be required to confirm these findings. Similar to previous reports, a higher proportion of the cases were male, although this was a reflection of the higher number of male children enrolled in the study. Overall, there was no significant difference in the prevalence by sex.

Most children infected with HBoV were also less than 2 years of age, which is similar to the Swedish (12 out of 14 cases), Japanese (16 out of 18), and Australian children. However, as acute respiratory infections in general mostly affect young children [Bryce et al., 2005], it is not surprising that most of the children with HBoV had this age distribution. Interestingly, different to RSV, which affects mostly children <6 months of age [Constantopoulos et al., 2002], HBoV seems to affect slightly older children, as has been observed with HMPV [Al-Sonboli et al., 2005].

Although, the study only enrolled children for 5 months, there seems to be a seasonal pattern during the late autumn and early winter, as reported from earlier studies and larger studies are warranted to confirm these findings.

TABLE I. Clinical Characteristics of the 21 Children With HBoV

Age (m)	Sex	Location	pO_2 %	Fever	Hyperpnoea	Wheeze	Crackles	Chest indrawing	Nasal congestion	Otitis media	Cough	Hoarseness	Sore throat	Co-infection
2	M	Hospitalized	96	No	No	No	Yes	No	Yes	No	Yes	No	No	No
4	M	Hospitalized	97	Yes	Yes	No	Yes	No	Yes	No	Yes	No	No	No
4	M	Hospitalized	97	No	Yes	No	Yes	No	Yes	No	Yes	No	No	No
9	M	Hospitalized	97	Yes	Yes	Yes	Yes	No	No	No	Yes	No	No	No
10	F	Hospitalized	96	No	Yes	Yes	Yes	No	No	No	Yes	No	No	No
12	M	Hospitalized	95	Yes	No	No	Yes	Yes	Yes	No	Yes	Yes	No	Adv
13	F	Hospitalized	96	Yes	Yes	No	Yes	Yes	Yes	No	Yes	No	No	No
13	F	Hospitalized	96	Yes	Yes	Yes	No	No	No	No	Yes	No	No	RSV
13	M	Hospitalized	96	Yes	Yes	Yes	No	No	No	No	Yes	No	No	No
16	F	Hospitalized	87	Yes	Yes	Yes	Yes	No	No	No	Yes	No	No	No
19	F	Hospitalized	96	Yes	No	Yes	No	No	No	No	Yes	No	No	No
21	M	Hospitalized	97	Yes	No	No	No	No	No	No	Yes	No	Yes	Adv
22	M	Hospitalized	89	Yes	Yes	No	Yes	Yes	No	No	Yes	No	No	No
26	M	Hospitalized	91	Yes	Yes	No	Yes	No	No	No	Yes	No	No	Inf A
40	M	Hospitalized	98	Yes	No	No	No	No	No	No	Yes	Yes	No	RSV
9	M	Outpatient	NA	Yes	No	Yes	No	No	No	Yes	Yes	Yes	No	No
9	M	Outpatient	NA	Yes	Yes	Yes	No	No	Yes	No	Yes	No	Yes	No
13	M	Outpatient	NA	Yes	No	No	No	No	No	Yes	Yes	No	No	No
20	F	Outpatient	NA	No	No	No	No	No	No	Yes	Yes	Yes	Yes	RSV
40	F	Outpatient	NA	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	No
56	M	Outpatient	NA	Yes	No	No	No	No	No	No	Yes	Yes	Yes	Adv

NA, not available; Adv, adenovirus; RSV, respiratory syncytial virus; Inf A, influenza A.

TABLE II. Clinical Presentation of Children With and Without HBoV

	Inpatients		Outpatients	
	HBoV (+) N = 15	HBoV (−) N = 107	HBoV (+) N = 6	HBoV (−) N = 133
Cough N (%)	15 (100)	106 (99)	6 (100)	127 (96)
Mean (SD) duration in day	4 (2)	4 (2)	3 (1)	2 (1)
Fever N (%)	12 (80)	75 (70)	5 (83)	98 (74)
Mean (SD) duration in day	2 (1)	3 (2)	1 (0.5)	3 (2)
Respiratory rate/min, mean (SD)	36 (8)	36 (10)	32 (13)	24 (5)
Rapid breathing N (%)	10 (67)	88 (82)	1 (17)	17 (13)
Breathing difficulty	11 (73)	91 (85)	1 (17)	20 (15)
Crackles	10 (67)	73 (68)	0	5 (4)
Nasal congestion	5 (33)	55 (51)	2 (33)*	97 (73)*
Chest indrawing	3 (20)	33 (31)	0	0
Hoarseness	2 (13)	5 (5)	4 (67)	65 (49)
Otitis media	0	2 (2)	4 (67)	48 (36)
Conjunctivitis	1 (7)	10 (9)	0	6 (5)
Sore throat	1 (7)	6 (6)	4 (67)	78 (59)
Pallor	0	7 (7)	1 (17)	5 (4)
Wheezing	6 (40)	43 (40)	2 (33)	53 (40)
pO_2 <94%	3 (20)	33 (31)	—	—
Chest X ray taken	14 (93)	99 (92)	—	—
Hyperinfiltration	11 (73)	73 (73)	—	—
Consolidation	3 (21)	37 (37)	—	—
Admission days, mean (SD)	5 (2)	5 (3)	—	—

*$P = 0.05$.

Co-infections with other respiratory viruses have been observed in previous studies [Allander et al., 2005; Bastien et al., 2006; Ma et al., 2006; Sloots et al., 2006] and a relatively high proportion of co-infections is reported in this study (33%). This noticeable proportion of co-infections needs to be further elucidated as it might suggest that HBoV is an incidental finding without a significant role in the causation of acute respiratory infections or, as suggested for HMPV [Greensill et al., 2003], it might play a role modifying the clinical presentation of children who have co-infections with other viruses.

Given the high frequency of HBoV in Iran, this virus might play a significant role as a cause of acute respiratory infections in children. However, given that up to now all the information is based on small case series, without a negative control group of healthy children to confirm that HBoV is indeed responsible for the clinical manifestation observed, the aetiological role of HBoV as a cause of acute respiratory infections still needs to be demonstrated conclusively.

ACKNOWLEDGMENTS

This study was supported by the Iranian Ministry of Health and Medical Education through a study scholarship for Dr. Naghipour.

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