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## Hospital-acquired human bocavirus in infants

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### SUMMARY

Human bocavirus (HBoV) is a respiratory pathogen that affects young children. We screened 511 nasopharyngeal aspirates for hospital-acquired HBoV from infants hospitalised with respiratory infection from January to December 2008. Among 55 children with HBoV infection, 10 cases were hospital-acquired. Compared with the community-acquired cases, coinfection with other respiratory viruses in these patients was uncommon. HBoV should be considered for inclusion in screening protocols for nosocomial childhood respiratory infections, especially in intensive care units.

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### Introduction

Human bocavirus (HBoV) is a newly recognised respiratory pathogen that mainly affects children aged <2 years.<sup>1</sup> It is a member of the Parvoviridae family, subfamily Parvovirinae, genus bocavirus. Since its first description in 2005 by Allander *et al.*, it has been found worldwide in respiratory specimens from children with lower respiratory tract (LRT) infection with a prevalence varying from 1.5% to 19%.<sup>2,3</sup> HBoV has also been isolated from faeces of children with gastroenteritis, with or without respiratory symptoms.<sup>4</sup> Recent studies have described two novel HBoV groups, named HBoV2 and HBoV3.<sup>5</sup>

Publications addressing the epidemiology of nosocomial respiratory virus infection are scarce. A few authors have reported cases of nosocomial HBoV infection, mostly acquired in intensive care units.<sup>6–8</sup> As a potential new agent of nosocomial childhood infection, we screened for HBoV in young children with respiratory tract infection.

### Methods

The data presented are part of a prospective study on the aetiology of childhood respiratory infections being carried out at Santa Casa de Misericórdia Hospital, São Paulo, Brazil. Santa Casa is

a tertiary reference hospital located in the central region of São Paulo City.

From January 2008 to December 2008, nasopharyngeal aspirates (NPAs) were collected from children aged <2 years and screened for respiratory viruses including HBoV. Two groups of children were screened: (1) children on the paediatric ward or intensive care unit (ICU) with clinically suspected LRT infection (either community- or hospital-acquired), and (2) infants who had been in the neonatal ICU (NICU) since birth. On the NICU, NPAs were collected from those with suspected LRT infection, and once a week from asymptomatic infants who were receiving mechanical ventilation.

NPAs were stored in liquid nitrogen and sent weekly to the virology laboratory of the University of São Paulo for detection of respiratory virus by polymerase chain reaction (PCR).<sup>9</sup> All samples were tested for the following respiratory viruses: respiratory syncytial virus, human metapneumovirus, parainfluenza virus 1, 2 and 3, influenza virus A and B and adenovirus. PCR and reverse transcription (RT)–PCR assays were developed based on previous publications using GeneScan analysis with primers previously described.<sup>10–12</sup> PCR for HBoV was performed using primers previously described targeting the NS1 gene.<sup>13</sup> All HBoV-positive samples were amplified by a second PCR using primers based on a conserved region of the VP1/VP2 gene: HBoVP1 (5' 4128 ACCACCAAGTACTAGAACTGG 4149 3') and HBoVP2 (3' 4785 AATAGTGCTGGAGGATGATCC 4765 5'). The expected product size was 657 bp. In most cases these products were sequenced for phylogenetic analysis. Sequences were edited with the sequence navigator program version 1.0 (Applied Biosystems, Inc., Foster City,

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CA, USA) and the phylogenetic tree was created by the clustal method using Megalign (Lacergene, DNA STAR, Inc., Madison, WI, USA).

The study was approved by the Research Ethics Committee of Santa Casa de Misericórdia Hospital and by the University of São Paulo. Written informed consent was obtained from the parent or guardian of each child enrolled in the study.

## Results

A total of 511 NPA were collected from 406 children: 381 samples were collected from 359 children in the paediatric ward and ICU, while 130 were collected from 47 infants in the NICU. Fifty-five samples (10.7%) were positive for HBoV, with a higher prevalence between May and August.

Ten of the HBoV infections were definitely or probably acquired in the hospital. Four infants had been in the NICU since birth (patients 1, 2, 3 and 10) while four infants admitted from the community had previously tested negative for HBoV (patients 4, 6, 7 and 9). One infant (patient 8) was in the paediatric ICU following liver transplantation and developed respiratory distress 28 days after admission. The final patient (patient 5) was in the paediatric ward being treated for visceral leishmaniasis and developed LRT infection 17 days after admission. The characteristics of the patients with hospital-acquired HBoV are described in Table 1.

Among the 45 presumed community-acquired HBoV infections, 23 (51.1%) were coinfecting with at least one other respiratory virus, particularly adenovirus and respiratory syncytial virus (RSV) (in nine cases each). Of the 10 hospital-acquired infections, only one was coinfecting (adenovirus).

Phylogenetic analysis of eight of the 10 HBoV amplicons revealed three different strains of HBoV group 1 with 99.6–99.8% similarity (Figure 1). Two amplicons (representing patients 8 and 10) were not sequenced.

## Discussion

Nosocomial HBoV infection has been recently described. Kesebir *et al.* reported three children who were infected in the hospital, all of whom had a history of premature birth and chronic lung disease.<sup>6</sup> Chow *et al.* reported three cases of nosocomial HBoV, two in the NICU.<sup>8</sup> Calvo *et al.* described two newborns with HBoV in the NICU, both premature and receiving mechanical ventilation.<sup>7</sup> In this paper we describe 10 further cases of nosocomial HBoV infection in children and infants. Sequence analysis of eight of the infecting viruses revealed three different strains of HBoV group 1, denominated A (six cases), B (one case) and C (one case).

Among these 10 children, only one was coinfecting with another respiratory virus (adenovirus). Previous reports of HBoV infection suggest rates of coinfection with other respiratory viruses of up to 75%.<sup>14</sup> The low rate of coinfection in hospital-acquired compared with community-acquired HBoV may reflect the diminished circulation of respiratory virus in the hospital setting. We did not screen our samples for rhinovirus or coronavirus.

One child had undergone liver transplantation 27 days before HBoV was detected and was under intense immunosuppression. The role of HBoV infection in immunocompromised children is not well-defined. Recent studies report persistence of HBoV DNA in NPA of healthy individuals up to four months after infection.<sup>15</sup> In this case, since no previous sample was available, it is possible that the infection represented reactivation of previous community HBoV infection due to immunosuppression.

The finding of HBoV in four NICU infants hospitalised since birth raises the question of maternal–fetal transmission. However, three of these children (patients 2, 3 and 10) had been in the NICU for more than two weeks when the virus was detected, and the fourth (patient 1) was seven months old, so it is more likely to have been acquired postnatally.

Hospital-acquired respiratory virus infection can increase morbidity and mortality in hospitalised children, mainly the younger ones and those with underlying conditions. We have

**Table 1**  
Clinical characteristics of infants with hospital-acquired human bocavirus (HBoV infection)

Patient number	Month of HBoV detection	Hospital days <sup>a</sup>	Age (months)	Sex	Hospital setting	Relevant clinical features	Relevant laboratory and radiographic features <sup>b</sup>	Mechanical ventilation/related to HBoV <sup>c</sup>	Underlying condition	Coinfection <sup>d</sup>	HBoV strain
1	Jan	238	7	F	NICU	Fever, respiratory distress, wheezing	None	Yes/No	CLD/Prem	No	A
2	Feb	19	0	F	NICU	Fever, respiratory distress	None	Yes/Yes	CLD/Prem/CHD	No	A
3	Jul	29	1	F	NICU	Respiratory distress	None	No/No	Prem	No	A
4	Jul	138	5	M	PICU 2	Respiratory distress	Leucocytosis, increased CRP	Yes/No	CLD	No	B
5	Jul	17	10	M	PWard	Cough, fever, fast breathing	Pneumonia	No/No	Visc Leish	No	A
6	Jul	65	8	M	PICU 1	Fever, respiratory distress, wheezing	Pneumonia	Yes/No	CLD/Prem/CHD	Adeno	A
7	Aug	81	3	M	PICU 2	Fever, respiratory distress	Leucocytosis, increased CRP	Yes/No	CHD	No	A
8	Sep	29	12	F	PICU 1	Fever, respiratory distress	Increased CRP	Yes/Yes	Liver TX	No	ND
9	Oct	526	25	M	PICU 2	Fever, respiratory distress, wheezing	None	Yes/No	Prem/CLD	No	C
10	Dec	20	0	F	NICU	Respiratory distress	Leucocytosis, increased CRP, pneumonia	Yes/Yes	CLD/CHD	No	ND

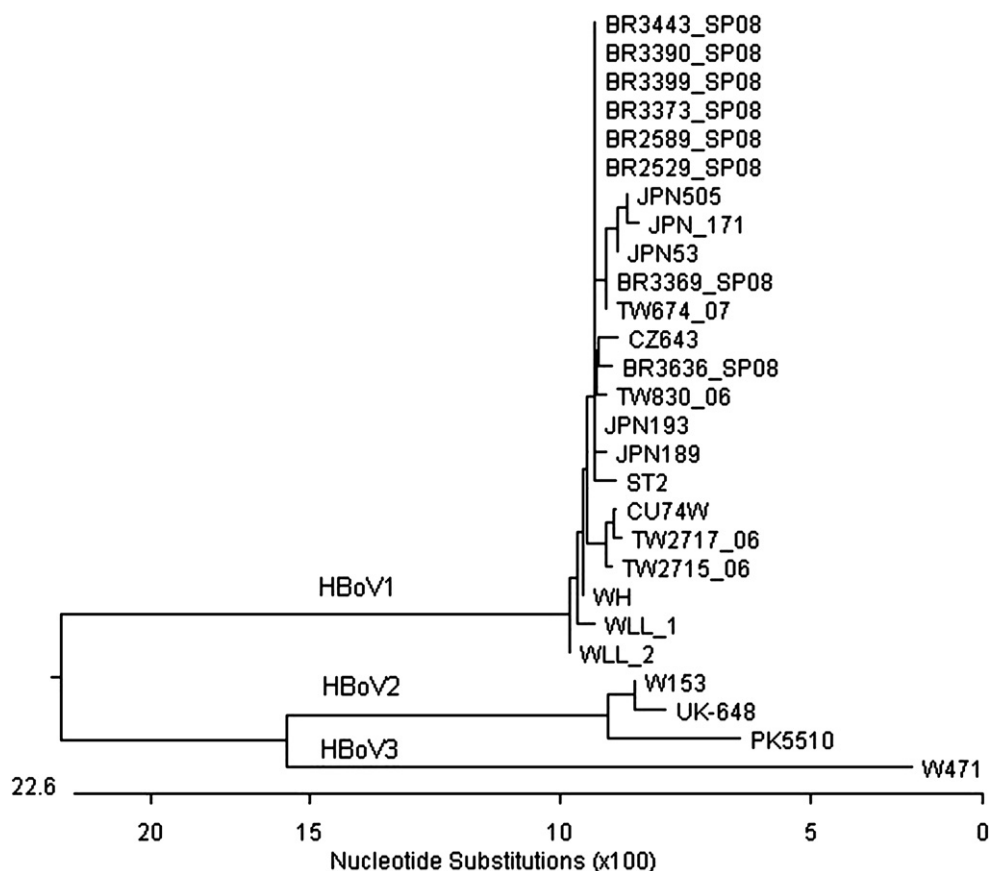
NICU, neonatal intensive care unit; PICU, paediatric intensive care unit; PWard, paediatric ward; CLD, chronic lung disease; Prem, history of prematurity; CHD, congenital heart disease; Visc Leish, visceral leishmaniasis; Liver TX, post liver transplantation; Adeno, adenovirus; HBoV, human bocavirus; ND, not done; CRP, C-reactive protein.

<sup>a</sup> Number of days between hospital admission and HBoV detection.

<sup>b</sup> Leucocytosis  $\geq 20\,000$  leucocytes; increased CRP  $\geq 5$  mg/dL (normal values  $<0.5$ ); alveolar and/or non-alveolar pneumonia detected by chest radiography.

<sup>c</sup> Patients under mechanical ventilation regardless of HBoV infection/related to acute nosocomial HBoV infection.

<sup>d</sup> Detection of other respiratory virus (respiratory syncytial virus, human metapneumovirus, parainfluenza virus 1, 2 and 3, influenza virus A and B and adenovirus) by polymerase chain reaction assay in the samples analysed.



**Figure 1.** Phylogenetic tree showing sequence analysis of eight human bocavirus strains found in this study (BR2529\_SP08, BR2589\_SP08, BR3373\_SP08, BR3369\_SP08, BR3390\_SP08, BR3399\_SP08, BR3443\_SP08, BR3636\_SP08) compared with 19 strains isolated previously (JPN: Japan, JPN505/AB480173, JPN\_171/AB480176, JPN53, JPN193/AB480172, JPN189/AB480171; TW: Taiwan, TW674\_07/EU984244, TW830\_06/EU984231, TW2717\_06/EU984233, TW2715\_06/EU984232; CZ, WH, WLL, W: China, CZ643/DQ457413, WH/FJ496754, WLL\_1/DQ778300, WLL\_2/EF441262, W153/EU082213, W471/NC012564; CU: Thailand, CU74 W/EF203922; UK: United Kingdom, UK-648; PK: Pakistan, PK5510/NC012042; ST-2: Stockholm, ST-2/DK000496). Partial VP1/VP2 gene fragments (453 nucleotide fragments) were detected during 2008 in Brazil (accession numbers GQ178063–GQ178070). Distance tree was created by using Megalign (Lacergene, DNA STAR, Inc., Madison, WI, USA).

demonstrated that HBoV can be detected from infants with clinical evidence of nosocomial respiratory infection as well as those with community-acquired infection.

#### Conflict of interest statement

None declared.

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