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Original Article

High prevalence of human bocavirus 1 in infants with lower acute respiratory tract disease in Argentina, 2007 – 2009

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ABSTRACT

Human bocavirus (HBoV) is a parvovirus whose association with respiratory disease is currently under investigation.
Objective: To determine HBoV prevalence in children with lower acute respiratory infection.
Methods: We investigated HBoV in 433 nasopharyngeal aspirates collected in 2007-2009 from children 0 to 5 years old hospitalized with bronchiolitis or pneumonia in Córdoba, Argentina.
Results: The general prevalence of HBoV was 21.5% and the positive cases (HBoV+) were more frequent during winter and spring. The mean age of HBoV+ patients was 6.9 months, with 87.1% of the detections corresponding to infants less than 1 year old (among which the prevalence of HBoV was 26.3% in patients < 3 months of age, 22.1% in 3 to 6 months, 25.3% in 6 to 9 months, and 18.8% in 9 to 12 months). The sequence analysis of the NP1 coding region of 15 isolates showed that all isolates from Cordoba were HBoV1 which exhibited a homology of nearly 100% both among themselves and with the originally discovered virus from 2005.
Conclusion: Overall, our results indicate that HBoV is a significant pathogen that contributes to acute respiratory infection both on its own and during coinfection with other viruses.

Introduction

Human bocavirus (HBoV) is a parvovirus first identified in 2005 in nasopharyngeal aspirates (NPA) of children with lower respiratory tract infection.[1] Since then, it has been associated with acute infection of the upper and lower respiratory tract (ARI), which is a major cause of doctor's office visits and hospitalization and has a high impact on morbidity and mortality, particularly among children in developing countries.[2,3] With a ubiquitous distribution,[4] the presence of HBoV DNA in patients with ARI has been reported mainly in children ranging from ~ 5 to 19%,[5-13] although a higher prevalence (106 of 318 cases of illness, i.e. 33%) has been described recently.[14] Even though the virus is often associated with upper and lower ARI, the high rates of coinfection with other respiratory viruses with well established pathogenic potential[6,9,10,12,15-18] and the possibility of a persistent infection[14,19,20] make it difficult to evaluate the etiological role of HBoV in respiratory disease.

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As the other members in the genus Bocavirus, the HBoV genome is organized in three open reading frames, which encode the non-structural proteins NP1 (genus-specific) and NS1 and the structural proteins VP1/VP2.[1] Currently 4 different species of human bocavirus have been proposed and the name HBoV1 has now been suggested for the originally discovered virus.[21]

The aim of this study was to determine the circulation of HBoV in Córdoba, Argentina, and to identify the epidemiological pattern of the infection in preschool-age children with lower ARI.

Materials and methods

Patients and clinical samples

The procedures of this study were evaluated and approved by the Ethics Committee of the Hospital Nacional de Clínicas, Universidad Nacional de Córdoba. Four hundred and thirty-three clinical specimens, consisting of NPA, from 433 patients aged 0 to 5 years old, hospitalized with a diagnosis of bronchiolitis or pneumonia of suspected viral etiology, were collected under a surveillance program of pneumonia in Córdoba (patients with immunosuppression, active pulmonary TBS or positive baciloscopy, cystic fibrosis and nosocomial pneumonia were not included). The study involved the detection of HBoV in NPA collected in 2007, 2008 and 2009. Samples from 2007 (n = 100), 2008 (n = 33), and 2009 (n = 300) were stored at -20ºC.

Nucleic acid extraction from clinical specimens

Nucleic acids were extracted from NPA using the method of guanidine buffer and silica by Boom et al.,[22] with modifications as described below. A 50-µL NPA aliquot was added to the preassembled reaction buffer, containing 10 µL silica and 100 µL lysis buffer (0.1 M Tris pH 6.4, 37 mM EDTA pH 8.0, 0.22 g/mL Triton X-100, 1.2 g/mL guanidine isothiocyanate), and then vortexed for 10 sec. After incubation for 10 min at room temperature, the mixture was vortexed again (5 sec) and briefly centrifuged for 5 sec to spin down (0 to 3,700 rpm) in a Labnet (Woodbridge, NJ, USA) microcentrifuge. The supernatant was discarded and next the silica-nucleic acid pellet was subsequently washed twice with ethanol 70% (v/v), and once with acetone. After disposal of the acetone, the pellets were dried at 56°C with open lids in a Labnet (Woodbridge, NJ, USA) heat block for 10 min. Elution buffer (25 µl TE buffer, pH 8.0, without RNase inhibitor) was added, and the vessel was closed, vortexed briefly, and incubated for 15 min at 56°C. The vessel was briefly vortexed again and centrifuged for 2.5 min at 16,000 x g, and the supernatant containing DNA and RNA was stored at -20°C for later use in the PCR reactions.

Detection of human bocavirus genome by PCR

A region of HBoV DNA comprising nucleotides 2354 to 2684 was amplified with previously published primers,[1] resulting in a fragment of 354 bp. Each PCR reaction consisted of 0.2 mM dNTPs, 0.4 µM forward and reverse primers mix, 2.5 mM $MgCl_2$, 0.02 U/µL Platinum Taq DNA polymerase (Invitrogen), and 2 µL of nucleic acid extract. The cycling conditions included 35 rounds of the sequence 94ºC, 30 sec; 48ºC, 30 sec; 72ºC, 1 min; with a previous cycle at 94ºC for 2 min and a final extension at 72ºC for 10 min. Appropriate negative and positive controls were included (the positive controls were kindly provided by Cristina Videla and Guadalupe Carballal, CEMIC, Buenos Aires). The PCR products were visualized on 8.5% polyacrylamide gels stained with silver solution (0.11 M AgNO3).

DNA sequencing of human bocavirus NP1 region

Fifteen isolates from 2007 and 2009 were used for sequence analysis from which a region corresponding to the NP1 protein of HBoV was amplified by nested PCR. Each PCR reaction was performed with 0.25 mM dNTP mix and 5 U/µL EX Taq Hot Start Version DNA polymerase (TaKaRa), in the buffer provided by the manufacturer, for a total volume of 50 µL. For the first round 5 µL template and 0.20 µM of each primer HBoV_2204F (5'GAG ACA TCG CAA GTG GAC TAT3') and HBoV_3101R (5'TTG AGC AGC GCG ATC AGC GTT A3') were used. For the nested reaction 5 µL template of the first round was used and 40 µM of each of the primers HBoV_2321F (5'GCA CAG CCA CGT GAC GAA GAT GA3') and HBoV_3056R (5'GGA TTA AAT GGC CCA AGA TA3'). The final product had an expected size of 736 nt. Amplified fragments were purified following agarose-gel electrophoresis by using a QIAquick gel extraction kit (Qiagen). Sequencing reactions were performed bidirectionally by using appropriate primers and cycle-sequencing kits (ABI PRISM BigDye Terminator v. 3.1; PE Applied Biosystems) and resolved by using a 3100 Genetic Analyzer (Applied Biosystems).

Sequence analysis

Phylogenetic analysis included our 15 isolates (3 from 2007 and 12 from 2009) and representative previous isolations available at GenBank (HBoV1 EF203921; HBoV1 DQ340570; HBoV1 DQ000495; HBoV1 DQ000496; HBoV2 GU048663; HBoV2 GU048662; HBoV2 GU048664; HBoV2 FJ170279; HBoV2 FJ170280; HBoV2 GQ200737; HBoV2 EU082214; HBoV2 FJ948860; HBoV3 HM132056; HBoV3 GU048665; HBoV3 FJ948861; HBoV3 EU918736; HBoV3 CQ867666; HBoV4 FJ973561; CnMV AB158475; CnMV AF495467; and BPV DQ335247). For cataloguing and storage, sequences were input into free online sequence-alignment software (ALIGN Query, GENESTREAM SEARCH network server IGH, Montpellier, France; http://xylian.igh.cnrs.fr/bin/align-guess.cgi). Phylogenetic analysis was conducted with MEGA version 5.03 software (available at http://www.megasoftware.net) using the neighbour-joining method.

Analysis of epidemiological and clinical features associated with HBoV infection

We analyzed clinical and epidemiological aspects associated with samples positive for HBoV (HBoV+), based on the information recorded in the official form of the network monitoring pneumonia. An approximation of the rate of coinfection with other respiratory viruses was obtained from diagnostic assays for respiratory syncytial virus (RSV),

parainfluenza 1/2/3 (PIV), influenza A and B (Flu) and adenovirus (AV) in the patients who were HBoV+. These assays (RSV, PIV, Flu and AV) were performed by direct immunofluorescence assay in the hospital facilities as part of the diagnostic testing required for the patients in the network reporting survey. We also analyzed epidemiological and clinical data of HBoV+ patients who at the same time were negative for RSV, PIV, Flu, AV, and blood culture. Quantitative and qualitative variables were compared using Student's t test or Chi square test, respectively, with a level of significance of 0.05.

Results

Characteristics of the study population

The average age of all patients included in the study was 8.84 months [standard deviation (SD): 11.1 months], with 345/433 (79.7%) of the samples from children ≤ 12 months. Out of 433 samples, 337 (77.8%) were collected during the fall and winter, while 245/433 (56.6%) were male patients.

Prevalence of HBoV

The general prevalence of HBoV was 93/433 (21.5%), with 16/100 (16%) positive samples (HBoV+) in 2007, 9/33 (27.3%) in 2008, and 68/300 (22.6%) in 2009 (Table 1). In an attempt to estimate the rate of coinfection among our HBoV+ specimens, we used the diagnostic test results available for RSV, PIV, Flu, and AV. Forty nine out of 93 HBoV+ samples (52.7%) were coinfected with at least one of the above viral agents (Table 1). The majority of the coinfections, 47/49 (95.9%) were single coinfections and 40/49 (81.6%) were HBoV-RSV coinfections. Two cases with multiple coinfecting viruses were detected (one NPA from 2007 and the other from 2009) and in both cases HBoV was detected in conjunction with RSV and PIV.

Other epidemiological features associated with HBoV infection

The mean age of the 93 HBoV+ patients was 6.9 months (SD: 8.9 months; median: 5 months). HBoV was detected in the entire age range included in the study (10 days to 60 months), but 81/93 (87.1%) were under 12 months (Fig. 1). The prevalence of HBoV per age group in children 0-1 year old was as follows: 30/114 (26.3%) in the 0 to 3 months group; 21/95 (22.1%) in the 3 to 6 months group, 17/67 (25.4%) in the 6 to 9 months group, and 13/69 (18.8%) in the 9 to 12 months group. HBoV infections were detected throughout the year except in the summer (a season during which only 11 NPA were collected in 2007 and none in the other years included in the study). However, HBoV+ cases were concentrated in the winter season (Fig. 2). The distribution of HBoV+ cases plotted together with RSV, PIV and Flu by

Table 1 - Percentage of HBoV+ clinical specimens and co-detection with other respiratory viruses (VRS, PIV, Flu A and/or AV)

Year	HBoV+	Patients with HBoV in coinfection	Coinfections			
			RSV	PIV	Flu A	AV
2007	16/100 (16%)	5/16 (31.3%)	2/5 (40%)	3/5 (60%)	-	-
2008	9/33 (27.3%)	4/9 (44.4%)	4/4 (100%)	-	-	-
2009	68/300 (22.7%)	40/68 (58.8%)	34/40 (85%)	2/40 (5%)	4/40 (10%)	-
Total	93/433 (21.5%)	49/93 (52.7%)	40/49 (81.6%)	5/49 (10.2%)	4/49 (8.2%)	0

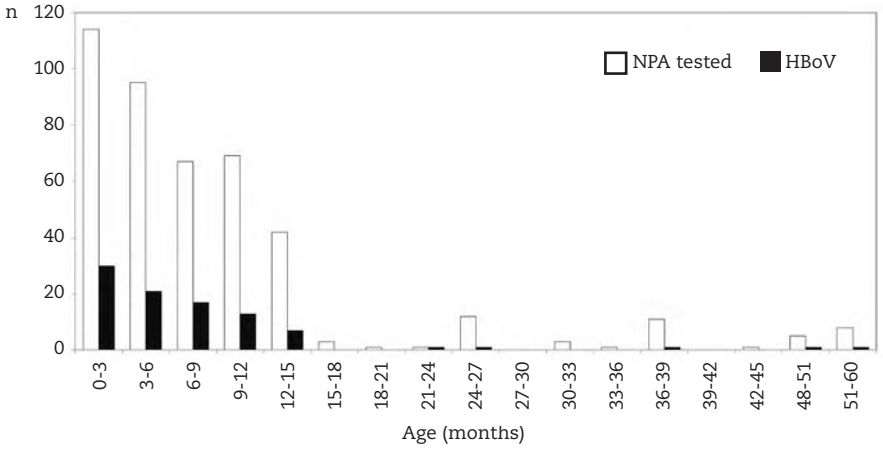

Fig. 1 - Distribution of HBoV+ cases by age groups, 2007-2009.

epidemiological week shows the overlapping circulation of these respiratory viruses mainly during the winter months (Fig. 2, epidemiological weeks 21 through 40), with a difference in the peaks of maximum frequency of detection between RSV (weeks 25-28) and HBoV (weeks 33-36).

Signs and symptoms associated with HBoV infection

We analyzed laboratory data and clinical manifestations of 17 patients whose NPA were HBoV+ but negative for all other diagnostic assays performed (RSV, PIV, Flu, AV, and blood culture, Table 2). Most patients had fever of 39ºC or higher

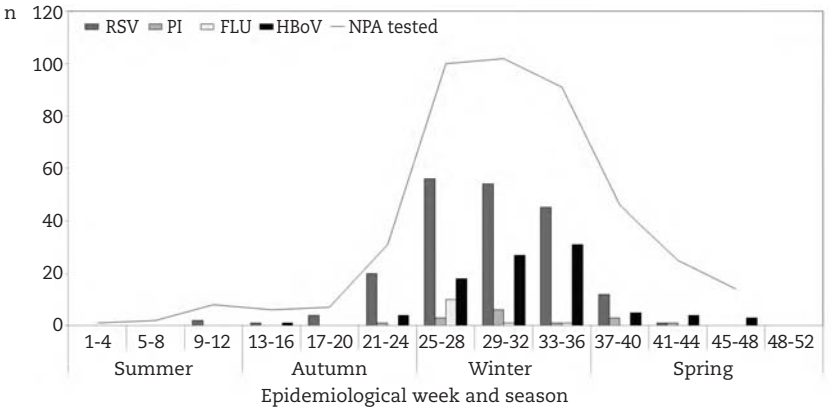

Fig. 2 - Distribution of HBoV+ cases and co-circulation with other respiratory viruses by range of epidemiological week and season, 2007-2009.

Table 2 - Clinical and epidemiological data of 17 HBoV+ patients -negative for RSV, PIV, Flu, AV, and blood culture

Nº	Year	EW	Sex	Age (mo)	T	W	C	RD	F ≥ 39ºC	R	D	Ts-cs (d)	L
1	2009	28	F	7	+	-	-	+	+	-	-	2	17200
2	2009	30	F	10	+	-	+	+	+	-	+	23	14500
3	2009	30	M	7	-	-	+	+	+	-	-	1	3100
4	2009	31	F	10	-	-	-	-	+	-	-	4	21500
5	2009	33	M	9	+	-	+	-	+	-	-	3	22200
6	2009	33	F	8	-	-	+	+	+	-	-	2	10300
7	2009	33	M	9	-	-	-	-	+	+	-	2	23300
8	2009	34	F	9	-	-	+	+	-	+	-	3	10800
9	2009	36	M	12	+	-	-	+	+	-	-	2	23100
10	2009	43	M	6	-	-	+	+	+	-	-	4	9300
11	2008	35	M	3	+	-	+	-	+	-	-	8	14100
12	2008	32	F	14	-	-	+	-	+	-	-	NA	NA
13	2007	28	M	3	+	+	-	-	+	+	-	8	22200
14	2007	28	M	12	+	+	+	+	+	-	+	1	19300
15	2007	28	M	3	-	-	-	-	-	-	-	NA	NA
16	2007	28	M	48	+	-	+	+	+	-	-	5	9200
17	2007	41	M	36	+	-	-	+	-	-	-	4	18000

EW, epidemiological week; T, tachypnea; W, wheezing; C, cough; RD, respiratory distress; R, rhinorrhoea; D, desnutrition; Ts-cs, time between onset of symptoms and clinical sample (NPA, at the moment of hospitalization) in days; L, leukocyte count; +, yes; -, no; NA, data not available.

and white blood cell count higher than the normal range (> 10.8 x 10^3/mm^3). HBoV detection was also associated with respiratory distress (59% of cases), cough (59%) and tachypnea (53%). Wheezing and rhinorrhea were observed in some cases. Three of the 4 cases with a more conspicuous illness had high fever, respiratory distress, cough and tachypnea (2 of these were associated with malnutrition). The average number of days between the onset of symptoms and hospitalization (at which time the respiratory sample for diagnosis was taken) was 4.8 days. In addition, these cases occurred in epidemiological weeks 28 through 43 (winter – spring) and the average age of the patients was 12.1 months [not significantly different from the average age in all HBoV+ cases (p = 0.051)].

To further assess the clinical picture associated with HBoV infection, the features of this group of 17 HBoV+ patients were compared to the features of 17 patients with a positive detection for RSV only (Table 3). The RSV+ group consisted of patients whose NPA had a positive detection for RSV and were negative for all other assays performed, taken randomly among the RSV+ clinical specimens in this study. Significant differences were found in the comparisons "range of epidemiological weeks" of occurrence of cases (as evidenced in Fig. 2) and "average number of days between onset of symptoms and hospitalization". Also, patients with RSV were more likely to present tachypnea and cough, whereas patients with HBoV were more likely to have high fever and leukocytosis (Table 3).

Sequence analyses

Fifteen HBoV isolates were sequenced to perform phylogenetic analysis and the genomic region analyzed consisted of the complete NP1 coding region between nucleotides 2321 and 3056 of the HBoV genome. All 15 isolates grouped with HBoV1 (Fig. 3). Only 3 isolates exhibited any degree of genetic diversity, namely a substitution at nt 2733 which changed a T to a C (genetic distance: 0.001), revealing an extremely high similarity among the isolates and also in comparison with the original HBoV1 sequence.

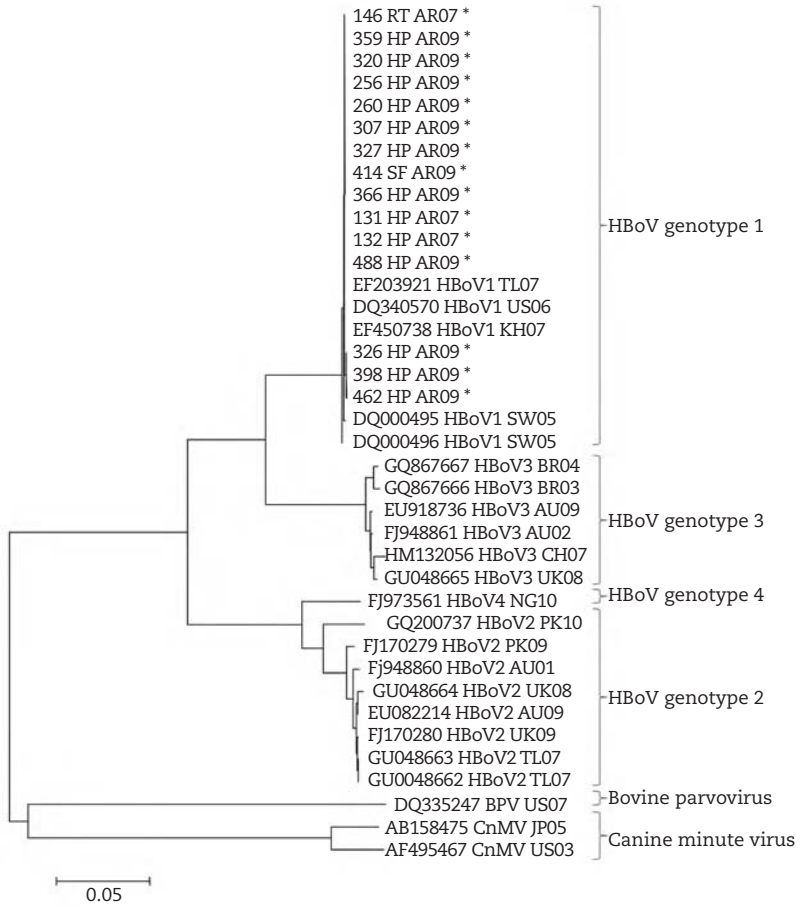

Fig. 3 - Neighbor-joining tree showing phylogenetic relationships among HBoV1, HBoV2, HBoV3, HBoV4, CnMV, BPV and 15 local HBoV sequences (*) amplified after nucleic acid extraction from nasopharyngeal aspirates of pediatric patients with bronchiolitis or pneumonia. The phylogenetic analysis was done using the region comprising nt 2321 through 3056 (NP1) of the HBoV1 genome.

Discussion

In this report, we describe epidemiological and clinical features associated with HBoV1 infection in 0 to 5 year-old children with bronchiolitis or pneumonia in Córdoba, Argentina, revealing a high prevalence of HBoV (21.5%) in the population studied.

The age range of HBoV+ cases extended from 10 days to 5 years, with an average age of 6.9 months. Most HBoV+ patients (87.1%) were less than 1 year old (Fig. 1); likewise other authors found the main prevalence of HBoV in approximately the same range.[6,11,23] Interestingly, HBoV1 prevalence was similar in the age ranges under 1 year (26.3% in < 3 months, 22.1% in 3 to 6 months, 25.4% in 6 to 9 months, and 18.8% in 9 to 12 months). Since initially HBoV had been detected in patients 5-6 months of age and over,[1,24,25] some authors proposed that maternal antibodies prevent neonatal infection by HBoV.[10,26,27] However, we found high and similar HBoV1 prevalence among 1-3 months and 3-6 months infants, suggesting an incidence of HBoV1 infection very early in life. Remarkably, we and others have detected HBoV as early as few days after birth[8,28] and a seroepidemiological study also provided evidence that HBoV infection is common during early infancy.[29]

HBoV+ cases occurred in fall through spring and peaked during the winter, overlapping with other respiratory viruses such as RSV, PIV and Flu (Fig. 2). Similar findings have been reported,[6,12,16,18,24,30] although some studies found HBoV cases throughout the year with spring outbreaks.[5,15,31,32] The higher frequency of detection in the winter months suggests a maximum incidence of HBoV1 infection in this season as well. However, most surveys on HBoV1 so far analyze samples collected for diagnosis of ARI, which is the reason for the predominance of samples collected during fall/winter, as in the present study.

The rate of coinfection was at least 52.7%. This can be taken as estimation, since HBoV was detected by PCR, while coinfection with other respiratory viruses was determined based on immunofluorescence assay. Even so, noticeably over 80% of the coinfections are HBoV1-RSV coinfections (Table 1). Others also recognized elevated percentages of coinfection with RSV among HBoV+ patients.[6,11,13,16,25] If the circulation pattern is confirmed as it is presumed (mainly in infants less than 1 year old and during winter) this high rate of coinfection could be an effect of cocirculation (Fig. 2), although an interaction between these viruses cannot be disregarded.

Clinical manifestations concomitant with HBoV1 detection included high fever (≥ 39ºC), leukocytosis, cough, and respiratory distress (Table 2). Also, fewer days were observed between onset of symptoms and hospitalization in HBoV+ cases compared to RSV+ cases (Table 3), which indicated a less insidious development of a severe form of the associated disease.

Finally, the phylogenetic analysis of 15 local isolates from 2007 and 2009 confirmed that all of them were genotype 1 (Fig. 3), showing an extremely high homology with the original sequence identified in 2005, in agreement with the low nucleotide substitution rate in the NP1 region of HBoV1 shown recently.[21] The primers used in the present work were designed to detect the four genotypes of HBoV identified to date, yet all of the isolations sequenced belonged to genotype 1. Thus, our results reflect the association of HBoV1 with acute respiratory infection.

Conclusion

This is the first report of HBoV1 in Argentina. Our results suggest that HBoV1 plays an important role in respiratory illness that causes significant disease both on its own and in conjunction with other coinfecting viruses.

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Table 3 - Comparison of clinical presentations and epidemiological features of 17 RSV+ patients versus 17 HBoV+ patients

Clinical / epidemiological aspects	RSV	HBoV	p
Range of epidemiological weeks (seasons)	17-38 (fall-winter)	28-43 (winter-spring)	0.005
Mean age (months)	7.1	12.1	0.050
Mean time between onset of symptoms and hospitalization (days)	9.2	4.8	0.047
Tachypnea	15/17 (88.2%)	9/17 (52.9%)	< 0.001
Wheezing	3/17 (17.6%)	2/17 (11.8%)	0.123
Cough	14/17 (82.4%)	10/17 (58.8%)	< 0.001
Respiratory distress	11/17 (64.7%)	10/17 (58.8%)	0.218
Fever > 39ºC	11/17 (64.7%)	14/17 (82.4%)	< 0.001
Rhinorrhoea	2/17 (11.8%)	3/17 (17.6%)	0.068
Leukocytosis	8/17 (47.1%)	10/15 (66.7%)	< 0.001

Conflict of interest

All authors declare to have no conflict of interest.

REFERENCES

1. Allander T, Tammi MT, Eriksson M, et al. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci USA. 2005;102:12891-6. Erratum in: Proc Natl Acad Sci USA 2005;102:15712.
2. Simoes EAF, Cherian T, Chow J, et al. Acute respiratory infections in children. In: Jamison DT, Breman JG, Measham AR, et al. Disease Control Priorities in Developing Countries. 2nd edition. Washington (DC): World Bank. 2006. p. 483-97.
3. World Health Organization. Acute Respiratory Infections (Update September 2009). Disease Burden. http://www.who.int/vaccine_research/diseases/ari/en/index1.html
4. Schildgen O, Müller A, Allander T, et al. Human bocavirus: passenger or pathogen in acute respiratory tract infections? Clin Microbiol Rev. 2008;21:291-304.
5. Choi EH, Lee HJ, Kim SJ, et al. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000-2005. Clin Infect Dis. 2006;43:585-92.
6. Kaplan N, Dove W, Abu-Zeid A, et al. Human bocavirus infection among children, Jordan. Emerg Infect Dis. 2006;12:1418–20.
7. Manning A, Russell V, Eastick K, et al. Epidemiological profile and clinical associations of human bocavirus and other human parvoviruses. J Infect Dis. 2006;194:1283-90.
8. Weissbrich B, Neske F, Schubert J, et al. Frequent detection of bocavirus DNA in German children with respiratory tract infections. BMC Infect Dis. 2006;6:109-15.
9. Allander T, Jartti T, Gupta S, et al. Human bocavirus and acute wheezing in children. Clin Infect Dis. 2007;44:904-10.
10. García-García ML, Calvo Rey C, Pozo Sánchez F, et al. Human bocavirus infections in Spanish 0-14 year-old: clinical and epidemiological characteristics of an emerging respiratory virus. An Pediatr (Barc). 2007;67:212-9.
11. Kleines M, Scheithauer S, Rackowitz A, et al. High prevalence of human bocavirus detected in young children with severe acute lower respiratory tract disease by use of a standard PCR protocol and a novel real-time PCR protocol. J Clin Microbiol. 2007;45:1032-4.
12. Pozo F, García-García ML, Calvo C, et al. High incidence of human bocavirus infection in children in Spain. J Clin Virol. 2007;40:224-8.
13. Cilla G, Oñate E, Perez-Yarza EG, et al. Viruses in community-acquired pneumonia in children aged less than 3 years old: High rate of viral coinfection. J Med Virol. 2008;80:1843-9.
14. Martin ET, Fairchok MP, Kuypers J, et al. Frequent and prolonged shedding of bocavirus in young children attending daycare. J Infect Dis. 2010;201:1625-32.
15. Fry AM, Lu X, Chittaganpitch M, et al. Human bocavirus: a novel parvovirus epidemiologically associated with pneumonia requiring hospitalization in Thailand. J Infect Dis. 2007;195:1038-45.
16. Gerna G, Piralla A, Campanini G, et al. The human bocavirus role in acute respiratory tract infections of pediatric patients as defined by viral load quantification. New Microbiol. 2007;30:383-92.
17. Christensen A, Nordbo SA, Krokstad S, et al. Human bocavirus commonly involved in multiple viral airway infections. J Clin Virol. 2008;41:34-7.
18. Hindiyeh MY, Keller N, Mandelboim M, et al. High rate of human bocavirus and adenovirus coinfection in hospitalized Israeli children. J Clin Microbiol. 2008;46:334-7.
19. Brieu N, Guyon G, Rodière M, et al. Human bocavirus infection in children with respiratory tract disease. Pediatr Infect Dis J. 2008;27:969-73.
20. von Linstow ML, Hogh M, Hogh B. Clinical and epidemiologic characteristics of human bocavirus in Danish infants: results from a prospective birth cohort study. Pediatr Infect Dis J. 2008;27:897-902.
21. Kapoor A, Simmonds P, Slikas E, et al. Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. J Infect Dis.2010;201:1633-43.
22. Boom R, Sol CJA, Salimans MMM, et al. Rapid and simple method for purification of nucleic acids. J Clin Microbiol. 1990;28:495-503.
23. Zappa A, Canuti M, Frati E, et al. Co-circulation of genetically distinct human metapneumovirus and human bocavirus strains in young children with respiratory tract infections in Italy. J Med Virol. 2011;83:156-64.
24. Sloots TP, McErlean P, Speicher DJ, et al. Evidence of human coronavirus HKU1 and human bocavirus in Australian children. J Clin Virol. 2006;35:99-102.
25. Canducci F, Debiaggi M, Sampaolo M, et al. Two-year prospective study of single infections and co-infections by respiratory syncytial virus and viruses identified recently in infants with acute respiratory disease. J Med Virol. 2008;80:716-23.
26. Ma X, Endo R, Ishiguro N, et al. Detection of human bocavirus in Japanese children with lower respiratory tract infections. J Clin Microbiol. 2006;44:1132-4.
27. Endo R, Ishiguro N, Kikuta H, et al. Seroepidemiology of human bocavirus in Hokkaido prefecture, Japan. J Clin Microbiol. 2007;45:3218-23.
28. Chow BD, Huang YT, Esper FP. Evidence of human bocavirus circulating in children and adults, Cleveland, Ohio. J Clin Virol. 2008;43:302-6.
29. Kahn JS, Kesebir D, Cotmore SF, et al. Seroepidemiology of human bocavirus defined using recombinant virus-like particles. J Infect Dis. 2008;198:41-50.
30. Kesebir D, Vazquez M, Weibel C, et al. Human bocavirus infection in young children in the United States: molecular epidemiological profile and clinical characteristics of a newly emerging respiratory virus. J Infect Dis. 2006;194:1276-82.
31. Bastien N, Brandt K, Dust K, et al. Human bocavirus infection, Canada. Emerg Infect Dis. 2006;12:848-50.
32. Chung JY, Han TH, Kim CK, et al. Bocavirus infection in hospitalized children, South Korea. Emerg Infect Dis. 2006;12:1254-6.