Prevalence and clinical aspects of human bocavirus infection in children

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Abstract

Human bocavirus (HBoV) was recently described as a new member of the *Parvoviridae*. In order to investigate the suggested association of HBoV with respiratory and gastric disease in infants and young children, sera of 357 paediatric patients hospitalized with infectious and non-infectious diseases were retrospectively analyzed for the presence of HBoV DNA and virus-specific antibodies using quantitative PCR and ELISA, respectively. HBoV seroprevalence was determined to range from 25% in infants younger than 1 year of age to 93% in children aged more than 3 years. Viral loads between 1×10^2 and 1.2×10^6 geq/mL were observed in 6.7% (20/297) of sera obtained preferentially from young children suffering from infectious diseases. HBoV genomes were furthermore detected in 5% (3/60) of sera collected from individuals with non-infectious illnesses. HBoV DNA was present most frequently in patients with respiratory disease (9.6%). Whereas only 5.2% of patients with upper respiratory tract disease were viraemic, HBoV DNA was found in 14.6% and 10.0% of patients with lower respiratory tract illness and pneumonia, respectively. Acute HBoV infections were also observed in 7.5% of patients with gastroenteritis and in one child with inflammatory bowel disease. None of 77 patients hospitalized for various other infectious diseases (e.g. rash, urinary tract infection, meningitis) displayed viraemia. In 60.9% and 47.8% of DNA-positive children, HBoV-specific IgM and IgG was observed, respectively. The present prospective study provides comprehensive data on the clinical association of acute HBoV infection with respiratory illness and on the seroprevalence of virus-specific antibodies in children.

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Introduction

Human bocavirus (HBoV), a recently discovered member of the *Parvoviridae*, was identified in children with respiratory infections [1,2]. Phylogenetic analysis of the viral genome has revealed a close relationship of HBoV to the canine minute virus and bovine parvovirus, which are both members of the genus *Bocavirus*. Several HBoV isolates with high sequence homology have been described [3]. HBoV was detected using PCR-based techniques not only in clinical respiratory samples, but also in faecal excretions of young children in Australia, North America, Asia, Europe, Africa and the Middle East, indicating a worldwide distribution of the virus [4–19]. The prevalence of HBoV-DNA in samples of children with respiratory illness is in the range 1.5–18.3% and may vary depending on seasonal fluctuations and the age of the patients. In children, HBoV tends to be linked with a high rate of co-infections with other respiratory viruses (e.g. picornaviruses, adenoviruses, respiratory syncytial virus and human metapneumovirus) [20]. HBoV has been estimated to be the fourth most prevalent single virus after respiratory syncytial virus (RSV), rhino- and adenovirus in children hospitalized with respiratory disease [21]. Because most studies did not include control samples from children without symptoms indicating the involvement of infections, HBoV has not been clearly identified as a pathogen responsible for respiratory diseases.

Studies of the seroprevalence of HBoV have described the use of recombinant VP1 and VP2 proteins and VP2 virus-like particles (VP2–VLP) for the detection of HBoV-specific IgG

in Japanese, Chinese, Finnish and German populations [22–25]. Thereby, ubiquitous antibody responses with a seroprevalence of up to 94–100% were detected in adults and in children aged more than 6 years, whereas seronegative individuals were predominantly found among small children aged approximately 1–2 years.

In the present study, we present a comprehensive overview concerning the frequency and impact of acute HBoV infection in 297 children and adolescents hospitalized with symptoms indicating the involvement of infectious diseases. In addition, sera derived from 60 children were included who were admitted because of various non-infectious conditions (e.g. planned surgery, bone fractures, accidents).

Materials and Methods

Patients and controls

Between December 2006 and May 2007, sera were collected from 297 patients (160 males, 137 females, age range 1–190 months, mean age 57.4 months) at the Children's Hospital St Hedwig (University of Regensburg). All samples were derived from immunocompetent caucasians with residence in Bavaria (Germany), who were hospitalized with characteristic symptoms and clinical parameters of infectious diseases (i.e. fever, elevated C-reactive protein, leukocytosis). Follow-up samples were available from six children. Sixty children (38 males, 22 females, age range 1–194 months, mean age 77.9 months) with non-infectious conditions (i.e. planned surgery, accidents, fractures) served as controls.

Detection of HBoV DNA and HBoV-specific antibodies

The HBoV DNA was isolated from serum using the QiaAmp DNA blood mini kit (Qiagen, Hilden, Germany) and amplified using real-time TaqMan PCR, as described previously [22,26]. For the detection of HBoV-specific antibodies, an ELISA based on recombinant HBoV VP2–VLP was used [22,26].

Laboratory analysis of infectious diseases

All patients were routinely tested for blood parameters indicative for infectious diseases and inflammatory processes (e.g. C-reactive protein, differential blood cell count). Depending upon the disease manifestation, throat swabs/respiratory aspirates and stool samples from patients with respiratory and gastrointestinal symptoms, respectively, were routinely analyzed using bacterial culture techniques; all samples from patients with respiratory symptoms were tested for the presence of RSV using routine antigen detection systems. Samples testing negative for bacterial pathogens were additionally analyzed for the presence of respiratory (influenza-, human metapneumo-, adenovirus) or gastrointestinal viruses (rota-, noro-, enterovirus) using PCR and/or routine antigen detection systems. In special cases, assays for the detection of further viral pathogens (e.g. Epstein–Barr and human cytomegalovirus) were performed.

Ethics

The study was approved by the ethics committee of the University of Regensburg (no. 06/85). Only sera drawn for diagnostic and/or clinical reasons were included in the study. Parental consent was obtained prior to sample processing.

Statistical data analysis

All data were evaluated by chi squared and the Mann–Whitney *U*-test for independent samples.

Results

Description of patients included in the study

A total of 297 children and adolescents with symptoms indicating infectious diseases were studied. With respect to their clinical diagnosis, the children were divided into three groups (Table 1). Group A comprised 156/297 patients (52.5%) with diseases of the respiratory tract, who were subdivided into 58 patients with symptoms involving the upper respiratory tract (i.e. rhinitis, otitis media, laryngitis, tonsillitis, cough), 48 patients with symptoms involving the lower respiratory tract (i.e. wheezing bronchitis and bronchiolitis) and 50 patients with pneumonia. The diagnosis of pneumonia was routinely confirmed by X-ray analysis. Of the 297 patients, 64 (21.5%, group B) presented with gastrointestinal symptoms (i.e. diarrhoea, nausea, vomiting). Eleven of them were diagnosed with chronic inflammatory bowel disease. The third patient group (group C) comprised 77 patients (77/297, 25.9%) with a broad range of infections involving neither the respiratory nor the gastrointestinal tract (e.g. meningitis, encephalitis and urinary tract infections). Furthermore, 60 children without infectious diseases were included in the study. Because respiratory aspirates were available only from a small subgroup of patients, analysis was focused on sera that were drawn during hospitalization and tested for the presence of HBoV-specific humoral immune reactions and viral DNA.

Age-related prevalence of HBoV DNA and of HBoV-specific antibodies

A seroprevalence of 71.4% (255/357 patients) was observed ranging from 23% at the age of 7–9 months to more than 90% in children aged 31–36 months or more (Fig. 1A).

TABLE 1. Human bocavirus (HBoV)-specific IgG antibodies and viral DNA present in serum samples of patients and controls

Clinical course/ symptoms	Number of patients	Mean age (months)	Age range (months)	HBoV-specific IgG	VP2	HBoV	DNA
Infectious disease	297	57.4	1–190	209/297	70.3%	20/297	6.7%
(A) Respiratory tract	156	40.5	1–178	95/156	60.9%	15/156	9.6%
Upper respiratory tract (rhinitis, otitis media, laryngitis, tonsillitis)	58	48.3	1–178	36/58	62.1%	3/58	5.2%
Lower respiratory tract (wheezing bronchitis, broncheolitis)	48	20.1	1–95	24/48	50.0%	7/48	14.6%
Pneumonia	50	51.8	5–167	35/50	70.0%	5/50	10.0%
(B) Gastrointestinal tract	64	68.5	1–173	49/64	76.6%	5/64	7.8%
Diarrhoea, nausea, vomiting	53	58.4	1–161	38/53	71.7%	4/53	7.5%
Chronic inflammatory bowel disease	11	117.5	41–173	11/11	100.0%	1/11	9.1%
(C) Others (febrile convulsion urinary tract infection, exanthema, meningitis, encephalitis)	77	82.7	4–190	65/77	84.4%	0/77	0.0%
Non-infectious condition (e.g. bone fracture, planned surgery)	60	77.9	1–194	44/60	73.3%	3/60	5.0%
Total	357	67.7	1–194	255/357	71.4%	23/357	6.4%

The number of patients in which specific antibodies or viral DNA could be detected is shown for each subgroup.

Gender-specific differences were not evident. A reduction of seroprevalence from 78% to 23% was observed in children up to the age of 7–9 months. In parallel, the median IgG titres declined from 741 relative units (RU) to 65 RU (Fig. 1B). Because acute HBoV infections were only occasionally observed in these age groups (Fig. 1C), the detected IgG mainly represent maternal antibodies. Starting at the age of 25 months, rising values of both seroprevalence and IgG-titres were observed as a consequence of the accumulating numbers of acute HBoV infections occurring in children aged 13–30 months (Fig. 1A,C).

HBoV genomes were observed preferentially in sera from patients aged younger than 30 months. In older patients, only sporadic HBoV-infections were observed (Fig. 1C). Whereas the mean age of all patients was 67.7 months, patients with acute HBoV infection had a lower mean age of 24.9 months (Table 2).

Viral genomes and HBoV-specific antibodies in patients and controls

HBoV DNA indicating acute infection were detected in 20/297 patients (6.7%) and three 60 controls (5.0%) (Table 1). Although these overall values were rather similar, differences became evident after correlating patient groups A, B and C with markers for acute HBoV infection: in the serum samples derived from 15/156 (9.6%) patients of group A (patients 1–15) presenting with symptoms of respiratory tract disease, HBoV DNA was detectable at concentrations varying between 10^2 and 10^3 and 1.2×10^6 geq/mL (Tables 1 and 3). Follow-up samples were available for patient 10, comprising a girl suffering from bronchitis. The reduction in the DNA load from 1.9×10^5 to 4.6×10^3 geq/mL within 3 days was observed in combination with increasing amounts of VP2-specific IgM (Table 3). The prevalence of HBoV DNA in serum was highest in children suffering from diseases of the lower respiratory tract (7/48, 14.6%) and from pneumonia (5/50, 10.0%) compared to upper respiratory tract disease (3/58, 5.2%) (Table 1). With respect to patients of group C (other infections, 0/77) and those with non-infectious diseases (3/60, 5.0%), the frequency of acute HBoV infection was significantly elevated in patients with lower respiratory tract disease and pneumonia (Table 4). Co-infections with other pathogens were observed in patients 4 (RSV) and 12 (streptococcus A). Low-level HBoV viraemia (1×10^2–3.3×10^3 geq/mL) was also observed in 5/64 patients (7.8%) with gastroenteritis, one of whom was diagnosed as having chronic inflammatory bowel disease. In patients 16–18 with gastroenteritis, rotavirus infection was diagnosed in addition.

With the exception of patients with lower respiratory tract disease, the mean age of the HBoV DNA-positive patients was lower compared to that of all patients of the respective groups (Table 2). This tendency was most evident in children with gastroenteritis (group B) with a mean age of 58.4 months for all patients and 15.5 months for HBoV-infected patients. Although young children preferentially presented with gastroenteritis or respiratory tract symptoms, particularly pneumonia was the lead diagnosis in children infected at an age of 22 months or more.

In children with non-infectious condition, HBoV DNA was detectable in three patients: one newborn boy (patient 21) suffering from haematemesis, one girl (patient 22) presenting with atonic seizures and a boy with a submandibular gland abscess (patient 23). Respiratory or gastrointestinal symptoms that possibly manifested during the preceding weeks were not reported.

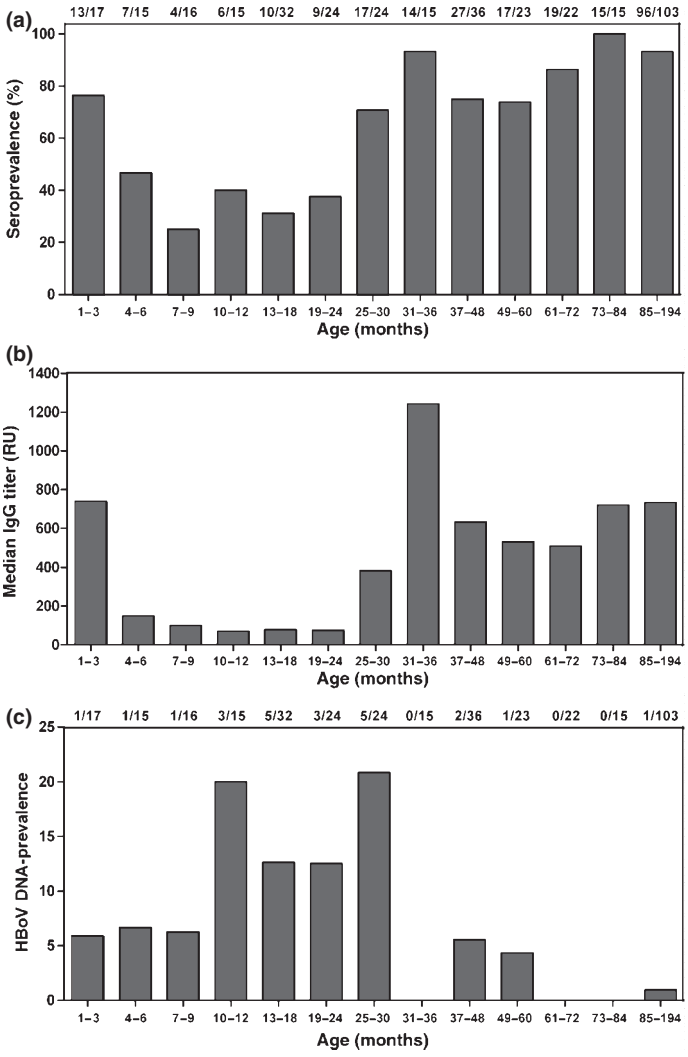

FIG. 1. Seroprevalence (a), median anti-human bocavirus (HBoV) IgG titres (b) and DNA prevalence (c) in children and adolescents. The numbers given above the columns representing the data obtained for various age groups in (a) and (c) indicate the number of individuals with positive results in relation to that of all individuals tested. (Ru; relative units).

TABLE 2. Mean patient age in the groups studied

Patients	Mean age of all patients (months)	Mean age of HBoV DNA-positive patients (months)
Total	67.7	24.9
Infectious diseases	57.4	22.5
Respiratory tract	40.5	22.1
Upper respiratory tract	48.3	20.6
Lower respiratory tract	20.1	18.7
Pneumonia	51.8	31.0
Gastrointestinal tract	68.5	20.6
Gastroenteritis	58.4	15.5
Non-infectious conditions	77.9	40.3

HBoV, human bocavirus.

HBoV-specific antibodies

IgG against HBoV VP2–VLP were detected in all patient groups, irrespective of the disease pattern. Overall, 255/357 children were IgG-positive: 209/297 (70.3%) of patients with infections and 44/60 (73.3%) of patients with non-infectious diseases (Table 1). The seroprevalence ranged from 50% in patients with lower respiratory tract disease to 84.4% in patient group C.

The majority of HBoV DNA-positive patients (14/23, 60.7%) produced virus-specific IgM as an additional marker for acute infection: 9/23 patients (39.1%; patients 3, 7, 8, 10, 14, 15, 18, 19 and 20) tested IgM-positive; furthermore, 5/23 patients (21.7%; patients 1, 2, 5, 12 and 17) displayed weakly positive IgM values (Table 3). VP2-specific IgG was observed in 11/23 patients with acute infection (47.8%; patients 1–4, 16–21 and 23) and borderline results were obtained in 2/23 patients (patients 13 and 23). In 8/23 patients (34.8%), IgG was detected in combination with IgM at positive or borderline values (patients 1, 2, 3, 8 and 7–20).

TABLE 3. Individual data of patients with acute human bocavirus (HBoV) infection

Patient	Age (months)/sex	Clinical course	HBoV serology		HBoV DNA (geq/mL)	Co-infection
			IgG	IgM		
Group A						
1	18/female	Fever, rhinitis, cough	Positive	Weakly positive	10^2–10^3	–
2	22/female	Acute laryngitis	Positive	Weakly positive	1.8×10^3	–
3	22/male	Otitis media	Positive	Positive	1.6×10^3	–
4	4/male	Acute bronchitis	Negative	Negative	1.0×10^2	RSV
5	12/male	Bronchitis, otitis media	Negative	Weakly positive	1.9×10^5	–
6	12/male	Bronchitis	Negative	Negative	3.1×10^5	–
7	17/male	Spastic bronchitis	Negative	Positive	1.2×10^6	–
8	27/male	Bronchitis	Positive	Positive	1.8×10^3	–
9	29/male	Peribronchitis, otitis media	Negative	Negative	2.3×10^4	–
10	30/female (0[a])	Bronchitis	Negative	Weakly positive	1.9×10^5	–
	30 (+2[a])	Bronchitis	Negative	Positive	1.1×10^5	–
	30 (+3[a])	Bronchitis	Negative	Positive	4.6×10^3	–
11	15/female	Bronchopneumonia	Negative	Negative	5.6×10^4	–
12	18/female	Pneumonia, respiratory insufficiency	Negative	Weakly positive	2.2×10^5	–
13	29/female	Bronchopneumonia	Weakly positive	Negative	10^2–10^3	–
14	42/female	Pneumonia	Negative	Positive	1.0×10^3	Streptococcus A
15	51/female	Atypical pneumonia	Negative	Positive	1.1×10^5	–
Group B						
16	8/female	Gastroenteritis	Positive	Negative	10^2–10^3	Rotavirus
17	12/male	Gastroenteritis	Positive	Weakly positive	10^2–10^3	Rotavirus
18	13/male	Gastroenteritis	Positive	Positive	10^2–10^3	Rotavirus
19	29/male	Gastroenteritis, diarrhoea	Positive	Positive	3.3×10^3	–
20	41/male	Inflammatory bowel disease	Positive	Positive	10^2–10^3	–
Non-infectious conditions						
21	1/male	Haematemesis	Positive	Negative	2.8×10^3	–
22	34/female	Atonic seizure	Weakly positive	Negative	4.9×10^3	–
23	86/male	Submanibular gland abscess	Positive	Negative	10^2–10^3	–

RSV: respiratory syncytial virus.
[a]Follow-up samples of patient 10 were taken 2 and 3 days after the first sample, respectively.

TABLE 4. p-Values describing the significance of acute HBoV infection in patients (groups A and B) compared to patients of group C and patients with non-infectious conditions

	Patients (group C) Other infectious disease	Patients with non-infectious conditions
Patients (group A)		
Respiratory tract disease	p 0.00491	p 0.27165
Upper respiratory tract	p 0.04357	p 0.96600
Lower respiratory tract	p 0.00056	p 0.08776
Pneumonia	p 0.00699	p 0.38904
Patients (group B)		
Gastrointestinal tract disease	p 0.01251	p 0.52407
Diarrhoea/nausea	p 0.01434	p 0.57511

Discussion

HBoV genomes have been detected in respiratory tract samples of children worldwide mainly in retrospective analyses [7,9,13,15,17]. Until recently, the serodiagnosis of HBoV infection was not possible because of the lack of suitable antigen preparations. Based on recombinant HBoV-like particles, HBoV-specific T-helper cell reactions have been detected in adults [26]. Furthermore, an ELISA was established that allowed the detection of HBoV-specific IgG and IgM in 94% and 1% of adult healthy blood donors in Germany, respectively [22]. These data point to the ubiquitous prevalence of HBoV infection in the population and to a seroconversion in childhood.

In the present study, we analyzed sera derived from 357 children and adolescents who presented with symptoms of various infectious and non-infectious conditions for HBoV-specific antibodies indicative of acute and past HBoV infection in combination with a PCR assay for the quantification of HBoV DNA. In this cohort, 88% of children aged 30–194 months displayed HBoV-specific IgG as a serological marker for previous infection. This value is consistent with previous data obtained in German and Japanese adults [22,25] and indicates that the incidence of HBoV infection is highest among young children. This assumption was confirmed when analyzing sera from children aged 10–30 months using PCR: 16 out of a total of 23 DNA-positive patients (69.6%) belonged to this age group. Because HBoV-specific IgM was detected in almost 70% of the DNA-positive patients, these individuals display the characteristic markers for acute infections. In newborns and young children up to the age of 9 months, HBoV DNA was detectable in only three patients. This low incidence is probably a result of the high prevalence of maternal IgG protective against acute HBoV infection. Together with declining concentrations of maternal IgG, a rising incidence of HBoV infection was

observed. Children aged 31–36 months displayed high titres of HBoV-specific IgG, with the titres declining in older age groups (Fig. 1B). As the consequence of the increasing IgG titres and seroprevalence, acute HBoV infections were rare in children aged more than 30 months: only 4/217 (1.8%) individuals showed markers of acute HBoV infection. These data are consistent with those obtained from human parvovirus B19 infections, with virus-specific IgG-titres peaking directly after acute infection and declining afterwards [27,28].

With respect to distinct patient groups, we observed the highest rate of acute HBoV infections in children with lower respiratory tract disease and pneumonia. Compared to patients with other infectious diseases (group C), this enhanced rate of acute HBoV infection was highly significant. By contrast to other reports, co-infectious agents were only observed in two HBoV DNA-positive patients: RSV infection was diagnosed in a 4-month-old boy with acute bronchitis, and a streptococcus A infection was evident in a girl suffering from pneumonia (Table 3). This is particularly surprising because RSV-infections were the main cause of bronchitis and pneumonia in patients belonging to group A (data not shown). The low rate of co-infections may be the result of the present study the focusing on the exclusive analysis of sera that were drawn at the time of patient admission to the clinic. In the case of pathogens causing systemic infections associated with respiratory tract disease, testing of sera for the presence of virus and of antiviral immune reactions represents a more reliable diagnostic procedure compared to the analysis of respiratory aspirates. The use of highly sensitive PCR methods for the detection of viral and/or bacterial nucleic acids in respiratory samples from hospitalized children may yield positive results because of aerogenic contaminations or nosocomially transmitted co-infections. These are frequent in paediatric wards, particularly during epidemic phases of respiratory infections in winter and early spring. As a member of the *Parvoviridae*, HBoV has to be considered as an extremely stable pathogen resistant against disinfectants and heat treatment [29, 31]. This property may facilitate nosocomial transmissions between children.

In addition to patients suffering from respiratory tract illness, acute HBoV infection was observed in four young children presenting with gastroenteritis (mean age 15.5 months), in three of whom rotavirus co-infection was observed.

A recent study described the occurrence of HBoV in stool samples of children with acute gastroenteritis [32]. Similar to our results, a high rate of rotavirus co-infections was reported. Therefore, the possible role of HBoV as a causative agent of acute gastroenteritis remains unclear.

Acute HBoV infection (IgM-/IgG-positive, DNA-positive) was also observed in a boy presenting with an acute episode of an underlying chronic inflammatory bowel disease. Markers for acute HBoV infection were not observed in any of the children presenting with symptoms of other infectious diseases (group C). When analyzing children with non-infectious diseases, low amounts of HBoV genomes were detectable in three patients, although no symptoms for either respiratory or gastrointestinal tract diseases were on record in any of these cases.

Together with the high HBoV seroprevalence in older children and adults, this observation indicates that HBoV infections may be frequently asymptomatic or associated with mild symptoms not requiring hospitalization.

In conclusion, the data obtained in the present study reveal that acute HBoV infection is most frequent in young children aged 10–30 months and that HBoV is as new infectious agent in the growing group of pathogens causing respiratory tract disease in young children.

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Transparency Declaration

The authors report no conflicting interests.

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