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REVIEW

Human bocavirus—the first 5 years

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SUMMARY

Four species of human bocavirus (HBoV) have been recently discovered and classified in the *Bocavirus* genus (family *Parvoviridae*, subfamily *Parvovirinae*). Although detected both in respiratory and stool samples worldwide, HBoV1 is predominantly a respiratory pathogen, whereas HBoV2, HBoV3, and HBoV4 have been found mainly in stool. A variety of signs and symptoms have been described in patients with HBoV infection including rhinitis, pharyngitis, cough, dyspnea, wheezing, pneumonia, acute otitis media, fever, nausea, vomiting, and diarrhea. Many of these potential manifestations have not been systematically explored, and they have been questioned because of high HBoV co-infection rates in symptomatic subjects and high HBoV detection rates in asymptomatic subjects. However, evidence is mounting to show that HBoV1 is an important cause of lower respiratory tract illness. The best currently available diagnostic approaches are quantitative PCR and serology. This concise review summarizes the current clinical knowledge on HBoV species. Copyright © 2011 John Wiley & Sons, Ltd.

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INTRODUCTION

Human bocavirus (HBoV, lately denoted HBoV1) was discovered by Allander *et al*. in 2005 [1]. It was the first virus identified by "molecular virus screening", a procedure based on DNase treatment of nasopharyngeal samples, random nucleic acid amplification and cloning, followed by large scale sequencing and bioinformatic analyses. After excluding human, bacterial and known viral sequences, a new parvovirus sequence was identified. Further analysis based on the deduced amino acid sequences showed that the closest relatives of the new parvovirus were bovine parvovirus and canine minute virus of the genus *Bocavirus*. The new virus was therefore named human bocavirus. In 2009–2010, this genus was expanded by three additional species of human bocaviruses, HBoV2, HBoV3 and HBoV4 (Figure 1) [2–4]. HBoV2 can further be divided in strains A and B based on a >5% divergence in the nonstructural gene nucleotide sequences [4].

Human bocaviruses have been detected worldwide by PCR not only in respiratory (Table 1) and stool samples (Table 2) but also in serum [5–10], tonsillar [11], saliva [12], and urine samples [13,14] as well as in river [15] and sewage water [16,17]. Contrary to HBoV1, HBoV2-4 seem to occur mainly in human stool (Table 2) [2–4,18,19] but rarely also in the respiratory tract [20,21]. Relatives of HBoV have further been detected in animals [22–27]. Although HBoV1 has been encountered in individuals of all ages, its predilection has been young children with respiratory symptoms. However, with molecular diagnostics of respiratory tract secretions, HBoV1 may often be seen also in asymptomatic children raising justified concerns on causality [28–35]. Recent analyses of blood samples and advances in

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Abbreviations used
AAV2, adeno-associated virus 2; BAL, bronchoalveolar lavage; B19V, human parvovirus B19; DNA, deoxyribonucleic acid; EIA, enzyme immunoassay; HBoV, human bocavirus; Ig, immunoglobulin; ML, maximum likelihood; NP, nuclear phosphoprotein; NPA, nasopharyngeal aspirate; NS, nonstructural protein; ORF, open reading frame; PCR, polymerase chain reaction; Th, T helper; VP, viral (structural) protein; VLP, virus-like particle.

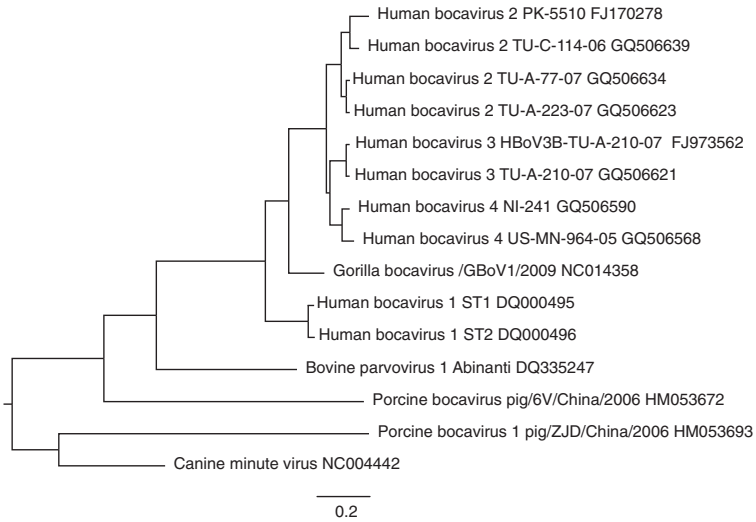

Figure 1. Maximum likelihood (ML) phylogenetic tree of representative members of the genus *Bocavirus* based on partial VP1 gene sequences (CDS 3282–3738 according to FJ170278 sequence). The alignment was constructed using Se-Al and regions that were difficult to align were removed. The final alignment was 480 nucleotides long. The ML tree was inferred using PhyML 3.0 with 5 random starting trees, the SPR (subtree pruning regrafting) and nearest NNI (neighbor interchange) search algorithms, and the GTR + G + I substitution model, which was identified as the best model by FindModel. The scale bar indicates nucleotide substitutions per site

serodiagnosis, however, have provided compelling evidence for HBoV1 being a true respiratory pathogen [10,14,36–40]. In this concise review, we update the current clinical knowledge on HBoV infections in humans.

SEARCH STRATEGY AND SELECTION CRITERIA

Since the discovery of HBoV, we have performed active research on HBoV and followed the literature. For this review, we searched the PubMed data base for articles published before 23.5.2011 with "bocavirus" as a search term (335 hits including 295 published in English). Of the latter, we include major molecular and clinical studies on HBoV.

VIRUS STRUCTURE

Human bocaviruses belong to the family *Parvoviridae*, subfamily *Parvovirinae*. They are minute DNA viruses that for replication are highly dependent on cellular functions including the DNA polymerase. Their linear single-stranded genome is only ~5 kb in length with still unknown terminal sequences [41]. The genome putatively encodes two forms of the nonstructural protein NS1 and a for bocaviruses unique, nuclear phosphoprotein NP1, as well as two major structural proteins, VP1 and VP2 (Figure 2) [42–44]. These latter capsid proteins share in common the C-terminal part except the very N-terminus of VP1, containing a phospholipase-A motif, unique (VP1u). By electron microscopy, the structure of HBoV is typical of *Parvoviridae*, that is, non-enveloped capsid of icosahedral symmetry and diameter of ~25 nm [10,39,45,46]. The 3-dimensional ultrastructure has been solved with recombinant HBoV VP2 capsids at 7.9-Å resolution, showing many characteristics in common with other parvoviruses, such as a depression at the twofold axis, a protrusion at the threefold axis and a channel at the fivefold axis surrounded by a canyon [46]. In smooth topology, the HBoV VP2 capsid resembles that of human parvovirus B19 (B19V). The fivefold channel of B19V is, however, closed, whereas that of HBoV, similarly to many other parvoviruses, may permit the VP1-N or VP2-N termini to be externalized. This might imply that VP1u in the virion could reside inside the capsid rather than on the surface. In line with this notion is our serological results showing, contrary to B19V, only minor antibody reactivity with HBoV VP1u compared with VP2 and a relative resistance of VP2 against denaturation by low concentrations of urea [36,40].

EPIDEMIOLOGY

The true prevalence of HBoV1 infection was difficult to determine until the development of serology

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Table 1. Major clinical studies on human bocavirus-1 DNA etiology of respiratory illnesses by PCR[a]

1st author	Study site	Study year	*n*	Age (years)	HBoV1+, *n* (%)	HBoV1 qPCR reported	Viruses tested	Mixed viral findings (%)	HBoV1 co-infection, *n* (%)
Allander [1]	Sweden	03–04	540	70% pediatric	17 (3)	no	8	n/g	3 (18)
Lu [158]	Thailand	04–05	1178	n/g	39 (3)	yes	n/g	n/g	n/g
Bastien [63]	Canada	03–04	1209	<6: 290 (24%)	8 (3)[b]	no	9[b]	n/g	n/g
				6–15: 149 (12%)	3 (2)[b]				
				16–50: 444 (37%)	6 (1)[b]				
				>50: 324 (27%)	1 (0.3)[b]				
Choi [53]	Korea	00–05	515	<6	58 (11)	no	11	36 (7)	22 (38)
Foulongne [127]	France	03–04	589	<6	26 (4)[b]	no	9[b]	n/g	9 (35)
Weissbrich [129]	Germany	02–05	835	<9	87 (10)	no	8[b]	n/g	34 (39)
Manning [52]	United Kingdom	05–06 + archive	924	<5: 624 (68%)	53 (6)	yes	8[b]	47 (5)	23 (43)
				≥5: 300 (32%)					
Allander [5]	Finland	00–02	259	<16	49 (19)	yes	16	89 (34)	37 (76)
Fry [6]	Thailand	04–05	1680	all ages	73 (4)	yes	14	n/g	42/53 (79%)[d]
Lau [99]	Hong Kong	04–05	400	all ages	20 (5)	no	12[b]	n/g	26/79 (33)
			1200	<18	83 (7)				
Kesebir [50]	USA	04	425	<2	22 (5)	no	8	n/g	n/g
Ma [159]	Japan	02–03	318	median 1.8	18 (6)	no	4[c]	—	—
Vicente [97]	Spain	05–06	520	<3	40 (8)	no	12	n/g	25 (63)
Monteny [160]	The Netherlands	05–06	257	<3	4 (2)	yes	14	n/g	3 (75)
Völz [133]	Germany	05–06	389	<2	11 (3)	no	10[b]	n/g	4 (36)
Pozo [13]	Spain	04–06	917	<14	123 (13)	no	16	n/g	74 (60)
Esposito [145]	Italy	04–05	1332	<15	99 (7)	no	15	230 (17)	50 (51)
Chieochansin [101]	Thailand	06–07	302	<15	20 (7)	no	7[b]	n/g	8 (40)
Christensen [161]	Norway	06–07	376	children	45 (12)	yes	17	103 (27)	35 (78)
Regamey [28]	Switzerland	99–04	112	<1	5 (4)	no	16	18 (16)	n/g
I.P. [162]	Hong Kong	05–06	1906	<15	95 (5)	no	8[b]	n/g	18 (19)
Calvo [89]	Spain	05–07	710	<14	99 (14)	no	16	127 (18)	64 (65)
Garcia-Garcia [60]	Spain	04–06	908	<14	153 (17)	no	16	187 (21)	n/g
von Linstow [32]	Denmark	04–06	697	<1	57 (8)	yes	13	n/g	27 (47)
Brieu [58]	France	06–07	507	<5	55 (11)	yes	9	n/g	22 (40)
Pierangeli [163]	Italy	04–07	214	children	34 (8)	no	multiple	n/g	21 (62)

Continues

Table 1. (Continued)

1st author	Study site	Study year	*n*	Age (years)	HBoV1+, *n* (%)	HBoV1 qPCR reported	Viruses tested	Mixed viral findings (%)	HBoV1 co-infection, *n* (%)
Longtin [61]	Canada	02–03	225	<4,	31 (14)	no	5[b]	n/g	22 (71)
			126	adults	1 (1)				0 (0)
Dina [87]	France	03–05	828	<15	11 (1)[c]	no	12–16	n/g	n/g 4 (57)
			448		7 (2)				
Tozer [8]	Australia	03–04	96	<5	17 (18)	yes	26	n/g	4 (24)
Han [20]	South Korea	08–09	212	<5	HBoV1 0 (0)	no	14	n/g	−4 (80)
					HBoV2 5 (2)				
Zeng [83]	China	06–07	351	<4	16 (5)	no	8[b]	n/g	3 (19)
Karalar [9]	Germany	06–07	156	<16	15 (10)	yes	5[b]	n/g	2 (13)
Yoshida [164]	Vietnam	07–08	958	<5	(2)[e]	no	13	(12)[e]	n/g
Wang [14]	China	06–08	817	0.5–9	96 (12)	yes	15	103 (13%)	49 (51)
Franz [165]	Germany	06–08	404	<16	28 (7)	no	13	67 (17%)	19 (68)
Calvo [166]	Spain	05–08	370	<2	42 (11)	no	16	79 (21)	28 (67)
Miron [167]	Israel	05–06	465	<3	31 (7)	no	8	140 (30)	28 (90)
Martin [35]	USA	06–08	318	0–2	106 (33)	yes	15	(24)[e]	76 (72)

n, number of samples; HBoV, human bocavirus; qPCR, quantitative polymerase chain reaction; n/g, not given.

[a]Selection criteria: Majority of other respiratory viruses were studied. The following studies had limited focus on specific respiratory illnesses: Choi (2006) [52], lower respiratory tract illness; Lu (2006) [158], pneumonia; Allander (2007) [5], acute wheezing; Longtin (2008) [60], adults had either chronic obstructive pulmonary disease or community-acquired pneumonia; Franz (2010) [165], acute wheezing and pneumonia; Calvo (2010) [166], bronchiolitis; and Miron (2010) [167], bronchiolitis.

[b]Rhinovirus PCR was not included.

[c]Only other virus negative samples were tested for HBoV.

[d]Only pneumonia cases were tested for co-infections.

[e]Only percentages were given.

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Table 2. Major clinical studies on human bocavirus etiology of gastrointestinal illnesses by PCR[a]

1st author	Study site	Study year	*n*	Age (years)	HBoV type tested	HBoV+, *n* (%)	HBoV qPCR reported	Viruses or microbes tested	Mixed viral or bacterial findings, *n* (%)	HBoV co-infection, *n* (%)
Vicente [97]	Spain	05–06	527	<3	HBoV1	48 (9%)	no	6	n/g	28 (58%)
Lau [99]	Korea	05–06	1435	<6	HBoV1	30 (2%)	no	6	n/g	14/25 (56%)
Albuquerque [98]	Brazil	03–05	705	<15	HBoV1	14 (2%)	yes	10	n/g	3 (21%)
Lee [96]	Korea	05–06	962	<6	HBoV1	8 (1%)	no	14	45 (5%)	3 (38%)
Yu [30]	China	06–07	1216	<5	HBoV1	67 (6%)	no	5	102 (8%)	52 (78%)
Chieochansin [101]	Thailand	05–06	225	0.3–4	HBoV1	2 (1%)	no	2	n/g	1 (50%)
Cheng [31]	China	06–07	397	<5	HBoV1	14 (4%)	yes	8	n/g	9 (64%)
Campe [33]	Germany	07	307	all ages	HBoV1	14 (5%)	yes	7	n/g	8 (57%)
Arthur [2]	Australia	01	186	<17	HBoV2 HBoV3	32 (17%)	no	multiple	51 (27%)	n/g
Kapoor [3]	Pakistan United Kingdom	archive	98 699	all ages	HBoV2	5 (5%) 3 (0.4%)	no	1	—	—
Nakanishi [168]	Japan	03–05	877	<15	HBoV1	4 (0.5%)	no	6	28 (3%)	1 (25%)
Han [67]	Korea	08–09	358	<17	HBoV1	2 (0.5%)	no	6	26 (7%)	2 (100%)
					HBoV2	13 (4%)				3 (23%)
Szomor [103]	Hungary	07–08	61	<5	HBoV1	2 (3%)	no	9	0 (0%)	—
Tozer [8]	Australia	03–04	136	children	HBoV1	13 (10%)	yes	9	3 (23%)	3 (23%)
			239	adults		5 (2%)			1 (25%)	1 (20%)
Räsänen [b] [17]	Finland	07	50	<15	HBoV1	4 (8%)	no	5–11	32 (64%)	3 (75%)
Nadji [169]	Iran	06–08	47	<17	HBoV1	6 (13%)	no	1	—	—
Pham [170]	Japan	07–08	247	0.2–15	HBoV1	3 (1%)	no	4	3 (1%)	2 (67%)
Santos [69]	Brazil	n/g	807	all ages	HBoV1	10 (1%)	no	multiple	n/g	2 (0.2%)
					HBoV2	30 (21%)				13 (43%)
					HBoV3	5 (1%)				2 (0.2%)
Chow [19]	USA	07–08	479	151 children 328 adults	HBoV1 HBoV2 HBoV3	9 (2%) 6 (1%) 0 (0%)	no	3	n/g	n/g
Karalar [9]	Germany	06–07	64	<15	HBoV1	5 (8%)	yes	4	n/g	3 (60%)
Kapoor [4]	Nepal Nigeria	n/g	641	children adults	HBoV1 HBoV2A	4 (1%) 4(1%)	no	5	n/g	—

Continues

Table 2. (Continued)

1st author	Study site	Study year	*n*	Age (years)	HBoV type tested	HBoV+, *n* (%)	HBoV qPCR reported	Viruses or microbes tested	Mixed viral or bacterial findings, *n* (%)	HBoV co-infection, *n* (%)
	Tunisia				HBoV2B	76 (12%)				
	USA				HBoV3	11(2%)				
					HBoV4	6 (1%)				
Kantola [68]	Finland	n/g	250	33 children	HBoV1	0 (0%)	yes	8	n/g	0 (0%)
				217 adults	HBoV2	4 (2%)				2 (50%)
					HBoV3	1 (0.4%)				1 (100%)
					HBoV4	0 (0%)				0 (0%)

n, number of samples; HBoV, human bocavirus; qPCR, quantitative polymerase chain reaction; n/g, not given.
[a]Selection criteria: PubMed search using key words "bocavirus" and "gastroenteritis" or "bocavirus" and "gastrointestinal".
[b]Recruited during a waterborne outbreak.

in 2007–2008 [36,47,48]. By PCR analysis of respiratory tract secretions, HBoV1 has been found worldwide in approximately 2–19% of patients with upper or lower respiratory disease (Table 1). HBoV1 DNA is mainly detectable in children aged 6–24 months, year-round, yet predominantly during the winter and spring [1,6,10,31,49–62]. HBoV1 is detectable less frequently in other age groups including adults [6,53,56,58,61,63–65]. Data are scarce on the occurrence and consequences of HBoV1 infection among the elderly.

HBoV2, HBoV3, and HBoV4 DNA occur mainly in stool [2–4,18,19,21,66–70], and HBoV2 and possibly HBoV3 are associated with gastroenteritis [2,19,67,69]. Of these enteric species, HBoV2 is the most common with incidences up to 26%, followed by HBoV3 (5%) and HBoV4 (2%). The detection rates in adults (respectively, 4%, 1%, and 1%) appear in some but not all studies to be lower than in children [2,4,19,67–69]. Of the enteric HBoVs, only HBoV2 has in two studies been detected also in nasopharyngeal samples [20,21]. Co-infections by other gastrointestinal viruses have been common (Table 2).

The seroepidemiological studies on HBoV1 are in line with the PCR data. HBoV1 infection is very common during early childhood (Table 3). Because of vertical antibody transfer, seropositivity is common in infants aged <2 months, after which it declines [47,48]. Low antibody detection rates persist up to age 6–12 months after which seropositivity increases until age 6 years, when almost all children have circulating HBoV1 antibodies [9,14,36,39,40,47,48,71,72]. The vast majority of adults seem to have antibodies to HBoV1, in line with the fact that HBoV1 infections are common [14,37,39,43,48,71,73,74]. Gender-specific differences have not been reported [9]. Most HBoV1 antibodies in human sera seem to target the viral capsid protein VP2 [36,37,39,40,48,71,75].

Serological studies have used recombinant HBoV1 capsid proteins as antigen, but have not ruled out cross-reactivity with the capsid proteins of the other three HBoV species [36,46,48,72,106]. Very recently, it has been shown that cross-reactivity with HBoV2-4 immunoglobulin (Ig) G partially accounts for the high HBoV1 seroprevalences of up to 96% that have been reported [76]. When measured after competition with HBoV2-4 virus-like particles (VLPs), only ~60% were IgG positive. Based on the competitive EIA, the prevalences of

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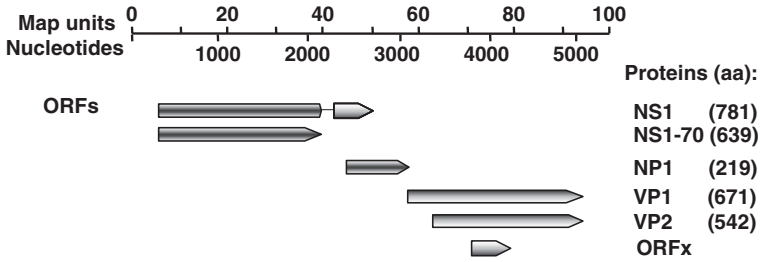

Figure 2. The genome of human bocavirus-1 with major open reading frames and proteins. The linear ssDNA genome is divided in map units and also given as nucleotides. The putative open reading frames (ORFs) are indicated as arrowed boxes, the thin line is an intron gap, and the corresponding proteins detected from transfection are shown on the right side with predicted amino acid lengths. Expression of ORFx has not been determined. Data are simplified from Dijkman *et al*. [42] and Chen *et al*. [44]. VP, viral (structural) protein; aa, aminoacids; NS, nonstructural protein; NP, nuclear phosphoprotein

HBoV infections in humans are likely to be in descending order, HBoV1, HBoV2, HBoV3, and HBoV4.

CO-INFECTIONS

HBoV DNA-positive subjects have a high co-infection rate: in respiratory samples up to 83% and in fecal samples up to 100% (Tables 1 and 2). Accordingly, HBoV1 DNA may be detectable in the nasopharynx of immunocompetent individuals for at least 6 months after infection [14,32,34,35]. Consequently, asymptomatic subjects may show high HBoV1 detection rates; in two studies up to 43–44% and in others lower 0–9% (Table 4). Approximately half of HBoV1-DNA findings have been of low copy number [5,54,77–79].

These data indicate that HBoV may exist in the respiratory or gastrointestinal tracts as a bystander without causality to the current symptoms. We observed that among wheezing children with HBoV1 detected together with other viruses by PCR in nasopharyngeal aspirate (NPA), 64% had serologically verified primary infection, whereas 36% had probably HBoV1 DNA persistence or mucosal contamination without viral replication/immunoactivation [39]. On the other hand, several PCR studies of children and/or adults including asymptomatic controls have shown an association between presence of the virus and symptomatic illness [6,10,50,58,60,62]. In many studies, a positive correlation was seen between respiratory illness and high copy numbers of HBoV1 DNA or the presence of HBoV1 monoinfection [5,10,14,40,58,77,79,80]. These multiple pieces of evidence strongly suggest that HBoV is an important respiratory pathogen in children.

TRANSMISSION

The transmission routes of human bocaviruses are unknown. However, many parvoviruses are transmitted by inhalation or contact with infectious sputum, feces, or urine [81]. It is likely that HBoV1 is transmitted similarly, as its DNA has been detected in many human secretions. Nosocomial respiratory acquisition may be as high as 18% among hospitalized HBoV1 cases, and up to 19% of nosocomial acute respiratory tract infections have been HBoV1 positive [50,56,82–84]. Intrauterine infection is unlikely because of the high degree of immunity among pregnant mothers [73,74].

TARGET ORGANS AND PATHOGENESIS

The pathogenesis of human bocaviruses is not known because no well established *in vitro* or animal models are available [41,42,44,85]. HBoV1 has, however, been cultured in primary airway epithelial cells differentiated into pseudo-stratified human airway epithelium [42]. Based on HBoV1 DNA sequences detected in that culture and in HBoV1-positive clinical samples, a hypothesis was proposed of HBoV1 having a different replication mechanism than parvoviruses in general [41]. This will, however, need further elucidation. The mechanisms of cell entry and the *in vivo* host range are unknown [41]. Multiple clinical studies suggest that HBoV1 is mainly a respiratory pathogen (Tables 1 and 3). Studies on children with pneumonia, acute wheezing, asthma and/or bronchiolitis suggest that HBoV1 is able to infect the lower airways down to the bronchioles [5,6,36,41,50,86–92]. Some reports show HBoV1 DNA in up to 3% of adult bronchoalveolar lavage (BAL) specimens

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Table 3. Clinical studies on acute human bocavirus-1 infection using serodiagnosis in patients with respiratory symptoms[a]

1st author	Study site	Study year	Age (years)	Methods	NPA PCR+, *n* (%)	Serodiagnosis of NPA PCR + cases, *n* (%)	Serum PCR+, *n* (%)	Serodiagnosis of serum PCR + cases, *n* (%)	Serodiagnosis of cases with respiratory symptoms, *n* (%)
Endo [47]	Japan	06–07	0.2–6	IgGsc	8	4/4 (100)	4	4/4 (100)	n/g
Lindner [37]	Germany	n/g	0.1–10	IgM	n/g	n/g	24	10 (42)	n/g
Lin [71]	China	06	children	IgGsc	5	3 (60)	n/g	n/g	n/g
Söderlund-Venermo [39]	Finland	00–02	0.3–6.1	IgM, IgGsc	49 (any) 28(high) 27 (low)	35 (71) 27 (96) 8 (38)	49	45 (92)	48/253 (19)[b]
Wang [14]	China	06–08	0.5–9	IgM	36 (sole) 43 (co-infected) 22 (high load)	26 (69) 18 (42) 16 (73)	21	15 (71)	44/79 (56)[c]
Don [38]	Italy	01–02	0.1–15	IgM, IgGsc	n/g	n/g	n/g	n/g	12/101 (12)[d]
Karalar [9]	Germany	06–07	0.1–15	IgM	n/g	n/g	22	16 (73)[e]	n/g

n, number of samples; NPA, nasopharyngeal aspirate; PCR, polymerase chain reaction; IgGsc, IgG seroconversion; n/g, not given.
[a]Selection criteria: PubMed search using key words "bocavirus" and "serology".
[b]Acute wheezing.
[c]Laryngitis 19%, bronchitis 5%, bronchiolitis 22%, bronchopneumonia 21%, asthma 21%, and other 12%.
[d]Pneumonia.
[e]IgM positive ($n = 10$) or weakly positive ($n = 6$).

Table 4. Human bocavirus-1 detection by PCR in respiratory samples in asymptomatic subjects[a]

1st author	Study site	Study year	*n*	Age (years)	Sample type	HBoV1 + *n* (%)	HBoV1 qPCR done	Viruses or microbes tested	Mixed viral or bacterial findings, *n* (%)	HBoV1 co-infection, *n* (%)	Comparison between HBoV1+ symptomatic and HBoV1+ asymptomatic subjects
Kesebir [50]	USA	04	96	<2	respiratory	0 (0)	no	8	n/g	—	—
Fry [6]	Thailand	04–05	280	all ages	NPS	3 (1)	yes	14	n/g	1 (33)	HBoV1 was found more often in pneumonia cases than controls. All controls had low viral loads.
Maggi [54]	Italy	01 03	30 21	infants and school-aged	respiratory	0 (0)	no	11	n/g	—	—
Chieochansin [101]	Thailand	05–06	202	0.2–5	stool	0 (0)	no	2[b]	n/g	—	Prevalence: NS
Longtin [61]	Canada	02–03	100	children	NPS	43 (43)	no	5[b]	n/g	n/g	HBoV1 was found more often in asymptomatic (43%) than symptomatic (14%) cases.
García-García [60]	Spain	04–06	116	<14	NPS	8 (7)	no	16	5 (3)	4 (50)	—
Cheng [31]	China	06–07	115	<5	stool	4 (4)	yes	8[b]	n/g	n/g	Prevalence: NS viral load: NS
von Linstow [32]	Denmark	04–06	152	<1	nasal	13 (9)	yes	13	n/g	n/g	Consecutive positive specimens contained decreasingly lower viral loads.
Brieu [58]	France	06–07	68	<5	NPS	0 (0%)	yes	9[b]	n/g	—	—

Continues

Table 4. (Continued)

1st author	Study site	Study year	*n*	Age (years)	Sample type	HBoV1 + *n* (%)	HBoV1 qPCR done	Viruses or microbes tested	Mixed viral or bacterial findings, *n* (%)	HBoV1 co-infection, *n* (%)	Comparison between HBoV1+ symptomatic and HBoV1+ asymptomatic subjects
Arthur [2]	Australia	01	186	<17	stool	15 (8%)	no	multiple	15 (8%)	n/g	HBoV1 was not associated with acute gastroenteritis.
Martin [12]	USA	07–08	56	2–4	saliva nasal	5 (9) 1 (2)	yes	0 8	n/g	n/g	Prevalence: NS
Martin [35]	USA	06–08	45	0–2	nasal	20 (44)	yes	15	n/g	n/g	Prevalence: NS qPCR: NS
Christensen [10]	Norway	07–09	162	children	NPS	27 (17)	yes	18	n/g	25 (93)	HBoV1 monoinfections were more prevalent in patients (29%) than controls (7%) but an adjusted analysis found no association to symptoms.

n, number of samples; HBoV, human bocavirus; n/g, not given; NPS, nasopharyngeal secretion; NS, non-significant.
[a]Selection criteria: PubMed search using key words "bocavirus" and "asymptomatic", or "bocavirus" and "control".
[b]Rhinovirus PCR was not included.

[54,64,65,93,94]. In follow-up of adult lung transplant recipients, BAL HBoV1-positive cases seem to be symptomatic (mainly pneumonia or acute respiratory insufficiency), with no difference in HBoV1 prevalence between immunocompetent and immunocompromised patients, whereas non-symptomatic surveillance samples seem to be HBoV1-DNA negative [64,95]. This would further indicate that HBoV1 is not a bystander but rather a real but rare, causative agent of adult respiratory symptoms.

HBoV1 infection appears to be systemic as suggested by the occurrence of its DNA in serum [5,9,10,14,36,39]. Although HBoV1 viremia/DNAemia has been linked closely to acute primary infection and moderate-to-severe illness [10,36,39], more detailed clinical correlates of HBoV1 dissemination are hitherto unknown.

Human bocaviruses have been detected in fecal samples without evidence of replication in the gastrointestinal epithelium. On the contrary, passive spread of HBoV1 from the respiratory to the gastrointestinal tract is likely because the levels of HBoV1 DNA in stool have been low, the detection rates between subjects with and without gastrointestinal symptoms have been similar, and the detection rates (of up to 100% in stool samples) of co-pathogens (gastrointestinal viruses and bacteria) have been high (Table 2) [2,4,19,30,31,33,68,69,96–102]. On the other hand, HBoV1 has been detected in stool also when the corresponding respiratory samples have been negative or there have been no symptoms other than gastroenteritis [97,103]. However, the more recently discovered HBoV species 2–4 may be true enteropathogens with detection rates in stool (especially for HBoV2) generally higher than HBoV1, and with predominant appearances in stool [2,4,68]. The notion that HBoV1, but not HBoV2, may spread from the respiratory to the gastrointestinal tract, is also supported by a study comparing stool samples of symptomatic patients and age-matched controls, with a difference in detection rate only for HBoV2 [2].

Aside of circulating antibodies, knowledge of other host defense mechanisms against HBoV is just beginning to emerge. Three studies have investigated cytokine responses either in NPA samples or in cultures of peripheral blood mononuclear cells stimulated by recombinant HBoV1 VP2 VLPs [104–106]. Because HBoV is difficult to cultivate [42,44], recombinant technology has been needed to engineer the antigens of interest. *In vitro*, HBoV1 VP2 VLPs have induced IL-13, interferon-gamma and IL-10 responses in CD4+ T cells [105,106]. HBoV1 DNA-positive NPAs of children with bronchiolitis relative to those of asymptomatic controls have shown increased levels of the T helper (Th) 1 and Th2 cell cytokines interferon-gamma, IL-2 and IL-4 [104]. However, the levels of IL-10 and TNF-alpha were lower in children with HBoV1-induced than RSV-induced bronchiolitis. These findings suggest that HBoV1 can elicit typical virus-induced immune responses involving both Th1 and Th2 cells.

PERSISTENCE

An interesting feature with HBoV1 is that its presence in the mucosa may be rather long-lasting; however, no in-depth studies regarding the mechanism are available [34,35,58,107]. Prolonged shedding or presence of virus in the airways could explain the high co-infection rate observed in numerous studies [34,35,58,107,108].

With HBoV1, unlike with other parvoviruses such as parvovirus B19V and the adeno-associated virus 2 (AAV2) [109–112], the available data speak against a disseminated genomic long-term tissue persistence in the human body [113,114]. HBoV1 might, however, have a more narrow tissue tropism for its persistence; in one study, high HBoV1 detection rates (up to 32%) were encountered among tonsillectomy patients suggesting that lymphatic tissue might be a persistence site [11]. Of note, a rather high detection rate (18%) of HBoV1 has been reported also in tissue samples of chronic sinusitis [115].

HBoV3 DNA has been detected in an episomal form in the ileum of a child with gastrointestinal symptoms [116]. Whether this episome is a storage form or a replicative intermediate in an alternative replication model [41,116], needs to be elucidated. Episomal genomes have been detected also in AAV2 *in vivo* persistence [111], even though genomic integration was common in cell culture [117]. It is further unknown, whether persistent HBoV, like AAV2, can establish latency and whether co-infection by other viruses in that case can be "helper viruses" and reactivate the dormant HBoV genomes, which also would contribute to the prevalent co-detections.

Another possible research topic is to assess whether proteins are produced without virus replication as observed for adenovirus, another DNA virus, in patients with chronic lung conditions

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[118–121]. Contrary to many other parvoviruses, however, bocaviruses seem to cause apoptosis only if replicating, and the genome itself is needed to cause a cell-cycle arrest at the G_2/M phase, whereas mere protein expression is not enough [122].

DIAGNOSIS

The diagnoses of HBoV infections reported in clinical association studies have been based almost exclusively on PCR. However, PCR is not an optimal diagnostic method because of prolonged positivity both in respiratory and gastrointestinal tracts especially in low copy numbers as discussed earlier, leading to high detection rates in asymptomatic subjects.

Primary infections diagnosed serologically or by the presence of HBoV1 DNA in serum have been linked to respiratory symptoms (Table 3) [5,9,10,14,36,39]. In one study, 94% of wheezing children with HBoV1 serodiagnosis were viremic, and 92% of viremic children showed a serodiagnosis [39]. In another study, 61% of viremic children had HBoV1-specific IgM [9]. The study also showed that 10% of patients with respiratory disease had HBoV1 viremia (upper respiratory tract disease 5%; lower respiratory tract illness 15%; pneumonia 10%), as had 8% of the patients with gastrointestinal disease. The diagnostic sensitivity of HBoV DNA detection in serum has, however, not yet been determined, nor has the precise duration of viremia/DNAemia in connection with primary infection. Serum PCR may necessitate precise timing of the sample collection.

Another question of importance is whether, or to what extent, DNA quantitation does improve HBoV diagnosis. Studies employing patient DNA stratification based on HBoV1 load in the nasopharynx have shown a correlation between load height and fewer co-infections as well as increased illness severity [5,10,58,77,79,80]. Especially very high viral loads in NPA ($>2 \times 10^8$ genomes/ml) were linked with respiratory symptoms [10]. In a study on wheezing children, 96% of those with a high load ($>10^4$ genomes/ml) of HBoV1 DNA in nasopharynx (100% with high load and sole HBoV1 infection) had in serum HBoV1 IgM or an IgG increase [39] compared with only 38% of those with a low DNA load. As some other studies failed to show an association between symptoms and HBoV copy numbers, further work is needed on the diagnostic utility of HBoV DNA quantification in the respiratory tract [9,35,79].

Serologic studies on children with acute respiratory symptoms have shown that the mere presence of HBoV1 DNA in the respiratory tract is not reliable proof of acute primary HBoV1 infection (Table 3). Even among a clinically selected cohort did only 71% of NPA HBoV1 PCR-positive children (and 6% of the PCR-negative) exhibit a serodiagnosis [39]. Although the clinical significance of HBoV1 in co-detection with other viruses in NPA has been questioned, over half of the NPA PCR-positive wheezing children co-infected with another virus did have an acute primary HBoV infection [39].

IgG avidity EIA has been set up for even more accurate diagnosis of HBoV1 infection [40]. By measuring IgG avidity that increases along the maturation of B cells, it is possible with a single serum sample to distinguish between acute and past infections, or between primary and secondary infections or immunoactivations. The latter were observed in large numbers among adults over several years of follow-up [40]. However, the contribution to past-immunity findings like these of antigenic cross-reactivity between the four bocaviruses must be kept in mind [76].

Serology and/or HBoV1-DNA detection in serum is essential for the verification of acute HBoV1 infection-induced illness. Application of these stringent diagnostic methods will be crucial for further studies on the impact of HBoV1.

CLINICAL FEATURES

Although a causal link between HBoV1 and respiratory disease has been reported in a number of studies [5,10,14,38,39], the exact clinical pictures await determination. A vast number of papers have reported HBoV1 in the context of acute respiratory illnesses including common cold, asthma, acute wheezing, bronchiolitis, pneumonia, acute otitis media, and even plastic bronchitis [5–7,13,14,34,49–53,55,61,62,83,86,87,123–138]. It is not possible to clinically differentiate between respiratory tract infections caused by different viruses such as rhinovirus, RSV, human metapneumovirus, influenza virus and HBoV1, or even bacteria [139]. Some distinct clinical features have, however, been reported: hypoxia and neutrophilia were more severe in HBoV1-positive than RSV-positive children with lower respiratory tract illness [62]. In a recent review, the prevalences of respiratory manifestations in NPA

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HBoV1 PCR-positive children included cough 79%, fever 67%, rhinorrhea 66%, oxygen therapy or hypoxia 40%, tachypnea 35%, wheeze 27%, pharyngitis 13% and other respiratory symptoms 48% (includes respiratory distress, increased work of breathing, cyanosis, apnea, rales and shortness of breath) [107]. However, because most studies are based on samples drawn for diagnostic purposes from children seeking hospital care for acute respiratory tract infection, these numbers to a large extent reflect the symptoms of the entire study population. In addition, most studies behind these numbers lack stringent criteria for HBoV1 laboratory diagnosis. In a study combining serum PCR and serodiagnostics in children, with various infectious and non-infectious diseases, only lower respiratory tract symptoms correlated with HBoV1 [9]. Among children with acute otitis media, HBoV1 DNA has been found in the nasopharyngeal aspirates in 6% and in the middle ear fluids in 3–4% of cases [140–142].

Signs of pneumonia, that is, patchy or interstitial infiltrates in chest radiography, and lung hyperinflation, peribronchial cuffing, or atelectasis appear to occur commonly (up to 43–83%) in association with pediatric HBoV1 infection, whereas lobar infiltrates or pleural effusion occur rarely (Figure 3) [1,38,50,83,127,133,138,143,144]. No radiographic sign, however, is pathognomonic for HBoV1. In one study, 18% of induced sputum samples from children with pneumonia contained HBoV1 DNA [146]. The problems discussed earlier associated with diagnosing HBoV1 infections by PCR in respiratory tract secretions must be taken into account when interpreting such data. Don *et al*. (2010) found serologically confirmed acute HBoV1 infection in 12% of children with pneumonia [38]. In all, the only symptoms that have been statistically associated with HBoV1 or confirmed by serodiagnosis are lower respiratory tract symptoms, in particular wheezing and pneumonia [5,6,10,38,39]. No study has so far provided statistical evidence for an association between HBoV1 and gastrointestinal disease, whereas one study indicated that HBoV2 is associated with gastroenteritis [2]. Other symptoms such as rash or exanthema, thrombopenia, clinical sepsis or life-threatening events have been rare in HBoV1-affected patients [50,137,145]. Blood counts and C-reactive protein levels are usually normal [49,50,125,133], whereas increased neutrophilia has been noted [62].

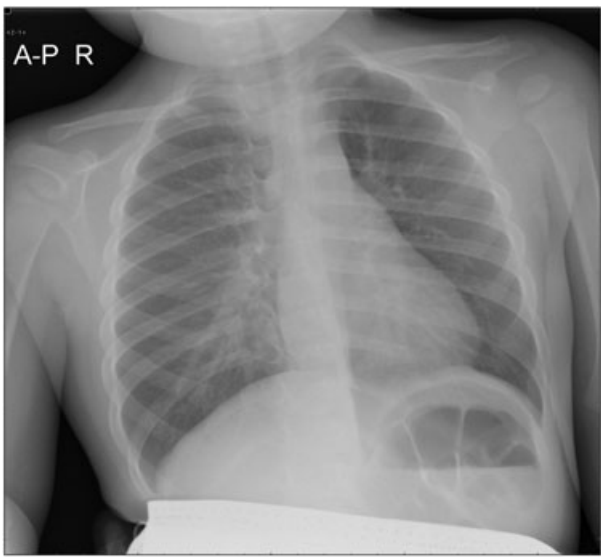

Figure 3. Radiograph of pneumonia and interstitial infiltrates in a 1.7-year old girl. Sole HBoV1-infection was serologically confirmed (16 respiratory viruses and four respiratory bacteria were searched by PCR from induced sputum samples, and Mycoplasma serology was performed). White blood cell count was 11.6×10^9/L, and serum C-reactive protein was 16 mg/L. Duration of respiratory symptoms was 6 days before hospitalization; during hospitalization, symptoms included fever, cough, and dyspnoea. The radiograph was kindly provided by Dr. Elina Lahti from the Department of Pediatrics, Turku University Hospital, Turku, Finland

In one study, 31% of 16 children with Kawasaki disease harbored HBoV1 DNA in serum, cerebrospinal fluid or stool compared with 5.5% of 579 controls [146]. This association was not confirmed in a more recent study in which HBoV1 infections were diagnosed serologically; comparable seroprevalences of HBoV1-specific IgG and IgA were found between the Kawasaki cases and controls [147]. Similarly, another study using HBoV1 PCR on serum samples showed no positive findings in 12 Kawasaki patients [20].

RISK FACTORS

HBoV infection seems to produce a long-lasting, high-avidity IgG antibody response [39,40]. Thus, a lack of protecting B-cell immunity might increase the risk of HBoV infection. Risk factors for severe HBoV1-associated illness have been considered to be similar to those for common respiratory viral infections: underlying chronic medical conditions such as cardiac disease (congenital heart lesions or heart failure) or pulmonary disease (asthma or chronic obstructive pulmonary disease), prematurity with chronic lung disease and immunosuppression

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[1,8,49,50,54,56,61,64,65,69,92,93,133,148–155]. In immunocompromised subjects, the detection of HBoV1 DNA in respiratory samples has been associated with fever, lower respiratory symptoms, seizures, hepatitis and gastrointestinal symptoms [149,151,155]. However, the extent of risk is not clear because the detection rates of HBoV1 in immunocompromized subjects have also been similar to those in immunocompetent subjects, and in most studies, the infections have not been confirmed by serum PCR or serodiagnostics. Young age can also be considered a risk factor because HBoV1 viremia occurs most commonly in children <2 years [10]. Among infants, risk factors for HBoV1-associated respiratory illness have included maternal smoking, winter birth time, and asthma predisposition [32,153]. Large day care groups increase the risk of HBoV infection because they likely are a main reservoir of HBoV. Maternal antibodies apparently protect young infants [47,48]. Atopy (i.e. allergen-specific sensitization) was not associated with HBoV1 infection among wheezing children [156]. Finally, contact with sewage or river water may also increase HBoV infection frequency [15–17].

TREATMENT

No comparative studies exist of antiviral agents for treatment of HBoV infection. Prednisolone was found not to be effective in a *post hoc* analysis of a randomized controlled trial on wheezing children with serologically confirmed HBoV1 infection [157]. Supportive care is the current treatment of choice. Although HBoV1 has been associated with lower respiratory symptoms, the course of HBoV disease is often self-limiting and uncomplicated. Moreover, no specific preventive measures are available.

CONCLUSIONS

Overall, a great deal of data is available on HBoV1, whereas little is known about the other human bocavirus species. Although most studies have been based on PCR detection of HBoV1 in respiratory tract secretions, only a few have confirmed the HBoV1 diagnosis by serology or PCR in serum, the diagnostic procedures of choice. If HBoV serology or serum samples for PCR are not available, the probably next best option is quantitative PCR with a cutoff of $>10^4$ HBoV1 genomes/ml of nasopharyngeal aspirate. Diagnosis should not be based on qualitative PCR in respiratory or gastrointestinal samples because of the persistence or recurrence of HBoV1 DNA. Despite these diagnostic challenges, it is becoming increasingly evident that HBoV1 is an important respiratory pathogen. Application of stringent diagnostic methods and criteria will be crucial not only for clinics but also for further research on HBoV.

CONFLICT OF INTEREST

The authors have no competing interest.

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REFERENCES

1. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proceedings of the National Academy of Sciences of the United States of America* 2005; **102**: 12891–12896.
2. Arthur JL, Higgins GD, Davidson GP, Givney RC, Ratcliff RM. A novel bocavirus associated with acute gastroenteritis in Australian children. *PLoS Pathogens* 2009; **5**: e1000391.
3. Kapoor A, Slikas E, Simmonds P, *et al*. A newly identified bocavirus species in human stool. *Journal of Infectious Diseases* 2009; **199**: 196–200.
4. Kapoor A, Simmonds P, Slikas E, *et al*. Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. *Journal of Infectious Diseases* 2010; **201**: 1633–1643.
5. Allander T, Jartti T, Gupta S, *et al*. Human bocavirus and acute wheezing in children. *Clinical Infectious Diseases* 2007; **44**: 904–910.
6. Fry AM, Lu X, Chittaganpitch M, *et al*. Human bocavirus: a novel parvovirus epidemiologically associated with pneumonia requiring hospitalization in Thailand. *Journal of Infectious Diseases* 2007; **195**: 1038–1045.
7. Neske F, Blessing K, Tollmann F, *et al*. Real-time PCR for diagnosis of human bocavirus infections and phylogenetic analysis. *Journal of Clinical Microbiology* 2007; **45**: 2116–2122.
8. Tozer SJ, Lambert SB, Whiley DM, *et al*. Detection of human bocavirus in respiratory, fecal, and blood samples by real-time PCR. *Journal of Medical Virology* 2009; **81**: 488–493.
9. Karalar L, Lindner J, Schimanski S, Kertai M, Segerer H, Modrow S. Prevalence and

DOI: 10.1002/rmv

clinical aspects of human bocavirus infection in children. *Clinical Microbiology and Infection* 2010; **16**: 633–639.

10. Christensen A, Nordbø SA, Krokstad S, Rognlien AG, Døllner H. Human bocavirus in children: mono-detection, high viral load and viraemia are associated with respiratory tract infection. *Journal of Clinical Virology* 2010; **49**: 158–162.
11. Lu X, Gooding LR, Erdman DD. Human bocavirus in tonsillar lymphocytes. *Emerging Infectious Diseases* 2008; **14**: 1332–1334.
12. Martin ET, Taylor J, Kuypers J, *et al*. Detection of bocavirus in saliva of children with and without respiratory illness. *Journal of Clinical Microbiology* 2009; **47**: 4131–4132.
13. Pozo F, García-García ML, Calvo C, Cuesta I, Pérez-Breña P, Casas I. High incidence of human bocavirus infection in children in Spain. *Journal of Clinical Virology* 2007; **40**: 224–228.
14. Wang K, Wang W, Yan H, *et al*. Correlation between bocavirus infection and humoral response, and co-infection with other respiratory viruses in children with acute respiratory infection. *Journal of Clinical Virology* 2010; **47**: 148–155.
15. Hamza IA, Jurzik L, Wilhelm M, Uberla K. Detection and quantification of human bocavirus in river water. *Journal of General Virology* 2009; **90**: 2634–2637.
16. Blinkova O, Rosario K, Li L, *et al*. Frequent detection of highly diverse variants of cardiovirus, cosavirus, bocavirus, and circovirus in sewage samples collected in the United States. *Journal of Clinical Microbiology* 2009; **47**: 3507–3513.
17. Räsänen S, Lappalainen S, Kaikkonen S, Hämäläinen M, Salminen M, Vesikari T. Mixed viral infections causing acute gastroenteritis in children in a waterborne outbreak. *Epidemiology and Infection* 2010; **138**: 1227–1234.
18. Chieochansin T, Kapoor A, Delwart E, Poovorawan Y, Simmonds P. Absence of detectable replication of human bocavirus species 2 in respiratory tract. *Emerging Infectious Diseases* 2009; **15**: 1503–1505.
19. Chow BD, Ou Z, Esper FP. Newly recognized bocaviruses (HBoV, HBoV2) in children and adults with gastrointestinal illness in the United States. *Journal of Clinical Virology* 2010; **47**: 143–147.
20. Han TH, Chung JY, Hwang ES. Human bocavirus 2 in children, South Korea. *Emerging Infectious Diseases* 2009; **15**: 1698–1700.
21. Song JR, Jin Y, Xie ZP, *et al*. Novel human bocavirus in children with acute respiratory tract infection. *Emerging Infectious Diseases* 2010; **16**: 324–327.
22. Cheung AK, Wu G, Wang D, Bayles DO, Lager KM, Vincent AL. Identification and molecular cloning of a novel porcine parvovirus. *Archives of Virology* 2010; **155**: 801–806.
23. Kapoor A, Mehta N, Esper F, *et al*. Identification and characterization of a new bocavirus species in gorillas. *PLoS One* 2010; **5**: e11948.
24. Sharp CP, LeBreton M, Kantola K, *et al*. Widespread infection with homologues of human parvoviruses B19, PARV4, and human bocavirus of chimpanzees and gorillas in the wild. *Journal of Virology* 2010; **84**: 10289–10296.
25. Blomström AL, Belák S, Fossum C, Fuxler L, Wallgren P, Berg M. Studies of porcine circovirus type 2, porcine boca-like virus and torque teno virus indicate the presence of multiple viral infections in postweaning multisystemic wasting syndrome pigs. *Virus Research* 2010; **152**: 59–64.
26. Zhai S, Yue C, Wei Z, *et al*. High prevalence of a novel porcine bocavirus in weanling piglets with respiratory tract symptoms in China. *Archives of Virology* 2010; **155**: 1313–1317.
27. Shan T, Lan D, Li L, *et al*. Genomic characterization and high prevalence of bocaviruses in swine. *PLoS One* 2011; **6**: e17292.
28. Regamey N, Frey U, Deffernez C, Latzin P, Kaiser L. Swiss Paediatric Respiratory Research Group. Isolation of human bocavirus from Swiss infants with respiratory infections. *Pediatric Infectious Disease Journal* 2007; **26**: 177–179.
29. Schildgen O, Müller A, Allander T, *et al*. Human bocavirus: passenger or pathogen in acute respiratory tract infections? *Clinical Microbiology Reviews* 2008; **21**: 291–304.
30. Yu JM, Li DD, Xu ZQ, *et al*. Human bocavirus infection in children hospitalized with acute gastroenteritis in China. *Journal of Clinical Virology* 2008; **42**: 280–285.
31. Cheng WX, Jin Y, Duan ZJ, *et al*. Human bocavirus in children hospitalized for acute gastroenteritis: a case-control study. *Clinical Infectious Diseases* 2008; **47**: 161–167.
32. von Linstow ML, Høgh M, Høgh B. Clinical and epidemiologic characteristics of human bocavirus in Danish infants: results from a prospective birth cohort study. *Pediatric Infectious Disease Journal* 2008; **27**: 897–902.
33. Campe H, Hartberger C, Sing A. Role of human bocavirus infections in outbreaks of gastroenteritis. *Journal of Clinical Virology* 2008; **43**: 340–342.
34. Blessing K, Neske F, Herre U, Kreth HW, Weissbrich B. Prolonged detection of human bocavirus DNA in nasopharyngeal aspirates of children with respiratory tract disease. *Pediatric Infectious Disease Journal* 2009; **28**: 1018–1019.
35. Martin ET, Fairchok MP, Kuypers J, *et al*. Frequent and prolonged shedding of bocavirus in young children attending daycare. *Journal of Infectious Diseases* 2010; **201**: 1625–1632.
36. Kantola K, Hedman L, Allander T, *et al*. Serodiagnosis of human bocavirus infection. *Clinical Infectious Diseases* 2008; **46**: 540–546.
37. Lindner J, Karalar L, Zehentmeier S, *et al*. Humoral immune response against human bocavirus VP2 virus-like particles. *Viral Immunology* 2008; **21**: 443–449.
38. Don M, Söderlund-Venermo M, Valent F, *et al*. Serologically verified human bocavirus pneumonia in children. *Pediatric Pulmonology* 2010; **45**: 120–126.
39. Söderlund-Venermo M, Lahtinen A, Jartti T, *et al*. Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. *Emerging Infectious Diseases* 2009; **15**: 1423–1430.
40. Hedman L, Söderlund-Venermo M, Jartti T, Ruuskanen O, Hedman K. Dating of human bocavirus infection with protein-denaturing IgG-avidity assays-secondary immune activations are ubiquitous in immunocompetent adults. *Journal of Clinical Virology* 2010; **48**: 44–48.
41. Lüsebrink J, Schildgen V, Tillmann RL, *et al*. Detection of head-to-tail DNA sequences of human bocavirus in clinical samples. *PLoS One* 2011; **6**: e19457.
42. Dijkman R, Koekkoek SM, Molenkamp R, Schildgen O, van der Hoek L. Human

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bocavirus can be cultured in differentiated human airway epithelial cells. *Journal of Virology* 2009; **83**: 7739–7748.

43. Cecchini S, Negrete A, Virag T, Graham BS, Cohen JI, Kotin RM. Evidence of prior exposure to human bocavirus as determined by a retrospective serological study of 404 serum samples from adults in the United States. *Clinical and Vaccine Immunology* 2009; **16**: 597–604.
44. Chen AY, Cheng F, Lou S, *et al*. Characterization of the gene expression profile of human bocavirus. *Virology* 2010; **403**: 145–154.
45. Brieu N, Gay B, Segondy M, Foulongne V. Electron microscopy observation of human bocavirus (HBoV) in nasopharyngeal samples from HBoVinfected children. *Journal of Clinical Microbiology* 2007; **45**: 3419–3420.
46. Gurda BL, Parent KN, Bladek H, *et al*. Human bocavirus capsid structure: insights into the structural repertoire of the *Parvoviridae*. *Journal of Virology* 2010; **84**: 5880–5889.
47. Endo R, Ishiguro N, Kikuta H, *et al*. Seroepidemiology of human bocavirus in Hokkaido prefecture, Japan. *Journal of Clinical Microbiology* 2007; **45**: 3218–3223.
48. Kahn JS, Kesebir D, Cotmore SF, *et al*. Seroepidemiology of human bocavirus defined using recombinant virus-like particles. *Journal of Infectious Diseases* 2008; **198**: 41–50.
49. Arnold JC, Singh KK, Spector SA, Sawyer MH. Human bocavirus: prevalence and clinical spectrum at a children's hospital. *Clinical Infectious Diseases* 2006; **43**: 283–288.
50. 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. *Journal of Infectious Diseases* 2006; **194**: 1276–1282.
51. Chung JY, Han TH, Kim CK, Kim SW. Bocavirus infection in hospitalized children, South Korea. *Emerging Infectious Diseases* 2006; **12**: 1254–1256.
52. Manning A, Russell V, Eastick K, *et al*. Epidemiological profile and clinical associations of human bocavirus and other human parvoviruses. *Journal of Infectious Diseases* 2006; **194**: 1283–1290.
53. 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. *Clinical Infectious Diseases* 2006; **43**: 585–592.
54. Maggi F, Andreoli E, Pifferi M, Meschi S, Rocchi J, Bendinelli M. Human bocavirus in Italian patients with respiratory diseases. *Journal of Clinical Virology* 2007; **38**: 321–325.
55. Bastien N, Chui N, Robinson JL, *et al*. Detection of human bocavirus in Canadian children in a 1-year study. *Journal of Clinical Microbiology* 2007; **45**: 610–613.
56. Chow BD, Huang YT, Esper FP. Evidence of human bocavirus circulating in children and adults, Cleveland, Ohio. *Journal of Clinical Virology* 2008; **43**: 302–306.
57. 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. *Journal of Medical Virology* 2008; **80**: 716–723.
58. Brieu N, Guyon G, Rodière M, Segondy M, Foulongne V. Human bocavirus infection in children with respiratory tract disease. *Pediatric Infectious Disease Journal* 2008; **27**: 969–973
59. Catalano-Pons C, Bue M, Laude H, *et al*. Human bocavirus infection in hospitalized children during winter. *Pediatric Infectious Disease Journal* 2007; **26**: 959–960.
60. García-García ML, Calvo C, Pozo F, *et al*. Human bocavirus detection in nasopharyngeal aspirates of children without clinical symptoms of respiratory infection. *Pediatric Infectious Disease Journal* 2008; **27**: 358–360.
61. Longtin J, Bastien M, Gilca R, *et al*. Human bocavirus infections in hospitalized children and adults. *Emerging Infectious Diseases* 2008; **14**: 217–221.
62. Moriyama Y, Hamada H, Okada M, *et al*. Distinctive clinical features of human bocavirus in children younger than 2 years. *European Journal of Pediatrics* 2010; **169**: 1087–1092.
63. Bastien N, Brandt K, Dust K, Ward D, Li Y. Human bocavirus infection, Canada. *Emerging Infectious Diseases* 2006; **12**: 848–850.
64. Costa C, Bergallo M, Cavallo R. Detection of human bocavirus in bronchoalveolar lavage from Italian adult patients. *Journal of Clinical Virology* 2009; **45**: 81–82.
65. Garbino J, Soccal PM, Aubert JD, *et al*. Respiratory viruses in bronchoalveolar lavage: a hospital-based cohort study in adults. *Thorax* 2009; **64**: 399–404.
66. Shan TL, Zhang W, Guo W, *et al*. The first detection of human bocavirus 2 infections in China. *Journal of Clinical Virology* 2009; **46**: 196–197.
67. Han TH, Kim CH, Park SH, Kim EJ, Chung JY, Hwang ES. Detection of human bocavirus-2 in children with acute gastroenteritis in South Korea. *Archives of Virology* 2009; **154**: 1923–1927.
68. Kantola K, Sadeghi M, Antikainen J, *et al*. Real-time quantitative PCR detection of four human bocaviruses. *Journal of Clinical Microbiology* 2010; **48**: 4044–4050.
69. Santos N, Peret TC, Humphrey CD, *et al*. Human bocavirus species 2 and 3 in Brazil. *Journal of Clinical Virology* 2010; **48**: 127–130.
70. Wang Y, Gonzalez R, Zhou H, *et al*. Detection of human bocavirus 3 in China. *European Journal of Clinical Microbiology and Infectious Diseases* 2011; **30**: 799–805.
71. Lin F, Guan W, Cheng F, Yang N, Pintel D, Qiu J. ELISAs using human bocavirus VP2 virus-like particles for detection of antibodies against HBoV. *Journal of Virological Methods* 2008; **149**: 110–117.
72. Neske F, Prifert C, Scheiner B, *et al*. High prevalence of antibodies against polyomavirus WU, polyomavirus KI, and human bocavirus in German blood donors. *BMC Infectious Diseases* 2010; **10**: 215.
73. Enders M, Lindner J, Wenzel JJ, *et al*. No detection of human bocavirus in amniotic fluid samples from fetuses with hydrops or isolated effusions. *Journal of Clinical Virology* 2009; **45**: 300–303.
74. Riipinen A, Väisänen E, Lahtinen A, *et al*. Absence of human bocavirus from deceased fetuses and their mothers. *Journal of Clinical Virology* 2010; **47**: 186–188.
75. Shirkoohi R, Endo R, Ishiguro N, Teramoto S, Kikuta H, Ariga T. Antibodies against structural and nonstructural proteins of human bocavirus in human

DOI: 10.1002/rmv

sera. *Clinical and Vaccine Immunology* 2010; **17**: 190–193.

76. Kantola K, Hedman L, Arthur JL, *et al*. Seroepidemiology of human bocaviruses 1–4. *Journal of Infectious Diseases* 2011; **204**: 1403–1412.
77. Gerna G, Piralla A, Campanini G, Marchi A, Stronati M, Rovida F. The human bocavirus role in acute respiratory tract infections of pediatric patients as defined by viral load quantification. *The New Microbiologica* 2007; **30**: 383–392.
78. Jartti T, Jartti L, Peltola V, Waris M, Ruuskanen O. Identification of respiratory viruses in asymptomatic subjects: asymptomatic respiratory viral infections. *Pediatric Infectious Disease Journal* 2008; **27**: 1103–1107.
79. Zheng LS, Yuan XH, Xie ZP, *et al*. Human bocavirus infection in young children with acute respiratory tract infection in Lanzhou, China. *Journal of Medical Virology* 2010; **82**: 282–288.
80. Dina J, Nguyen E, Gouarin S, *et al*. Development of duplex real-time PCR for detection of two DNA respiratory viruses. *Journal of Virological Methods* 2009; **162**: 119–125.
81. Burns K, Parrish CR. Parvoviridae. In *Fields' Virology*, 5th edition, Fields BN, Knipe DM, Howley PM (eds). Wolters Kluwer Health/Lippincott Williams & Wilkins: Philadelphia, 2007; 2437–2466.
82. Calvo C, García-García ML, Blanco C, *et al*. Human bocavirus infection in a neonatal intensive care unit. *Journal of Infection* 2008; **57**: 269–271.
83. Zeng M, Zhu QR, Wang XH, Yu H, Shen J. Human bocavirus in children with respiratory tract infection in Shanghai: a retrospective study. *World Journal of Pediatrics* 2010; **6**: 65–70.
84. Durigon GS, Oliveira DB, Vollet SB, *et al*. Hospital-acquired human bocavirus in infants. *Journal of Hospital Infection* 2010; **76**: 171–173.
85. Chung JY, Han TH, Kim SW, Kim CK, Hwang ES. Detection of viruses identified recently in children with acute wheezing. *Journal of Medical Virology* 2007; **79**: 1238–1243.
86. Terrosi C, Fabbiani M, Cellesi C, Cusi MG. Human bocavirus detection in an atopic child affected by pneumonia associated with wheezing. *Journal of Clinical Virology* 2007; **40**: 43–45.
87. Dina J, Vabret A, Gouarin S, *et al*. Detection of human bocavirus in hospitalised children. *Journal of Paediatrics and Child Health* 2009; **45**: 149–153.
88. Zhang LL, Tang LY, Xie ZD, *et al*. Human bocavirus in children suffering from acute lower respiratory tract infection in Beijing Children's Hospital. *Chinese Medical Journal* 2008; **121**: 1607–1610.
89. Calvo C, García-García ML, Pozo F, Carvajal O, Pérez-Breña P, Casas I. Clinical characteristics of human bocavirus infections compared with other respiratory viruses in Spanish children. *Pediatric Infectious Disease Journal* 2008; **27**: 677–680.
90. Smuts H, Workman L, Zar HJ. Role of human metapneumovirus, human coronavirus NL63 and human bocavirus in infants and young children with acute wheezing. *Journal of Medical Virology* 2008; **80**: 906–912.
91. García-García ML, Calvo C, Falcón A, *et al*. Role of emerging respiratory viruses in children with severe acute wheezing. *Pediatric Pulmonology* 2010; **45** 585–591.
92. Antunes H, Rodrigues H, Silva N, *et al*. Etiology of bronchiolitis in a hospitalized pediatric population: prospective multicenter study. *Journal of Clinical Virology* 2010; **48**: 134–136.
93. Garbino J, Inoubli S, Mossdorf E, *et al*. Respiratory viruses in HIV-infected patients with suspected respiratory opportunistic infection. *AIDS* 2008; **22**: 701–705.
94. Müller A, Klinkenberg D, Vehreschild J, *et al*. Low prevalence of human metapneumovirus and human bocavirus in adult immunocompromised high risk patients suspected to suffer from Pneumocystis pneumonia. *Journal of Infection* 2009; **58**: 227–231.
95. Miyakis S, van Hal SJ, Barratt J, Stark D, Marriott D, Harkness J. Absence of human bocavirus in bronchoalveolar lavage fluid of lung transplant patients. *Journal of Clinical Virology* 2009; **44**: 179–180.
96. Lee JI, Chung JY, Han TH, Song MO, Hwang ES. Detection of human bocavirus in children hospitalized because of acute gastroenteritis. *Journal of Infectious Diseases* 2007; **196**: 994–997.
97. Vicente D, Cilla G, Montes M, Pérez-Yarza EG, Pérez-Trallero E. Human bocavirus, a respiratory and enteric virus. *Emerging Infectious Diseases* 2007; **13**: 636–637.
98. Albuquerque MC, Rocha LN, Benati FJ, *et al*. Human bocavirus infection in children with gastroenteritis, Brazil. *Emerging Infectious Diseases* 2007; **13**: 1756–1758.
99. Lau SK, Yip CC, Que TL, *et al*. Clinical and molecular epidemiology of human bocavirus in respiratory and fecal samples from children in Hong Kong. *Journal of Infectious Diseases* 2007; **196**: 986–993.
100. Schildgen O, Müller A, Simon A. Human bocavirus and gastroenteritis. *Emerging Infectious Diseases* 2007; **13**: 1620–1621.
101. Chieochansin T, Thongmee C, Vimolket L, Theamboonlers A, Poovorawan Y. Human bocavirus infection in children with acute gastroenteritis and healthy controls. *Japanese Journal of Infectious Diseases* 2008; **61**: 479–481.
102. Huang Y, Mao P, Wang H. Detection of, and frequent co-infection with, human bocavirus in faecal specimens from children in Wuhan, China. *Clinical Microbiology and Infection* 2010; **16**: 490–492.
103. Szomor KN, Kapusinszky B, Rigó Z, *et al*. Detection of human bocavirus from fecal samples of Hungarian children with acute gastroenteritis. *Intervirology* 2009; **52**: 17–21.
104. Chung JY, Han TH, Kim JS, Kim SW, Park CG, Hwang ES. Th1 and Th2 cytokine levels in nasopharyngeal aspirates from children with human bocavirus bronchiolitis. *Journal of Clinical Virology* 2008; **43**: 223–225.
105. Kumar A, Filippone C, Lahtinen A, *et al*. Comparison of Th-cell immunity against human bocavirus and parvovirus b19: proliferation and cytokine responses are similar in magnitude but more closely interrelated with human bocavirus. *Scandinavian Journal of Immunology* 2011; **73**: 135–140.
106. Lindner J, Zehentmeier S, Franssila R, Barabas S, Schroeder J, Deml L, Modrow S. CD4+ T helper cell responses against human bocavirus viral protein 2 viruslike particles in healthy adults. *Journal of Infectious Diseases* 2008; **198**: 1677–1684.

DOI: 10.1002/rmv

107. Chow BD, Esper FP. The human bocaviruses: a review and discussion of their role in infection. *Clinics in Laboratory Medicine* 2009; **29**: 695–713.
108. Don M, Söderlund-Venermo M, Hedman K, Ruuskanen O, Allander T, Korppi M. Don't forget serum in the diagnosis of human bocavirus infection. *Journal of Infectious Diseases* 2011; **203**: 1031–1032.
109. Söderlund M, von Essen R, Haapasaari J, Kiistala U, Kiviluoto O, Hedman K. Persistence of parvovirus B19 DNA in synovial membranes of young patients with and without chronic arthropathy. *Lancet* 1997; **349**: 1063–1065.
110. Norja P, Hokynar K, Aaltonen LM, *et al*. Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue. *Proceedings of the National Academy of Sciences of the United States of America* 2006; **103**: 7450–7453.
111. Söderlund-Venermo M, Hokynar K, Nieminen J, Rautakorpi H, Hedman K. Persistence of human parvovirus B19 in human tissues. *Pathol Biol (Paris)* 2002; **50**: 307–316.
112. Schnepp BC, Jensen RL, Chen CL, Johnson PR, Clark KR. Characterization of adeno-associated virus genomes isolated from human tissues. *Journal of Virology* 2005; **79**: 14793–14803.
113. Manning A, Willey SJ, Bell JE, Simmonds P. Comparison of tissue distribution, persistence, and molecular epidemiology of parvovirus B19 and novel human parvoviruses PARV4 and human bocavirus. *Journal of Infectious Diseases* 2007; **195**: 1345–1352.
114. Kuethe F, Lindner J, Matschke K, *et al*. Prevalence of parvovirus B19 and human bocavirus DNA in the heart of patients with no evidence of dilated cardiomyopathy or myocarditis. *Clinical Infectious Diseases* 2009; **49**: 1660–1666.
115. Falcone V, Ridder GJ, Panning M, Bierbaum S, Neumann-Haefelin D, Huzly D. Human bocavirus DNA in paranasal sinus mucosa. *Emerging Infectious Diseases* 2011; **17**: 1564–1565.
116. Kapoor A, Hornig M, Asokan A, Williams B, Henriquez JA, Lipkin WI. Bocavirus episome in infected human tissue contains non-identical termini. *PLoS One* 2011; **6**: e21362.
117. Kotin RM, Siniscalco M, Samulski RJ, *et al*. Site-specific integration by adeno-associated virus. *Proceedings of the National Academy of Sciences of the United States of America* 1990; **87**: 2211–2215.
118. Keicho N, Elliott WM, Hogg JC, Hayashi S. Adenovirus E1A upregulates interleukin-8 expression induced by endotoxin in pulmonary epithelial cells. *American Journal of Physiology* 1997; **272**: L1046–L1052.
119. Yamada K, Elliott WM, Hayashi S, *et al*. Latent adenoviral infection modifies the steroid response in allergic lung inflammation. *The Journal of Allergy and Clinical Immunology* 2000; **106**: 844–851.
120. Retamales I, Elliott WM, Meshi B, *et al*. Amplification of inflammation in emphysema and its association with latent adenoviral infection. *American Journal of Respiratory and Critical Care Medicine* 2001; **164**: 469–473.
121. Meshi B, Vitalis TZ, Ionescu D, *et al*. Emphysematous lung destruction by cigarette smoke. The effects of latent adenoviral infection on the lung inflammatory response. *American Journal of Respiratory Cell and Molecular Biology* 2002; **26**: 52–57.
122. Chen AY, Luo Y, Cheng F, Sun Y, Qiu J. Bocavirus infection induces mitochondrion-mediated apoptosis and cell cycle arrest at G2/M phase. *Journal of Virology* 2010; **84**: 5615–5626.
123. Hindiyeh MY, Keller N, Mandelboim M, *et al*. High rate of human bocavirus and adenovirus coinfection in hospitalized Israeli children. *Journal of Clinical Microbiology* 2008; **46**: 334–337.
124. Arden KE, McErlean P, Nissen MD, Sloots TP, Mackay IM. Frequent detection of human rhinoviruses, paramyxoviruses, coronaviruses, and bocavirus during acute respiratory tract infections. *Journal of Medical Virology* 2006; **78**: 1232–1240.
125. Ma X, Endo R, Ishiguro N, *et al*. Detection of human bocavirus in Japanese children with lower respiratory tract infections. *Journal of Clinical Microbiology* 2006; **44**: 1132–1134.
126. Smuts H, Hardie D. Human bocavirus in hospitalized children, South Africa. *Emerging Infectious Diseases* 2006; **12**: 1457–1458.
127. Foulongne V, Olejnik Y, Perez V, Elaerts S, Rodière M, Segondy M. Human bocavirus in French children. *Emerging Infectious Diseases* 2006; **12**: 1251–1253.
128. Weissbrich B, Neske F, Schubert J, *et al*. Frequent detection of bocavirus DNA in German children with respiratory tract infections. *BMC Infectious Diseases* 2006; **6**: 109.
129. Kaplan NM, Dove W, Abu-Zeid AF, Shamoon HE, Abd-Eldayem SA, Hart CA. Human bocavirus infection among children, Jordan. *Emerging Infectious Diseases* 2006; **12**: 1418–1420.
130. Naghipour M, Cuevas LE, Bakhshinejad T, Dove W, Hart CA. Human bocavirus in Iranian children with acute respiratory infections. *Journal of Medical Virology* 2007; **79**: 539–543.
131. Qu XW, Duan ZJ, Qi ZY, *et al*. Human bocavirus infection, People's Republic of China. *Emerging Infectious Diseases* 2007; **13**: 165–168.
132. Khetsuriani N, Kazerouni NN, Erdman DD, *et al*. Prevalence of viral respiratory tract infections in children with asthma. *The Journal of Allergy and Clinical Immunology* 2007; **119**: 314–321.
133. Völz S, Schildgen O, Klinkenberg D, *et al*. Prospective study of Human Bocavirus (HBoV) infection in a pediatric university hospital in Germany 2005/2006. *Journal of Clinical Virology* 2007; **40**: 229–235.
134. Ringshausen FC, Tan AY, Allander T, *et al*. Frequency and clinical relevance of human bocavirus infection in acute exacerbations of chronic obstructive pulmonary disease. *International Journal of Chronic Obstructive Pulmonary Disease* 2009; **4**: 111–117.
135. Ruohola A, Waris M, Allander T, Ziegler T, Heikkinen T, Ruuskanen O. Viral etiology of common cold in children, Finland. *Emerging Infectious Diseases* 2009; **15**: 344–346.
136. Oikawa J, Ogita J, Ishiwada N, *et al*. Human bocavirus DNA detected in a boy with plastic bronchitis. *Pediatric Infectious Disease Journal* 2009; **28**: 1035–1036.
137. Ursic T, Steyer A, Kopriva S, Kalan G, Krivec U, Petrovec M. Human bocavirus as the cause of a life-threatening infection. *Journal of Clinical Microbiology* 2011; **49**: 1179–1181.
138. Ruuskanen O, Lahti E, Jennings LC, Murdoch DR. Viral pneumonia. *Lancet* 2011; **377**: 1264–1275.

DOI: 10.1002/rmv

139. Pavia AT. Viral infections of the lower respiratory tract: old viruses, new viruses, and the role of diagnosis. *Clinical Infectious Diseases* 2011; **52**: S284–S289.
140. Ruohola A, Meurman O, Nikkari S, *et al*. Microbiology of acute otitis media in children with tympanostomy tubes: prevalences of bacteria and viruses. *Clinical Infectious Diseases* 2006; **43**: 1417–1422.
141. Beder LB, Hotomi M, Ogami M, *et al*. Clinical and microbiological impact of human bocavirus on children with acute otitis media. *European Journal of Pediatrics* 2009; **168**: 1365–1372.
142. Kleines M, Scheithauer S, Rackowitz A, Ritter K, Häusler M. 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. *Journal of Clinical Microbiology* 2007; **45**: 1032–1034.
143. Schenk T, Huck B, Forster J, Berner R, Neumann-Haefelin D, Falcone V. Human bocavirus DNA detected by quantitative real-time PCR in two children hospitalized for lower respiratory tract infection. *European Journal of Clinical Microbiology and Infectious Diseases* 2007; **26**: 147–149.
144. Lahti E, Peltola V, Waris M, *et al*. Induced sputum in the diagnosis of childhood community-acquired pneumonia. *Thorax* 2009; **64**: 252–257.
145. Esposito S, Bosis S, Niesters HG, *et al*. Impact of human bocavirus on children and their families. *Journal of Clinical Microbiology* 2008; **46**: 1337–1342.
146. Catalano-Pons C, Giraud C, Rozenberg F, Meritet JF, Lebon P, Gendrel D. Detection of human bocavirus in children with Kawasaki disease. *Clinical Microbiology and Infection* 2007; **13**: 1220–1222.
147. Lehmann C, Klar R, Lindner J, Lindner P, Wolf H, Gerling S. Kawasaki disease lacks association with human coronavirus NL63 and human bocavirus. *Pediatric Infectious Disease Journal* 2009; **28**: 553–554.
148. Kupfer B, Vehreschild J, Cornely O, *et al*. Severe pneumonia and human bocavirus in adult. *Emerging Infectious Diseases* 2006; **12**: 1614–1616.
149. Schenk T, Strahm B, Kontny U, Hufnagel M, Neumann-Haefelin D, Falcone V. Disseminated bocavirus infection after stem cell transplant. *Emerging Infectious Diseases* 2007; **13**: 1425–1427.
150. Jartti T, Waris M, Niesters HG, Allander T, Ruuskanen O. Respiratory viruses and acute asthma in children. *The Journal of Allergy and Clinical Immunology* 2007; **120**: 216.
151. Koskenvuo M, Möttönen M, Waris M, Allander T, Salmi TT, Ruuskanen O. Human bocavirus in children with acute lymphoblastic leukemia. *European Journal of Pediatrics* 2008; **167**: 1011–1015.
152. Koskenvuo M, Möttönen M, Rahiala J, *et al*. Respiratory viral infections in children with leukemia. *Pediatric Infectious Disease Journal* 2008; **27**: 974–980.
153. Vallet C, Pons-Catalano C, Mandelcwajg A, *et al*. Human bocavirus: a cause of severe asthma exacerbation in children. *Journal of Pediatrics* 2009; **155**: 286–288.
154. Schenk T, Maier B, Hufnagel M, *et al*. Persistence of human bocavirus DNA in immunocompromised children. *Pediatric Infectious Disease Journal* 2011; **30**: 82–84.
155. Kainulainen L, Waris M, Söderlund-Venermo M, Allander T, Hedman K, Ruuskanen O. Hepatitis and human bocavirus primary infection in a child with T-cell deficiency. *Journal of Clinical Microbiology* 2008; **46**: 4104–4105.
156. Jartti T, Kuusipalo H, Vuorinen T, *et al*. Allergic sensitization is associated with rhinovirus-, but not other virus-, induced wheezing in children. *Pediatric Allergy and Immunology* 2010; **21**: 1008–1014.
157. Jartti T, Söderlund-Venermo M, Allander T, Vuorinen T, Hedman K, Ruuskanen O. No efficacy of prednisolone in acute wheezing associated with human bocavirus infection. *Pediatric Infectious Disease Journal* 2011; **30**: 521–523.
158. Lu X, Chittaganpitch M, Olsen SJ, *et al*. Real-time PCR assays for detection of bocavirus in human specimens. *Journal of Clinical Microbiology* 2006; **44**: 3231–3235.
159. Ma X, Endo R, Ishiguro N, *et al*. Detection of human bocavirus in Japanese children with lower respiratory tract infections. *Journal of Clinical Microbiology* 2006; **44**: 1132–1134.
160. Monteny M, Niesters HG, Moll HA, Berger MY. Human bocavirus in febrile children, The Netherlands. *Emerging Infectious Diseases* 2007; **13**: 180–182.
161. Christensen A, Nordbø SA, Krokstad S, Rognlien AG, Døllner H. Human bocavirus commonly involved in multiple viral airway infections. *Journal of Clinical Virology* 2008; **41**: 34–37.
162. I P M, Nelson EA, Cheuk ES, Leung E, Sung R, Chan PK. Pediatric hospitalization of acute respiratory tract infections with Human Bocavirus in Hong Kong. *Journal of Clinical Virology* 2008; **42**: 72–74.
163. Pierangeli A, Scagnolari C, Trombetti S, *et al*. Human bocavirus infection in hospitalized children in Italy. *Influenza and Other Respiratory Viruses* 2008; **2**: 175–179.
164. Yoshida LM, Suzuki M, Yamamoto T, *et al*. Viral pathogens associated with acute respiratory infections in central Vietnamese children. *Pediatric Infectious Disease Journal* 2010; **29**: 75–77.
165. Franz A, Adams O, Willems R, *et al*. Correlation of viral load of respiratory pathogens and co-infections with disease severity in children hospitalized for lower respiratory tract infection. *Journal of Clinical Virology* 2010; **48**: 239–245.
166. Calvo C, Pozo F, García-García ML, *et al*.. Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. *Acta Paediatrica* 2010; **99**: 883–887.
167. Miron D, Srugo I, Kra-Oz Z, *et al*. Sole pathogen in acute bronchiolitis: is there a role for other organisms apart from respiratory syncytial virus? *Pediatric Infectious Disease Journal* 2010; **29**: e7–e10.
168. Nakanishi K, Tsugawa T, Honma S, *et al*. Detection of enteric viruses in rectal swabs from children with acute gastroenteritis attending the pediatric outpatient clinics in Sapporo, Japan. *Journal of Clinical Virology* 2009; **46**: 94–97.
169. Nadji SA, Poos-Ashkan L, Khalilzadeh S, *et al*. Phylogenetic analysis of human bocavirus isolated from children with acute respiratory illnesses and gastroenteritis in Iran. *Scandinavian Journal of Infectious Diseases* 2010; **42**: 598–603.
170. Pham NT, Trinh QD, Chan-It W, *et al*. Human bocavirus infection in children with acute gastroenteritis in Japan and Thailand. *Journal of Medical Virology* 2011; **83**: 286–290.

DOI: 10.1002/rmv