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Journal of Clinical Virology 43 (2008) 391–395

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Clinical and epidemiological aspects of human bocavirus infection

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ARTICLE INFO

Article history:
Received 28 May 2008
Accepted 14 August 2008

Keywords:
Human bocavirus
Respiratory Disease
Gastroenteritis

ABSTRACT

Human bocavirus was recently described as a novel member of the *Parvoviridae* to infect humans. Based on accumulating clinical and epidemiological data the virus is currently being associated with respiratory infections in young children and infants and is furthermore discussed as causative agent of gastrointestinal illness.

1. Introduction

Acute respiratory tract infections (ARTIs) caused by viruses represent a major cause of hospitalization and morbidity in young children and infants worldwide. Pathogens associated with this clinical condition include the respiratory syncytial virus (RSV), human adenovirus, human metapneumovirus and coronaviruses NL63 and HKU1.[1] Although a constantly growing number of pathogens is being associated with ARTIs, a high percentage of infections still remain uncharacterized and their causative agents unknown.

In 2005 Allander et al. described a previously uncharacterized virus in pools of human nasopharyngeal aspirates obtained from children suffering from diseases of the respiratory tract.[2] Comprehensive sequence and phylogenetic analyses revealed a close relation of the new virus with the bovine parvovirus (BPV) and the canine minute virus (CnMV), both members of the *Bocavirus* genus of the *Parvoviridae* family. It was therefore provisionally named human bocavirus (HBoV).

Parvoviruses represent a large family of small, non-enveloped viruses characterized by linear single-stranded DNA-genomes and an exceptional structural simplicity. Besides HBoV two additional parvoviruses, parvovirus B19 (B19V) and PARV4 including its second genotype termed PARV5, are currently known or discussed to infect humans.[3–5] For almost three decades B19V has represented the only member of the virus family to cause illness in humans, e.g. the self-limiting childhood disease *Erythema infectiosum*.[6] Additionally, B19V infections during pregnancy are known to frequently result in intrauterine infections of the fetus, occasionally leading to miscarriage or *Hydrops fetalis*.[7] While the clinical relevance of PARV4 remains unclear up to date, evidence for an influence of HBoV infections in the manifestation of respiratory and gastric symptoms is accumulating.

2. Material and methods

2.1. Detection of HBoV-specific antibodies by ELISA

For detection of HBoV VP2-specific IgG and IgM, 100 ng of purified HBoV VP2 virus-like particles (VP2–VLP) were generated as previously described[8] and coated onto Nunc-Immuno™ MediSorp™ plates (Nunc GmbH, Wiesbaden, Germany) in coating buffer (0.2 M Na_2CO_3, 0.2 M $NaHCO_3$, pH 9.5) overnight at 4 °C. The plates were subsequently washed six times with washing buffer (PBS, 0.05% Tween 20) and blocked with dilution buffer (PBS, 2% Tween 20, 3% FCS) for 1 h at 37 °C. After incubation with respective serum samples for 2 h at 37 °C, the plates were washed and rabbit anti-human IgG- or IgM-specific HRP-coupled secondary antibodies were added for 1 h at 37 °C (1:6000 and 1:1000 in dilution buffer, respectively; both Dako Deutschland GmbH, Hamburg, Germany). Development was performed using the BD OptEIA™ Substrate (BD Biosciences, Heidelberg, Germany) according to the manufacturer's instructions.

As an international IgG standard for HBoV is not yet available, serially diluted sera of a healthy adult male (age: 28 years) and of a boy (age: 22 months) both exhibiting strong HBoV-specific IgG-

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doi:10.1016/j.jcv.2008.08.009

and IgM-responses, respectively, were introduced for internal reference and used for the calculation of HBoV-specific antibody titers in all performed experiments. Sera with background optical densities were considered negative and used for the determination of respective IgG/IgM cut-off values, which were additionally confirmed by Western blot analysis.

2.2. Reviewed literature

All reports listed in the PubMed-database of the National Library of Medicine (Rockville Pike, MD, USA) until May 2008 have been considered and evaluated in this review.

3. Diagnosis of HBoV infections

Up to date, no cell culture systems for the *in vitro* replication of HBoV have been described. Therefore, diagnosis of HBoV infection has so far mainly been based on the detection of viral genomes present in human respiratory, serum, stool, and urine samples using different PCR techniques employing numerous sets of primers specific for the viral genes NP1,[2,9–12] NS1[12–15] and VP1/2.[11,12,16–18]

Recent reports describe the detection of HBoV-specific antibodies directed against the viral capsid proteins VP1 and/or VP2 in serum samples using ELISA,[19,20] Western blot,[21] and immunofluorescence assays.[22] Up to date, no cross-reactions of HBoV- and B19V-specific humoral and/or cellular immune responses have been described.

4. HBoV epidemiology

4.1. Prevalence of HBoV-DNA

HBoV-DNA has been frequently detected worldwide in respiratory,[2,9–11,13–16,18,23–66] serum,[30,43] fecal,[17,30,33,35,38,67,68] and urine samples[38] obtained from infants mainly around 2 years of age. The prevalence of HBoV-DNA has been described to vary considerably between 2.7–19% in children suffering from ARTIs and 0.8–9.1% in patients with gastroenteritis.[17,25,35,43] However, since the majority of currently published studies have been performed retrospectively, these variations in viral prevalence may be explained by differences in the study populations and patient characteristics. In infected infants, viral loads have been described to range between <500 to 10^{10} and $<10^3$ to 5.9×10^5 genome copies in nasopharyngeal aspirates and fecal samples, respectively.[25,37,43,52] In serum, we have detected viral loads of up to 1.2×10^6/ml.[69]

Only limited data is available on the prevalence of HBoV viremia in asymptomatic individuals, since most of the studies have focused on children with distinct clinical symptoms of infectious diseases. In a first study a total of 96 healthy controls were included for diagnostic analysis of HBoV, yet no viral DNA was observed in respiratory samples from these individuals.[16] Furthermore, we were unable to detect HBoV-DNA in sera collected from 298 healthy adult blood donors. However, a recent publication describes the detection of viral genomes in 5% of respiratory samples obtained from asymptomatic children.[24]

While most studies have detected the virus during the winter season,[2,28,33,38,46,70] single reports describe increased numbers of viral infections in spring or summer.[9,31,40] No information is currently available on the routes of viral transmission. However, since HBoV can be frequently detected in respiratory and fecal samples, a transmission of the virus via aerosols or direct contact has to be presumed. Thereby, the contagiousness of virus-containing body secretions might be potentiated by the exceptional stability of parvoviral virions and might facilitate increased frequencies of nosocomial infections.

4.2. Prevalence of HBoV-specific antibodies and cellular immune reactions

Up to date, only a limited number of studies have been focused on the analysis of HBoV-specific adaptive immune responses in healthy individuals and infants suffering from ARTIs, mainly due to the initial lack of recombinant viral antigens and standardized diagnostic methodologies.

In the first report published on HBoV seroprevalence Endo and co-workers describe ubiquitous IgG-responses against the viral capsid protein VP1 in up to 100% of children aged $\geq$2 years with respiratory infections.[22] The overall seroprevalence of HBoV-specific IgG in the Japanese population aged between 0 months and 41 years was 71.1%, while seronegative patients were observed most frequently in infants with 6–12 months of age.

In a subsequent study the prevalence of HBoV-specific antibodies in Finnish infants suffering from ARTIs was assessed using Western blot.[21] In children determined positive for HBoV-DNA, IgG- and IgM-antibodies against the viral VP2 protein were observed in 73% and 49% of analyzed samples, respectively. The mean age of these children was 2.1 years. The overall prevalence of HBoV-specific IgG and IgM in children without detectable viral genomes in nasopharyngeal samples was 35% and 13%, respectively. Antibodies against the aminoterminal domain of the viral VP1 protein, termed VP1-unique region, were detected rarely: only 7% (IgG) and 2% (IgM) of the patients showed positive results. In contrast to the data provided by Endo and colleagues and by our group (see below), the prevalence of HBoV-specific IgG was shown to decline from 52% in 1–2 year old infants to 29% in children aged over 5 years in the Finnish study.[21] Furthermore, maternal VP2-specific IgG were not observed in children <6 months of age despite a seemingly high seroprevalence of HBoV in adults. This finding may be due to the maturation of IgG-specificity in the time period of up to 6 months following an acute infection, during which antibodies against linear epitopes get replaced by those preferentially recognizing conformational antigen structures. This process has been well documented for B19V-specific humoral immune responses,[71] and therefore it may be assumed that similar changes in IgG affinity take place during HBoV infections.

More recently, we and others have established ELISA assays based on the use of recombinant HBoV VP2–VLP for the detection of HBoV-specific antibodies in human serum samples.[19,20] Herein, our group observed the prevalence of IgG_1 subclass antibodies against HBoV VP2–VLP to rise from 24% in children with 7–9 months of age to 98.3% adult blood donors (mean age: 42 years).

In addition to humoral immune reactions the presence of HBoV-specific T-cells in healthy adults supports a high prevalence of HBoV-specific immunity in adults. Thereby, frequent interferon-gamma (IFN-γ) mediated $CD4^+$ T helper cell reactions were observed against HBoV capsid proteins.[8] Similar data have been previously described for B19V-specific cellular immune responses.[72–75]

5. Clinical associations

HBoV infections are frequently linked to high rates of co-infections with viral and bacterial pathogens of the respiratory and/or gastrointestinal system. Together with the fact that most of the studies have been performed retrospectively and long-term follow-up studies with detailed clinical characterization of

symptomatic individuals are rare, it is currently difficult to clearly determine HBoV as sole infectious agent of human illnesses.

5.1. HBoV and respiratory disease

Up to date, HBoV infections have been detected in young children around the age of 2 years with acute diseases of the upper and lower respiratory tract,[2,9–11,13,14,16,18,25–29,31–60,62–66] frequently in combination with interstitial lung infiltrates and abnormal radiologic findings.[2,28,37,45,55] In HBoV positive individuals, we detected both virus-specific IgG and IgM in 42% of studied sera, whereas no IgM were observed in samples obtained from children without detectable amounts of HBoV genomes in blood.

Symptoms and disease manifestations observed in HBoV infected children include pneumonia, bronchiolitis, wheezing, respiratory distress, hypoxia, fever, rhinitis, laryngeal croup and, more rarely, conjunctivitis or rashes. In adults, acute HBoV infections leading to ARTIs seem to be rare and have been currently detected mainly in immunocompromised[13,49,51,76] and only in single immunocompetent individuals.[13,16]

Recently, the presence of elevated viral loads in nasopharyngeal aspirates (>10^4 genome copies/ml) has been suggested to correlate with the severity of respiratory symptoms during HBoV infection, whereas low viral loads (<10^4 genome copies/ml) may represent viral persistence.[43] These data are in contrast to those published by Kleines et al. who could not find a relation between the viral load and the severity of HBoV associated illness,[37] indicating that further work is necessary to study the influence of the viral load on respiratory disease manifestation. However, infections with the related parvovirus B19 often result in a prolonged replication of the virus in infected individuals[5] and therefore mechanisms of persistence may also apply for HBoV.

Individuals found positive for HBoV-DNA in nasopharyngeal aspirates are frequently found to be co-infected with a multitude of additional viral and/or bacterial respiratory pathogens. Thereby, high rates of co-infections reaching up to 91% have been observed.[26] Commonly detected additional pathogens include RSV, human adenovirus, rhinovirus, and *Streptococcus* sp. Despite these high rates of co-infection, HBoV viremia has been frequently described to be significantly more prevalent in infants suffering from ARTIs than in age-matched asymptomatic control groups,[24,26,28,43,51] and therefore a role of HBoV in the development of human respiratory diseases is to be presumed. This finding is supported by our data, which show a significantly higher prevalence of HBoV infections in young children with lower respiratory tract infections (14.6%, 7/48) as compared to a control group of age-matched individuals hospitalized due to non-infectious conditions such as bone fractures or planned surgeries (5.0%, 3/60).

5.2. HBoV and gastrointestinal disease

In addition to respiratory symptoms, HBoV is currently discussed to be associated with gastroenteritic symptoms. Similar features are known from veterinary infections with the closely related BPV and CnMV, which are known to induce gastric illness in their respective hosts.[77,78]

First reports have described the prevalence of HBoV genomes to range between 0.8% and 9.1% in fecal samples obtained from children suffering from acute gastroenteritis, often in combination with ARTIs.[17,28,31,33,35,53,63,67] In a recent prospective study we detected HBoV-DNA in 7.8% (5/64) fecal samples obtained from young children exhibiting gastrointestinal symptoms, e.g. diarrhea, nausea and vomiting. An additional child tested positive for HBoV was diagnosed with inflammatory bowel disease.

As co-infections with intestinal pathogens, e.g. human rota- and noroviruses, enteropathogenic strains of *Escherichia coli* or *Salmonella* sp., have been frequently observed in up to 77.6% of HBoV positive individuals, an association of HBoV with gastroenteritis remains unclear.[68] Since in many cases HBoV-DNA has been detected concurrently in both stool samples and nasopharyngeal aspirates obtained from young children with ARTIs,[11] the presence of HBoV in fecal samples might represent natural viral shedding during an acute HBoV infection and not play an active role in the pathogenesis of gastric disease.

6. Conclusions

Although HBoV was detected only three years ago, both epidemiological and clinical data establishing the virus as the second member of the *Parvoviridae* pathogenic to humans are accumulating. Based on current reports it seems most likely that HBoV may be associated with respiratory infections in young children and infants, while a further connection between HBoV and gastrointestinal symptoms has been suggested. However, as acute HBoV infections are often accompanied by infections with additional pathogens of both the respiratory and gastrointestinal tracts, a final establishment of HBoV as the causative agent of infectious disease in humans needs to be confirmed by additional prospective studies. The methodological heterogeneity used for the diagnosis of HBoV infection raises questions about the specificity and comparability of many published studies, highlighting the urgent need of internationally standardized diagnostic guidelines and reference samples for the detection of HBoV genomes and virus-specific immune responses in human samples. As serological diagnostics of HBoV infection will become more important in the future, standardized viral DNA and antibody specimen should be provided as a basis to establish comparable test systems.

Nevertheless, first data obtained from healthy control individuals and children with symptoms of non-infectious disease indicate distinctly lower rates of HBoV infections in comparison to patients suffering from ARTIs. Whether HBoV might require the presence of helper-viruses to establish human illness or may even act as the provider of such co-factors for other respiratory viruses, remains to be assessed in further studies.

References

1. Kesson AM. Respiratory virus infections. *Paediatr Respir Rev* 2007;**8**:240–8.
2. 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. *Proc Natl Acad Sci USA* 2005;**102**:12891–6.
3. Fryer JF, Delwart E, Hecht FM, Bernardin F, Jones MS, Shah N, et al. Frequent detection of the parvoviruses, PARV4 and PARV5, in plasma from blood donors and symptomatic individuals. *Transfusion* 2007;**47**:1054–61.
4. Fryer JF, Kapoor A, Minor PD, Delwart E, Baylis SA. Novel parvovirus and related variant in human plasma. *Emerg Infect Dis* 2006;**12**:151–4.
5. Young NS, Brown KE. Parvovirus B19. *N Engl J Med* 2004;**350**:586–97.
6. Anderson MJ, Lewis E, Kidd IM, Hall SM, Cohen BJ. An outbreak of *erythema infectiosum* associated with human parvovirus infection. *J Hyg (Lond)* 1984;**93**:85–93.
7. Woernle CH, Anderson LJ, Tattersall P, Davison JM. Human parvovirus B19 infection during pregnancy. *J Infect Dis* 1987;**156**:17–20.
8. Lindner J, Zehentmeier S, Franssila R, Barabas S, Schroeder J, Deml L, et al. CD4 + T helper cell responses against human bocavirus VP2 virus-like particles in healthy adults. *J Infect Dis* 2008.
9. Choi EH, Lee HJ, Kim SJ, Eun BW, Kim NH, Lee JA, et al. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000–2005. *Clin Infect Dis* 2006;**43**:585–92.
10. Simon A, Groneck P, Kupfer B, Kaiser R, Plum G, Tillmann RL, et al. Detection of bocavirus DNA in nasopharyngeal aspirates of a child with bronchiolitis. *J Infect* 2007;**54**:e125–7.
11. Neske F, Blessing K, Tollmann F, Schubert J, Rethwilm A, Kreth HW, et al. Real-time PCR for diagnosis of human bocavirus infections and phylogenetic analysis. *J Clin Microbiol* 2007;**45**:2116–22.

12. Choi JH, Chung YS, Kim KS, Lee WJ, Chung IY, Oh HB, et al. Development of real-time PCR assays for detection and quantification of human bocavirus. *J Clin Virol* 2008.
13. Manning A, Russell V, Eastick K, Leadbetter GH, Hallam N, Templeton K, et al. Epidemiological profile and clinical associations of human bocavirus and other human parvoviruses. *J Infect Dis* 2006;**194**:1283–90.
14. Regamey N, Frey U, Deffernez C, Latzin P, Kaiser L. Isolation of human bocavirus from Swiss infants with respiratory infections. *Pediatr Infect Dis J* 2007;**26**:177–9.
15. Lu X, Chittaganpitch M, Olsen SJ, Mackay IM, Sloots TP, Fry AM, et al. Real-time PCR assays for detection of bocavirus in human specimens. *J Clin Microbiol* 2006;**44**:3231–5.
16. Bastien N, Brandt K, Dust K, Ward D, Li Y. Human bocavirus infection, Canada. *Emerg Infect Dis* 2006;**12**:848–50.
17. Lee JI, Chung JY, Han TH, Song MO, Hwang ES. Detection of human bocavirus in children hospitalized because of acute gastroenteritis. *J Infect Dis* 2007;**196**:994–7.
18. Bastien N, Chui N, Robinson JL, Lee BE, Dust K, Hart L, et al. Detection of human bocavirus in Canadian children in a 1-year study. *J Clin Microbiol* 2007;**45**:610–3.
19. Kahn JS, Kesebir D, Cotmore SF, D'Abramo Jr A, Cosby C, Weibel C, et al. Seroepidemiology of human bocavirus defined using recombinant virus-like particles. *J Infect Dis* 2008.
20. 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. *J Virol Methods* 2008;**149**:110–7.
21. Kantola K, Hedman L, Allander T, Jartti T, Lehtinen P, Ruuskanen O, et al. Serodiagnosis of human bocavirus infection. *Clin Infect Dis* 2008;**46**:540–6.
22. Endo R, Ishiguro N, Kikuta H, Teramoto S, Shirkoohi R, Ma X, et al. Seroepidemiology of human bocavirus in Hokkaido prefecture, Japan. *J Clin Microbiol* 2007;**45**:3218–23.
23. Chieochansin T, Chutinimitkul S, Payungporn S, Hiranras T, Samransamruajkit R, Theamboolers A, et al. Complete coding sequences and phylogenetic analysis of Human bocavirus (HBoV). *Virus Res* 2007;**129**:54–7.
24. Garcia-Garcia ML, Calvo C, Pozo F, Perez-Brena P, Quevedo S, Bracamonte T, et al. Human bocavirus detection in nasopharyngeal aspirates of children without clinical symptoms of respiratory infection. *Pediatr Infect Dis J* 2008;**27**:358–60.
25. Lin F, Zeng A, Yang N, Lin H, Yang E, Wang S, et al. Quantification of human bocavirus in lower respiratory tract infections in China. *Infect Agent Cancer* 2007;**2**:3.
26. Fry AM, Lu X, Chittaganpitch M, Peret T, Fischer J, Dowell SF, et al. Human bocavirus: a novel parvovirus epidemiologically associated with pneumonia requiring hospitalization in Thailand. *J Infect Dis* 2007;**195**:1038–45.
27. Arden KE, McErlean P, Nissen MD, Sloots TP, Mackay IM. Frequent detection of human rhinoviruses, paramyxoviruses, coronaviruses, and bocavirus during acute respiratory tract infections. *J Med Virol* 2006;**78**:1232–40.
28. Kesebir D, Vazquez M, Weibel C, Shapiro ED, Ferguson D, Landry ML, et al. Human bocavirus infection in young children in the United States: molecular epidemiological profile and clinical characteristics of a newly emerging respiratory virus. *J Infect Dis* 2006;**194**:1276–82.
29. Catalano-Pons C, Bue M, Laude H, Cattan F, Moulin F, Menager C, et al. Human bocavirus infection in hospitalized children during winter. *Pediatr Infect Dis J* 2007;**26**:959–60.
30. Catalano-Pons C, Giraud C, Rozenberg F, Meritet JF, Lebon P, Gendrel D. Detection of human bocavirus in children with Kawasaki disease. *Clin Microbiol Infect* 2007;**13**:1220–2.
31. Arnold JC, Singh KK, Spector SA, Sawyer MH. Human bocavirus: prevalence and clinical spectrum at a children's hospital. *Clin Infect Dis* 2006;**43**:283–8.
32. Ma X, Endo R, Ishiguro N, Ebihara T, Ishiko H, Ariga T, et al. Detection of human bocavirus in Japanese children with lower respiratory tract infections. *J Clin Microbiol* 2006;**44**:1132–4.
33. Lau SK, Yip CC, Que TL, Lee RA, Au-Yeung RK, Zhou B, et al. Clinical and molecular epidemiology of human bocavirus in respiratory and fecal samples from children in Hong Kong. *J Infect Dis* 2007;**196**:986–93.
34. Chieochansin T, Samransamruajkit R, Chutinimitkul S, Payungporn S, Hiranras T, Theamboonlers A, et al. Human bocavirus (HBoV) in Thailand: clinical manifestations in a hospitalized pediatric patient and molecular virus characterization. *J Infect* 2008;**56**:137–42.
35. Vicente D, Cilla G, Montes M, Perez-Yarza EG, Perez-Trallero E. Human bocavirus, a respiratory and enteric virus. *Emerg Infect Dis* 2007;**13**:636–7.
36. Naghipour M, Cuevas LE, Bakhshinejad T, Dove W, Hart CA. Human bocavirus in Iranian children with acute respiratory infections. *J Med Virol* 2007;**79**:539–43.
37. Kleines M, Scheithauer S, Rackowitz A, Ritter K, Hausler 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. *J Clin Microbiol* 2007;**45**:1032–4.
38. Pozo F, Garcia-Garcia ML, Calvo C, Cuesta I, Perez-Brena P, Casas I. High incidence of human bocavirus infection in children in Spain. *J Clin Virol* 2007;**40**:224–8.
39. Chung JY, Han TH, Kim SW, Kim CK, Hwang ES. Detection of viruses identified recently in children with acute wheezing. *J Med Virol* 2007;**79**:1238–43.
40. Chung JY, Han TH, Kim CK, Kim SW. Bocavirus infection in hospitalized children, South Korea. *Emerg Infect Dis* 2006;**12**:1254–6.
41. Gendrel D, Guedj R, Pons-Catalano C, Emerian A, Raymond J, Rozenberg F, et al. Human bocavirus in children with acute asthma. *Clin Infect Dis* 2007;**45**:404–5.
42. Kaplan NM, Dove W, Abu-Zeid AF, Shamoon HE, Abd-Eldayem SA, Hart CA. Human bocavirus infection among children, Jordan. *Emerg Infect Dis* 2006;**12**:1418–20.
43. Allander T, Jartti T, Gupta S, Niesters HG, Lehtinen P, Osterback R, et al. Human bocavirus and acute wheezing in children. *Clin Infect Dis* 2007;**44**:904–10.
44. Sloots TP, McErlean P, Speicher DJ, Arden KE, Nissen MD, Mackay IM. Evidence of human coronavirus HKU1 and human bocavirus in Australian children. *J Clin Virol* 2006;**35**:99–102.
45. Foulongne V, Olejnik Y, Perez V, Elaerts S, Rodiere M, Segondy M. Human bocavirus in French children. *Emerg Infect Dis* 2006;**12**:1251–3.
46. Weissbrich B, Neske F, Schubert J, Tollmann F, Blath K, Blessing K, et al. Frequent detection of bocavirus DNA in German children with respiratory tract infections. *BMC Infect Dis* 2006;**6**:109.
47. Smuts H, Hardie D. Human bocavirus in hospitalized children. *South Africa Emerg Infect Dis* 2006;**12**:1457–8.
48. Khetsuriani N, Kazerouni NN, Erdman DD, Lu X, Redd SC, Anderson LJ, et al. Prevalence of viral respiratory tract infections in children with asthma. *J Allergy Clin Immunol* 2007;**119**:314–21.
49. Kupfer B, Vehreschild J, Cornely O, Kaiser R, Plum G, Viazov S, et al. Severe pneumonia and human bocavirus in adult. *Emerg Infect Dis* 2006;**12**:1614–6.
50. 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. *Eur J Clin Microbiol Infect Dis* 2007;**26**:147–9.
51. Maggi F, Andreoli E, Pifferi M, Meschi S, Rocchi J, Bendinelli M. Human bocavirus in Italian patients with respiratory diseases. *J Clin Virol* 2007;**38**:321–5.
52. Qu XW, Duan ZJ, Qi ZY, Xie ZP, Gao HC, Liu WP, et al. Human bocavirus infection. People's Republic of China. *Emerg Infect Dis* 2007;**13**:165–8.
53. Monteny M, Niesters HG, Moll HA, Berger MY. Human bocavirus in febrile children, The Netherlands. *Emerg Infect Dis* 2007;**13**:180–2.
54. Terrosi C, Fabbiani M, Cellesi C, Cusi MG. Human bocavirus detection in an atopic child affected by pneumonia associated with wheezing. *J Clin Virol* 2007;**40**:43–5.
55. Volz S, Schildgen O, Klinkenberg D, Ditt V, Müller A, Tillmann RL, et al. Prospective study of Human bocavirus (HBoV) infection in a pediatric university hospital in Germany 2005/2006. *J Clin Virol* 2007;**40**:229–35.
56. Hindiyeh MY, Keller N, Mandelboim M, Ram D, Rubinov J, Regev L, et al. High rate of human bocavirus and adenovirus coinfection in hospitalized Israeli children. *J Clin Microbiol* 2008;**46**:334–7.
57. Villa L, Melon S, Suarez S, Alvarez-Argüelles ME, Gónzalez D, Morilla A, et al. Detection of human bocavirus in Asturias, Northern Spain. *Eur J Clin Microbiol Infect Dis* 2008;**27**:237–9.
58. Christensen A, Nordbo SA, Krokstad S, Rognlien AG, Dollner H. Human bocavirus commonly involved in multiple viral airway infections. *J Clin Virol* 2008;**41**:34–7.
59. 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. *New Microbiol* 2007;**30**:383–92.
60. Redshaw N, Wood C, Rich F, Grimwood K, Kirman JR. Human bocavirus in infants, New Zealand. *Emerg Infect Dis* 2007;**13**:1797–9.
61. Schenk T, Strahm B, Kontny U, Hufnagel M, Neumann-Haefelin D, Falcone V. Disseminated bocavirus infection after stem cell transplant. *Emerg Infect Dis* 2007;**13**:1425–7.
62. Longtin J, Bastien M, Gilca R, Leblanc E, de Serres G, Bergeron MG, et al. Human bocavirus infections in hospitalized children and adults. *Emerg Infect Dis* 2008;**14**:217–21.
63. Esposito S, Bosis S, Niesters HG, Tremolati E, Sabatini C, Porta A, et al. Impact of human bocavirus on children and their families. *J Clin Microbiol* 2008;**46**:1337–42.
64. Canducci F, Debiaggi M, Sampaolo M, Marinozzi MC, Berrè S, Terulla C, et al. Two-year prospective study of single infections and co-infections by respiratory syncytial virus and viruses identified recently in infants with acute respiratory disease. *J Med Virol* 2008;**80**:716–23.
65. Smuts H, Workman L, Zar HJ. Role of human metapneumovirus, human coronavirus NL63 and human bocavirus in infants and young children with acute wheezing. *J Med Virol* 2008;**80**:906–12.
66. Rihkanen H, Ronkko E, Nieminen T, Komsi KL, Räty R, Saxen H, et al. Respiratory viruses in laryngeal croup of young children. *J Pediatr* 2008;**152**:661–5.
67. Albuquerque MC, Rocha LN, Benati FJ, Soares CC, Maranhao AG, Ramírez ML, et al. Human bocavirus infection in children with gastroenteritis, Brazil. *Emerg Infect Dis* 2007;**13**:1756–8.
68. Yu JM, Li DD, Xu ZQ, Cheng WX, Zhang Q, Li HY, et al. Human bocavirus infection in children hospitalized with acute gastroenteritis in China. *J Clin Virol* 2008.
69. Lindner J, Karalar L, Zehentmeier S, Plentz A, Pfister H, Struff W, et al. Humoral immune responses against human bocavirus VP2 virus-like particles. *Viral Immunol* 2008.
70. 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. *J Infect Dis* 2007;**195**:1345–52.
71. Soderlund M, Brown CS, Spaan WJ, Hedman L, Hedman K. Epitope type-specific IgG responses to capsid proteins VP1 and VP2 of human parvovirus B19. *J Infect Dis* 1995;**172**:1431–6.
72. Franssila R, Auramo J, Modrow S, Möbs M, Oker-Blom C, Käpylä P, et al. T helper cell-mediated interferon-gamma expression after human parvovirus

B19 infection: persisting VP2-specific and transient VP1u-specific activity. *Clin Exp Immunol* 2005;**142**:53–61.
73. Franssila R, Hedman K. T-helper cell-mediated interferon-gamma, interleukin-10 and proliferation responses to a candidate recombinant vaccine for human parvovirus B19. *Vaccine* 2004;**22**:3809–15.
74. Franssila R, Hokynar K, Hedman K. T helper cell-mediated in vitro responses of recently and remotely infected subjects to a candidate recombinant vaccine for human parvovirus b19. *J Infect Dis* 2001;**183**:805–9.
75. Lindner J, Barabas S, Saar K, Altmann D, Pfister A, Fleck M, et al. CD4(+) T-cell responses against the VP1-unique region in individuals with recent and persistent parvovirus B19 infection. *J Vet Med B Infect Dis Vet Public Health* 2005;**52**:356–61.
76. Garbino J, Inoubli S, Mossdorf E, Weber R, Tamm M, Soccal P, et al. Respiratory viruses in HIV-infected patients with suspected respiratory opportunistic infection. *AIDS* 2008;**22**:701–5.
77. Freeman KP, Castro AE, Kautz CE. Unusual characteristics of a parvovirus isolated from a clinically ill steer. *Vet Microbiol* 1986;**11**:61–8.
78. Binn LN, Lazar EC, Eddy GA, Kajima M. Recovery and characterization of a minute virus of canines. *Infect Immun* 1970;**1**:503–8.