Journal of Medical Virology 84:253–258 (2012)

Human Bocavirus Infection Diagnosed Serologically Among Children Admitted to Hospital With Community-Acquired Pneumonia in a Tropical Region

Cristiana M. Nascimento-Carvalho,[1]* Maria-Regina A. Cardoso,[2] Mira Meriluoto,[3] Kaisa Kemppainen,[3] Kalle Kantola,[3] Olli Ruuskanen,[4] Klaus Hedman,[3,5] and Maria Söderlund-Venermo[3]

[1]*Department of Pediatrics, Federal University of Bahia School of Medicine, Salvador, Brazil*
[2]*Department of Epidemiology, Faculty of Public Health, São Paulo University, São Paulo, Brazil*
[3]*Department of Virology, Haartman Institute, University of Helsinki, Helsinki, Finland*
[4]*Department of Pediatrics, University of Turku, Turku, Finland*
[5]*Department of Virology, Helsinki University Hospital Laboratory Division, Helsinki, Finland*

Human bocavirus (HBoV) is a human virus associated with respiratory disease in children. Limited information is available on acute infection with HBoV among children admitted to hospital with community-acquired pneumonia in tropical regions and the current diagnosis is inadequate. The aims were to diagnose and describe acute HBoV infections among children hospitalized for community-acquired pneumonia. In Salvador, Brazil, 277 children with community-acquired pneumonia were prospectively enrolled. Paired serum samples were tested by IgG, IgM, and IgG-avidity enzyme immunoassays (EIAs) using recombinant HBoV VP2. HBoV DNA was detected in nasopharyngeal aspirates and serum by a quantitative polymerase-chain reaction (PCR). HBoV DNA was detected in nasopharyngeal aspirates of 62/268 (23%) children and 156/273 (57%) were seropositive. Acute primary HBoV infection was reliably diagnosed (bearing at least two acute markers: Positive IgM, a four-fold increase/conversion of IgG, low IgG avidity or viremia) in 21 (8%) of 273 patients, 90% of 20 had HBoV DNA in nasopharyngeal aspirates, 83% with a high DNA load. The median age of infection with HBoV was 16 months, range 5–36. Community-acquired pneumonia was confirmed radiographically in 85% of 20 patients with acute HBoV infection diagnosed serologically. HBoV DNA was found in nasopharyngeal aspirates of 42/246(17%) children without an acute primary HBoV infection and available nasopharyngeal aspirate. Four children with HBoV secondary immune responses were detected, lacking both IgM and viremia. HBoV infection was diagnosed accurately in children aged 5–36 months with community-acquired pneumonia confirmed radiographically. PCR of nasopharyngeal aspirates is not a reliable marker of acute HBoV infection. ***J. Med. Virol. 84:253–258, 2012.***

KEY WORDS: acute respiratory infection; lower respiratory tract infection; respiratory virus; respiratory tract infection

INTRODUCTION

Human bocavirus (HBoV) has, since its discovery in 2005 [Allander et al., 2005], been detected in the respiratory tract, in symptomatic [Fry et al., 2007] and in healthy children [Martin et al., 2009; Christensen et al., 2010; Martin et al., 2010]. In addition, due to prolonged shedding or persistence in the mucosa co-infections of HBoV with other viruses are diagnosed commonly [Wang et al., 2010] and viral DNA can be detected recurrently for weeks and even months in the respiratory tract of immunocompetent children [von Linstow et al., 2008; Blessing et al., 2009; Martin et al., 2010]. In the light of these findings, the causal

Conflict of interest: None.

*Correspondence to: Cristiana M. Nascimento-Carvalho, Rua Prof. Aristides Novis, No. 105/1201B, Salvador, CEP 40210-630, Bahia, Brazil. E-mail: nascimentocarvalho@hotmail.com

Accepted 28 September 2011

DOI 10.1002/jmv.22268
Published online in Wiley Online Library (wileyonlinelibrary.com).

role of HBoV in respiratory or digestive diseases has been debated [Schildgen et al., 2008]. In order to clarify this issue, the use of serologic analysis or polymerase-chain reaction (PCR) of serum has been advocated [Allander, 2008; Söderlund-Venermo et al., 2009]. In addition to HBoV, three additional HBoVs have been detected mainly in stool [Arthur et al., 2009; Kapoor et al., 2009; Kapoor et al., 2010]. To the best of the authors' knowledge, in Brazil, HBoV infection has only been diagnosed by PCR, either in fecal specimens from children with gastroenteritis [Albuquerque et al., 2007] or in nasopharyngeal aspirates from children with respiratory infections [Gagliardi et al., 2009].

The results on the etiology of Brazilian children admitted to hospital with community-acquired pneumonia have been published recently, and a potential etiological agent was detected in 78% of the reported cases [Nascimento-Carvalho et al., 2008]. Nevertheless, HBoV has not been investigated yet. The aims of this investigation were to describe HBoV carriage, acute infections and possible re-infections, among children admitted to hospital with community-acquired pneumonia.

MATERIALS AND METHODS

Patients and Samples

From September 2003 to May 2005, every child under 5 years of age admitted to hospital with community-acquired pneumonia in the Pediatric Center Professor Hosannah de Oliveira, Salvador, North-East Brazil, was evaluated prospectively after written parental consent. The pediatrician on duty diagnosed community-acquired pneumonia initially on the basis of the report of respiratory complaints and pulmonary infiltrates or pleural effusion in a chest X-ray taken on admission. Later, the chest X-rays were read by the pediatric radiologist blinded to clinical information. Exclusion of a patient was due to refusal of informed consent (n = 28), child born to an HIV-infected woman (n = 6), chronic lung disease except asthma (n = 6), varicella (n = 3), and immunodeficiency (n = 2). Every enrolled child was invited to return 2–4 weeks later for a follow-up visit, when another clinical evaluation was performed and a second chest X-ray was taken.

On admission, nasopharyngeal aspirates were collected through the nostrils and the samples were kept at -70°C until testing. Blood was collected and serum was kept at -20°C until testing. During the follow-up visit, a second blood sample was taken and serum was stored in the same conditions.

Fever was defined as axillary temperature $>37.5^{\circ}$C [El-Radhi & Barry, 2006] and tachypnea as respiratory rate $\geq$50 breaths/minute in children aged 2–11 months and respiratory rate $\geq$40 breaths/minute in children from 12 months of age onwards [WHO, 2008a]. Nutritional evaluation was performed using the software Anthro, version 1.02 (CDC and WHO), and severe malnutrition was defined as Z-score for weight-for-age index under -3.00, according to the National Center for Health Statistics (NCHS) standard [WHO, 2008b]. Carriage was defined as detection of virus in the nasopharyngeal aspirates without a corresponding serodiagnosis, that is, presence of IgM, seroconversion or fourfold titer increase in serum IgG [Allander, 2008].

Diagnosis of Infectious Diseases

The HBoV IgG, the μ-capture IgM and the IgG-avidity enzyme immunoassays (EIAs) have been described [Söderlund-Venermo et al., 2009; Hedman et al., 2010]. Recombinant virus-like particles of VP2 were used as antigen in the three EIAs. The cutoff absorbance values for antibody presence (mean of negative controls $\pm$ 4 SD) were 0.188 for IgG and 0.167 for IgM, and for antibody absence (mean of negative controls $\pm$ 3 SD) were 0.154 for IgG and 0.136 for IgM [Söderlund-Venermo et al., 2009]. In the IgG-avidity EIA, a post-serum urea elution was employed, with cutoffs for low and high avidity values of 15 and 25%, respectively [Hedman et al., 2010]. Borderline results in all EIAs were considered indeterminate. HBoV-multiplex qPCR of serum was performed to study further the children with a diagnostic IgG increase without IgM or low IgG avidity [Kantola et al., 2010]. The diagnostic criteria of an acute primary HBoV infection were the presence of two or more of the following markers: Presence of IgM, a fourfold or greater increase or conversion of IgG in paired sera, low avidity of IgG, or positive qPCR in serum. In nasopharyngeal samples, HBoV DNA was detected by quantitative PCR [Allander et al., 2007]. High HBoV DNA load was defined as $\geq$10E + 4 copies/ml and the technique for isolating and quantifying the viral DNA was described elsewhere [Kantola et al., 2008]. The HBoV investigation was carried out at the University of Helsinki, Finland. Infections caused by *Influenza A* and *B virus*, *Respiratory Syncytial virus*, *Parainfluenza virus types 1, 2,* and *3*, *Adenovirus*, *Rhinoviruses*, *Enterovirus*, *Human metapneumovirus*, *Streptococcus pneumoniae*, nontypable *Haemophilus influenzae, Moraxella catarrhalis, Chlamydia pneumoniae, Chlamydia trachomatis, Mycoplasma pneumoniae* and *Simkania negevensis* were also searched for and the methods for their detection and the results have been published [Nascimento-Carvalho et al., 2008; Nascimento-Carvalho et al., 2011].

Statistical Analysis

For categorical variables, the results are presented as proportions and the Fisher's exact test was used to identify associations. For continuous variables, summary measures are reported and comparison was performed by the Student's *t*-test or Mann–Whitney U-test as appropriate. The study protocol was

approved by the Ethics Committee of the Federal University of Bahia and the Brazilian Ethics Committee on Research.

RESULTS

Out of 277 recruited children, 268 (97%) had nasopharyngeal samples studied; 62 (23%) were positive for HBoV DNA, 26 of which with a high DNA load ($>10^4$ copies/ml) and 36 with a low load. HBoV EIA serology was done for 273 (99%) children, of whom 156 (57%) were IgG positive (in one or both samples) and 114 (42%) were negative; 3 were indeterminate. Overall, according to the strict criteria (two or more of the following acute-phase markers: Presence of IgM, >fourfold increase or conversion of IgG in paired samples, low avidity of IgG, viremia), 21 (8% of 273) children had a definitive acute HBoV infection. Among them, 19 (90%) exhibited IgM, 8 (38%) an IgG increase or seroconversion, and all 21 (100%) showed low IgG avidity. Altogether, 7/21 (33%) children presented with all three serological acute-phase markers, four of whom were also PCR positive in serum (Table I). On the contrary, four children had a diagnostic increase in high-avidity IgG and were negative for IgM; 2/4 were nasopharyngeal aspirates PCR positive, but none was viremic (Table I). Of 20 children with a serodiagnosis for acute HBoV infection and nasopharyngeal aspirates available, 18 (90%) had HBoV DNA in the nasopharyngeal aspirate, 83% with a high DNA load. Out of 62 children with positive PCR in the nasopharyngeal aspirate, only 18 (29%) had a genuine acute HBoV infection that was confirmed serologically; 15/18 (83%) with a high DNA load. Among the same 62 children with positive PCR in the nasopharyngeal aspirate, 26 and 36 had a high and a low DNA load, respectively, and 15 (58%) of 26 with high load and 3 (8%) of 36 with low load had an acute HBoV infection. Twenty (7%) patients had only single serum samples available for testing. Among the 246 children without acute HBoV infection and with nasopharyngeal aspirates available, 42 (17%) had HBoV DNA in nasopharyngeal aspirates, 11/42 (26%) with a high load.

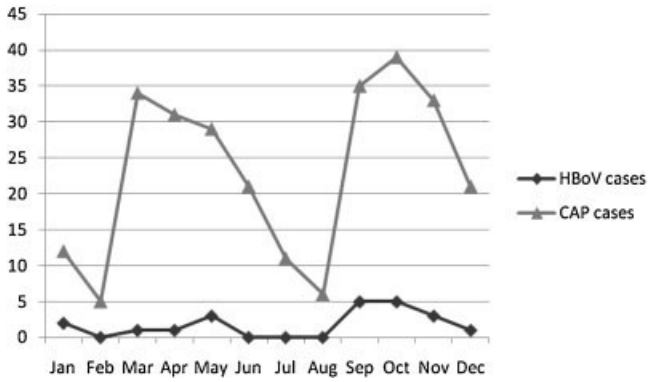

Fig. 1. Monthly distribution (n) of acute HBoV infection among community-acquired pneumonia cases

The median interval between the first and second serum sample was 18 days (mean = 19 ± 4, range: 14–32). Figure 1 shows the monthly occurrence of acute HBoV infection; the distribution (%) between months was not significantly different ($P = 0.4$ by Fisher's exact test). Three (14%) cases had sole HBoV acute infection. The other etiological agents detected among the remaining 18 cases with acute primary HBoV infection serologically diagnosed are presented in Table II.

Overall, the mean age ± standard deviation of those 21 children with acute HBoV infection was 19 ± 9 months (median = 16, range: 5–36) and the mean duration of disease before hospitalization was 7 ± 4 (median 6 days; range: 2–15 days). There were 15 (71%) males. The frequencies of symptoms and findings were: Reported fever (100%), cough (100%), difficulty breathing (90%), tachypnea (74%), chest indrawing (62%), chest retraction (62%), wheezing

TABLE I. Children With Community-Acquired Pneumonia and Diagnostic (≥fourfold) Increases in HBoV IgG Titres Between Acute (1st) and Convalescent (2nd) Serum Samples

	IgG absorbance values							
	1st sample		2nd sample					
Case	Value	Interpr.	Value	Interpr.	IgG avidity (<15 low >25 high)	IgM (1st or 2nd)	PCR naso-pharyngeal aspirate	PCR serum copies/ml (1st or 2nd)
7	0.023	Neg	0.949	Pos	Low	Pos	Pos HL	4.20E + 03
10	0.049	Neg	2.213	Pos	Low	Pos	Pos LL	Neg
39	0.107	Neg	0.413	Pos	High	Neg	Pos LL	Neg
42	0.012	Neg	0.601	Pos	Low	Pos	Neg	1.10E + 04
84	0.273	Pos	1.496	Pos	High	Neg	Neg	Neg
123	0.266	Pos	2.941	Pos	Low	Pos	Pos LL	Neg
141	0.677	Pos	3.259	Pos	High	Neg	Neg	Neg
214	0.021	Neg	0.988	Pos	Low	Neg	Neg	Neg
223	0.789	Pos	3.19	Pos	High	Neg	Pos HL	Neg
233	0.753	Pos	2.143	Pos	Low	Pos	Pos HL	5.10E + 03
266	0.141	Neg	1.941	Pos	Low	Pos	Pos HL	2.20E + 04
267	0.328	Pos	1.308	Pos	Low	Pos	Pos HL	Neg

HL, high load (>10,000 copies/ml); LL, low load (≤10,000 copies/ml). All tested sera were PCR negative for HBoV2, 3, and 4.

TABLE II. Co-Infections Among the 21 Patients With Acute HBoV Infection and Community-acquired Pneumonia

Co-infections	HBoV cases
None	3
S. pneumoniae	1
Rhinovirus	4
Parainfluenza 1/3 virus	2
Influenza A virus	1
S. pneumoniae + *Rhinovirus*	1
S. pneumoniae + RSV	1
S. pneumoniae + *Parainfluenza 3 virus*	2
C. trachomatis + *Parainfluenza 1/3 virus*	1
Influenza B virus + *Adenovirus*	1
Parainfluenza 1/3 virus + *Adenovirus*	1
S. pneumoniae + *M. catarrhalis* + *Enterovirus*	1
S. pneumoniae + *M. pneumoniae* + *Influenza A virus*	1
RSV + *Rhinovirus* + *Metapneumovirus*	1

(62%), crackles (52%), vomiting (48%), documented fever on admission (48%), rales (48%), and malnutrition (19%). Nineteen patients were discharged after improvement and two were transferred to another hospital after 1 or 2 days of hospitalization. The median duration of hospital stay for the discharged patients was 7 days (mean 8 ± 6; range: 2–31).

Alveolar (n = 15) or interstitial (n = 1) pulmonary infiltrates and pleural effusion (n = 1) were detected by the pediatric radiologist in the first chest X-ray; three patients had normal and one had unreadable chest X-ray. Therefore, pneumonia was confirmed in the final radiographic evaluation in 17 (85%) out of 20 evaluated cases with acute HBoV infection diagnosed serologically. Additional findings were atelectasis and hyperinflation (n = 2 each). At the follow-up chest X-ray, all pneumonia cases that were confirmed radiographically showed resolution of the radiographic findings. Two out of the three sole acute HBoV infection cases had alveolar infiltrates and one presented with normal chest X-ray. No significant differences were found when children with acute HBoV sole infection diagnosed serologically or co-infected cases were compared in regard to the clinical presentation (data not shown).

DISCUSSION

This paper presents evidence that acute HBoV infection can be detected serologically among children under the age of 5 years admitted to hospital with community-acquired pneumonia in a tropical region. Two out of the three cases with probable sole acute HBoV infection diagnosed serologically had pneumonia confirmed radiologically presenting with alveolar infiltrates. Although co-infections are found very commonly in children with acute HBoV infection, HBoV might be a sole causative agent of community-acquired pneumonia among children. Malawian children with community-acquired pneumonia were submitted to lung aspiration and PCR was performed in the lung aspirate fluid; HBoV was detected in 4% of the patients, either as single or co-infection [Carrol et al., 2011].

HBoV was identified in nasopharyngeal aspirates in a frequency three times higher than that of acute infections verified serologically, suggesting a state of carriership, that is, persistent shedding, for two thirds of the children with HBoV-DNA present in nasopharyngeal aspirates. Another study conducted in North-East Brazil reported that 18.7% of children in the under-5 age group with acute respiratory infection presented HBoV in nasopharyngeal aspirates by PCR [Bezerra et al., 2011], which is near to the frequency reported herein (23%). Among Italian children with community-acquired pneumonia, HBoV was diagnosed serologically in 12% [Don et al., 2010], a frequency similar to ours (8%). No cases were detected during the winter in Brazil (June, July, and August) (Fig. 1) and the occurrence of cases peaked in spring and fall (September, October, and May). Thus, despite the small number of cases, it is noteworthy that no cases were detected during the winter months in the region. In Hong Kong, HBoV infections were detected from September to February [Margareth et al., 2008] and in Spain they peaked in November and December [Pozo et al., 2007], that is, during the respective fall and winter. It is possible that the concomitant circulation of HBoV between the Northern and Southern hemispheres induces an apparent seasonality detected in the findings presented herein, which is different from genuine seasonality when respiratory infections peak during the respective winter in the region. The affected children were below three years of age, in accordance with the literature, including cases detected by PCR or serology [Margareth et al., 2008; Söderlund-Venermo et al., 2009; Karalar et al., 2010].

The reported frequency of HBoV infection in a population-based study on severe or very severe community-acquired pneumonia in Kenya (2.1%) was lower than ours [Berkley et al., 2010]. Such difference may be due to different selection criteria: In that study, community-acquired pneumonia was diagnosed only clinically. No clinical difference was found between the cases with acute sole HBoV infection diagnosed serologically or co-infections, perhaps because of sample size. The impact of co-infections on disease severity has been reported [Cilla et al., 2008]. The development of preventive measures for these viruses is challenging and the affected age strata must be taken into account when implementing them.

IgM has been significantly more prevalent in viremic patients and in those with high load of HBoV DNA in nasal swabs [Söderlund-Venermo et al., 2009; Wang et al., 2010]. On the contrary, of wheezing children with negative nasopharyngeal aspirates for HBoV DNA, 6% exhibited IgM and were also viremic [Söderlund-Venermo et al., 2009]. Viremia has been shown to be an excellent marker of acute HBoV infection, regardless of its magnitude [Söderlund-Venermo et al., 2009]. Since it has been shown that serology is

accurate [Kantola et al., 2008; Söderlund-Venermo et al., 2009] HBoV viremia was not searched for in order to confirm all clear serodiagnoses. However, PCR on the sera of those children with discordant IgM and IgG results was done in order to detect secondary infections. Furthermore, IgG-avidity tests that have been shown to differentiate between primary and secondary infections were performed [Hedman et al., 1993; Hedman et al., 2010]. Four children with secondary infections or anamnestic immune responses were detected. The fact that two of them harbored HBoV DNA in nasopharyngeal aspirates (one with a high load) might suggest a genuine re-infection rather than a passive boosting of the immune response. These two patients were not viremic which could point to a local infection as opposed to a systemic one. All four children with secondary HBoV immune responses harbored also other pathogenic agents that could account for the symptoms.

To the best of the authors' knowledge, this is the first report of HBoV infection diagnosed by serology in a tropical developing country. As 4 (1.4%) of the 277 patients studied did not have serum samples and 20 (7.2%) had only single serum samples available for testing, the reported frequency may be underestimated. The results show that 8% of all 273 children admitted to hospital with community-acquired pneumonia had an ongoing acute primary HBoV infection diagnosed by serology and PCR of serum. Even using the strict criteria to detect acute HBoV infection as in this study, the role of HBoV in the pathogenesis of community-acquired pneumonia remains unclear.

ACKNOWLEDGMENTS

We thank Lea Hedman for the IgG-avidity determinations. This study was supported by the Fundação de Amparo à Pesquisa no Estado da Bahia (FAPESB), Salvador, Brazil and the Academy of Finland (project 1122539), the Medical Society of Finland, the Research Fund of the University of Helsinki and the Sigrid Jusélius Foundation, Helsinki, Finland.

REFERENCES

Albuquerque MC, Rocha LN, Benati FJ, Soares CC, Maranhão AG, Ramírez ML, Erdman D, Santos N. 2007. Human bocavirus infection in children with gastroenteritis, Brazil. Emerg Infect Dis 13:1756–1758.

Allander T, Jartti T, Gupta S, Niesters HG, Lehtinen P, Osterback R, Vuorinen T, Waris M, Bjerkner A, Tiveljung-Lindell A, van den Hoogen BG, Hyypiä T, Ruuskanen O. 2007. Human bocavirus and acute wheezing in children. Clin Infect Dis 44:904–910.

Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. 2005. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci USA 102:12891–12896.

Allander T. 2008. Human bocavirus. J Clin Virol 41:29–33.

Arthur JL, Higgins GD, Davidson GP, Givney RC, Ratcliff RM. 2009. A novel bocavirus associated with acute gastroenteritis in Australian children. Plos Pathog 5:e1000391.

Berkley JA, Munywoki P, Ngama M, Kazungu S, Abwao J, Bett A, Lassauniére R, Kresfelder T, Cane PA, Venter M, Scott JA, Nokes DJ. 2010. Viral etiology of severe pneumonia among Kenyan infants and children. JAMA 303:2051–2057.

Bezerra PG, Britto MC, Correia JB, Duarte MC, Fonceca AM, Rose K, Hopkins MJ, Cuevas LE, McNamara PS. 2011. Viral and atypical bacterial detection in acute respiratory infection in children under five years. Plos One 6:e18928.

Blessing K, Neske F, Herre U, Kreth HW, Weissbrich B. 2009. Prolonged detection of human bocavirus DNA in nasopharyngeal aspirates of children with respiratory tract disease. Pediatr Infect Dis J 28:1018–1019.

Carrol ED, Mankhambo LA, Guiver M, Banda DL, The IPD Study Group, Denis B, Dove W, Jeffers G, Molyneux EM, Molyneux ME, Hart CA, Graham SM. 2011. PCR improves diagnostic yield from lung aspiration in Malawian children with radiologically confirmed pneumonia. 2011. Plos One 6:e21042.

Christensen A, Nordbo SA, Krokstad S, Rognlien AG, Dollner H. 2010. Human bocavirus in children: Mono-detection, high viral load and viraemia are associated with respiratory tract infection. J Clin Virol 49:158–162.

Cilla G, Oñate E, Perez-Yarza EG, Montes M, Vicente D, Perez-Trallero E. 2008. Viruses in community-acquired pneumonia in children aged less than 3 years old: High rate of viral coinfection. J Med Virol 80:1843–1849.

Don M, Söderlund-Venermo M, Valent F, Lahtinen A, Hedman L, Canciani M, Hedman K, Korppi M. 2010. Serologically verified human bocavirus pneumonia in children. Pediatr Pulmonol 45:120–126.

El-Radhi AS, Barry W. 2006. Thermometry in paediatric practice. Arch Dis Child 91:351–356.

Fry AM, Lu X, Chittaganpitch M, Peret T, Fischer J, Dowell SF, Anderson LJ, Erdman D, Olsen SJ. 2007. Human bocavirus: A novel parvovirus epidemiologically associated with pneumonia requiring hospitalization in Thailand. J Infect Dis 195:1038–1045.

Gagliardi TB, Iwamoto MA, Paula FE, Proença-Modena JL, Saranzo AM, Criado MF, Acrani GO, Camara AA, Cintra OA, Arruda E. 2009. Human bocavirus respiratory infections in children. Epidemiol Infect 137:1032–1036.

Hedman K, Lappalainen M, Söderlund M, Hedman L. 1993. Avidity of IgG in serodiagnosis of infectious diseases. Rev Med Microbiol 4:123–129.

Hedman L, Soderlund-Venermo M, Jartti T, Ruuskanen O, Hedman K. 2010. Dating of human bocavirus infection with protein-denaturing IgG-avidity assays - Secondary immune activations are ubiquitous in immunocompetent adults. J Clin Virol 48:44–48.

Kantola K, Hedman L, Allander T, Jartti T, Lehtinen P, Ruuskanen O, Hedman K, Söderlund-Venermo M. 2008. Serodiagnosis of human bocavirus infection. Clin Infect Dis 46:540–546.

Kantola K, Sadeghi M, Antikainen J, Kirveskari J, Delwart E, Hedman K, Söderlund-Venermo M. 2010. Real-time quantitative PCR detection of four human bocaviruses. J Clin Microbiol 48: 4044–4050.

Kapoor A, Simmonds P, Slikas E, Li L, Bodhidatta L, Sethabutr O, Triki H, Bahri O, Oderinde BS, Baba MM, Bukbuk DN, Besser J, Bartkus J, Delwart E. 2010. Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. J Infect Dis 201:1633–1643.

Kapoor A, Slikas E, Simmonds P, Chieochansin T, Naeem A, Shaukat S, Alam MM, Sharif S, Angez M, Zaidi S, Delwart E. 2009. A newly identified bocavirus species in human stool. J Infect Dis 199:196–200.

Karalar L, Lindner J, Schmanski S, Kertai M, Segerer H, Modrow S. 2010. Prevalence and clinical aspects of human bocavirus infection in children. Clin Microbiol Infect 16:633–639.

Margareth IP, Nelson EA, Cheuk ES, Leung E, Sung R, Chan PK. 2008. Pediatric hospitalization of acute respiratory tract infections with Human Bocavirus in Hong Kong. J Clin Virol 42:72–74.

Martin ET, Fairchok MP, Kuypers J, Magaret A, Zerr DM, Wald A, Englund JA. 2010. Frequent and prolonged shedding of bocavirus in young children attending daycare. J Infect Dis 201:1625–1632.

Martin ET, Taylor J, Kuypers J, Magaret A, Wald A, Zerr D, Englund JA. 2009. Detection of bocavirus in saliva of children with and without respiratory illness. J Clin Microbiol 47:4131–4132.

Nascimento-Carvalho CM, Cardoso MR, Ruuskanen O, Lappalainen M. 2011. Sole infection by human metapneumovirus among children with radiographically diagnosed community-acquired pneumonia in a tropical region. Influenza Other Respi Viruses 5:285–287.

Nascimento-Carvalho CM, Ribeiro CT, Cardoso MR, Barral A, Araújo-Neto CA, Oliveira JR, Sobral LS, Viriato D, Souza AL, Saukkoriipi A, Paldanius M, Vainionpää R, Leinonen M, Ruuskanen O. 2008. The role of respiratory viral infections among children hospitalized for community-acquired pneumonia in a developing country. Pediatr Infect Dis J 27:939–941.

Pozo F, García-García ML, Calvo C, Cuesta I, Pérez-Breña P, Casas I. 2007. High incidence of human bocavirus infection in children in Spain. J Clin Virol 40:224–228.

Schildgen O, Müller A, Allander T, Mackay IM, Völz S, Kupfer B, Simon A. 2008. Human bocavirus: Passenger or pathogen in acute respiratory tract infections? Clin Microbiol Rev 21:291–304.

Söderlund-Venermo M, Lahtinen A, Jartti T, Hedman L, Kemppainen K, Lehtinen P, Allander T, Ruuskanen O, Hedman K. 2009. Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. Emerg Infect Dis 15:1423–1430.

von Linstow ML, Hogh M, Hogh B. 2008. Clinical and epidemiologic characteristics of human bocavirus in Danish infants: results from a prospective birth cohort study. Pediatr Infect Dis J 27: 897–902.

Wang K, Wang W, Yan H, Ren P, Zhang J, Shen J, Deubel V. 2010. Correlation between bocavirus infection and humoral response, and co-infection with other respiratory viruses in children with acute respiratory infection. J Clin Virol 47:148–155.

World, Health Organization. 2008a. Integrated Management of Childhood Illness chart booklet. (WC 503.2). Geneva: WHO. [WHO website]. Available at: http://whqlibdoc.who.int/publications/2008/9789241597289_eng.pdf. Accessed January 15, 2009.

World Health Organization. 2008b. Training Course on Child Growth Assessment. Geneva: WHO, 2008. [WHO website]. Available at: http://http://whqlibdoc.who.int/publications/2008/9789241595070_A_eng.pdf. Accessed July 13, 2009.