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Clinical and epidemiological aspects of human bocavirus infection

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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. 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. 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 (HBoV).

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 and coated onto Nunc-ImmunoMediSorp plates (Nunc GmbH, Wiesbaden, Germany) in coating buffer (0.2 M Na2CO3, 0.2 M NaHCO3, 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 OptEIATM 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-
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 NS112–15 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 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.3,31,40 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 ≥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.9,26,49 Herein, our group observed the prevalence of IgG1 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 Similarly 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 a 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, respira
tory distress, hypoxia, fever, rhinitis, laryngeal croup and, more rarely, conjunctivitis or rashes. In adults, acute HBoV infec
tions leading to ARTIs seem to be rare and have been currently detected mainly in immunocompromised 13,49,51,76 and only in sin
gle 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 respira
tory disease manifestation. However, infections with the related parvovirus B19 often result in a prolonged replication of the virus in infected individuals 3 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 5 and therefore mechanisms of persistence may also apply for HBoV.

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.

5.2. HBoV and gastrointestinal disease

In addition to respiratory symptoms, HBoV is currently dis
cussed 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.

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 accumulat
ing. 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 gastroin
testinal 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 individu
al 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.

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