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Human bocavirus

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Abstract

Human bocavirus (HBoV) was first described in 2005 in nasopharyngeal aspirates of children with respiratory tract infection. Multiple studies have confirmed the presence of HBoV in respiratory tract samples of children world-wide. HBoV has recently also been detected in blood and fecal samples. Most studies so far have studied virus prevalence, and only a few reports provide data regarding the linkage of HBoV to disease. These reports indicate that HBoV infection is indeed associated with acute respiratory tract symptoms, but also that HBoV may persist in the respiratory tract for a longer time than other respiratory agents, resulting in frequent detection of low load HBoV carriage. This phenomenon has complicated the use of PCR diagnostics, which has been the only available diagnostic method. Development of alternative diagnostic strategies such as serology will be important for future studies of HBoV and its association with disease.

Keywords: Diagnostics; Gastroenteritis; Parvoviridae; Pediatrics; Respiratory tract infection

1. Introduction

Human bocavirus (HBoV) was first described in 2005 (Allander et al., 2005). It was the first virus identified by "molecular virus screening", a procedure based on DNase treatment of the samples, random amplification and cloning, followed by large scale sequencing and bioinformatic analyses. Identical or highly similar procedures have since then led to the identification also of KI and WU polyomaviruses (Allander et al., 2007a; Gaynor et al., 2007). The fact that HBoV was first detected in nasopharyngeal aspirates of patients with respiratory tract infections suggested that the virus may induce respiratory tract disease. In the first study this hypothesis was supported by the finding that HBoV was primarily found in patients where no other respiratory virus was detected (Allander et al., 2005). Multiple studies have investigated the presence of HBoV in respiratory tract samples, and the virus is prevalent in children world-wide (Arden et al., 2006; Arnold et al., 2006; Choi et al., 2006; Kesebir et al., 2006; Ma et al., 2006; Manning et al., 2006; Smuts and Hardie, 2006; Bastien et al., 2007; Fry et al., 2007; Naghipour et al., 2007; Qu et al., 2007). Recent studies have also identified HBoV in blood and fecal samples (Allander et al., 2007b; Fry et al., 2007; Neske et al., 2007; Vicente et al., 2007). However, most studies so far have only studied virus prevalence, and the issue of disease association has only more recently started to be more systematically addressed. A model is emerging in which HBoV infection is associated with acute respiratory symptoms, but also persists after primary infection for a longer time than other respiratory agents. HBoV can therefore frequently be detected in respiratory secretions by PCR also when it is not likely acting as a pathogen. This phenomenon has complicated the use of PCR diagnostics, and made it difficult to draw rapid conclusions about the pathogenicity of HBoV from available prevalence data.

2. Biology and diagnostics of human bocavirus

Human bocavirus is as yet known only by its nucleotide sequence. By genetic organization and sequence homology it has been provisionally classified in the family *Parvoviridae*, subfamily *Parvovirinae*, genus *Bocavirus* (Allander et al., 2005). Thus, it is a parvovirus and distantly related to parvovirus B19, the agent of fifth disease and other

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human disorders. Other parvoviruses infecting humans are the adeno-associated viruses and the recently discovered human parvovirus 4, which have not yet been associated with a disease (Jones et al., 2005). The genus bocavirus presently has two additional members apart from HBoV, the canine minute virus and bovine parvovirus 1. Both have been associated with respiratory and enteric symptoms of young animals (Durham et al., 1985; Carmichael et al., 1994). Parvoviruses in general infect proliferating cells, cause systemic infection and may persist for a long time after resolution of symptoms. Long term persistence has been documented in humans for both parvovirus B19 and human parvovirus 4 (Lefrere et al., 2005; Lindblom et al., 2005; Manning et al., 2007).

The only so far published diagnostic method for human bocavius is PCR. One advancement has been the application of quantitative PCR, which has proven useful for clinical studies. However, sampling of respiratory tract secretions is not a standardized procedure, so great care must be taken in collecting and interpreting quantitative data. HBoV sometimes reaches high viral loads in respiratory tract secretions with more than 10^{10} copies/ml of respiratory tract sample, but a large proportion of positive samples have low viral loads in most studies (Lu et al., 2006; Allander et al., 2007b; Kleines et al., 2007; Neske et al., 2007). High viral loads seem statistically associated with symptoms, but the diagnostic value of the viral load in individual patients has not been investigated (Allander et al., 2007b). HBoV DNA can also be detected in the blood stream of symptomatic children. Detection of HBoV in blood may correlate better with symptoms than does investigation of respiratory secretions (Allander et al., 2007b).

Human bocavirus has not been replicated *in vitro*, and no animal model has been reported. This is a frequent problem with newly identified viruses, since the very reason for them being undetected until now is their resistance to detection by traditional, culture-based methods. As of now, there are no published reports on the detection of HBoV antigens, and there are only preliminary unpublished results regarding detection of antibodies to HBoV (M. Söderlund-Venermo, Personal communication). The diagnostic limitations have been a severe problem for the study of HBoV. However, many of these problems will likely be solved within the nearest years by production of recombinant antigens enabling serology and generation of antibodies for antigen detection.

3. Epidemiology

HBoV has been detected world-wide (Allander et al., 2005; Arden et al., 2006; Arnold et al., 2006; Choi et al., 2006; Kesebir et al., 2006; Ma et al., 2006; Manning et al., 2006; Smuts and Hardie, 2006; Allander et al., 2007b; Bastien et al., 2007; Fry et al., 2007; Naghipour et al., 2007; Qu et al., 2007). Nearly all prevalence studies so far have been made on respiratory tract secretions from patients (mainly children) with acute respiratory tract disease, and prevalence rates between 1.5% and 19% have been observed in these studies. It is possible that differences of study populations, sampling techniques, and assay sensitivity between the studies account for these discrepancies rather than actual differences of HBoV prevalence.

Variable results have been reported regarding the seasonality of HBoV infection (Kesebir et al., 2006; Manning et al., 2006; Weissbrich et al., 2006; Allander et al., 2007b; Bastien et al., 2007; Fry et al., 2007; Maggi et al., 2007; Naghipour et al., 2007; Neske et al., 2007). Taken together, these results indicate that there is no obvious regular seasonal occurrence of HBoV. However, the presently available published studies have been based on PCR detection in respiratory secretions, and have not taken the possibility of asymptomatic persistence into account, which would seriously affect incidence estimates. Also, most studies are based on collections of samples submitted to a clinical laboratory, and suffering from the fact that sampling for respiratory tract viruses is highly seasonal per se and often guided by the occurrence of influenza and RSV epidemics. In a study of prospectively enrolled children admitted for acute wheezing, where we identified cases of likely primary symptomatic HBoV infections, these cases appeared to occur throughout the year (Allander et al., 2007b).

HBoV is usually detected in children under 2 years of age (Allander et al., 2005; Kesebir et al., 2006; Manning et al., 2006; Fry et al., 2007; Maggi et al., 2007). Many studies show a comparably low prevalence among the youngest children below 6 months (Foulongne et al., 2006; Ma et al., 2006; Allander et al., 2007b; Naghipour et al., 2007; Qu et al., 2007), suggesting some degree of protection from maternal antibodies. Together with the high prevalence rate, these findings suggest that HBoV is an endemic virus with a high attack rate in susceptible children, likely followed by some degree of immunity. It is thus possible that nearly 100% of the population is infected by HBoV during childhood. Future serological studies will be needed to confirm this hypothesis.

The genetic variability of HBoV is low. Phylogenetic studies indicate that two slightly different genetic lineages circulate in parallel world-wide (Kesebir et al., 2006; Bastien et al., 2007; Neske et al., 2007). It is questionable whether these limited sequence differences have any medical relevance, or can be used to understand the epidemiology or trace the spread of HBoV.

4. Human bocavirus and respiratory tract disease

A large number of studies on HBoV have been published, but only a few have to date addressed whether HBoV infection is associated with respiratory tract symptoms. The fact that HBoV is often co-detected with other respiratory viruses has made it difficult to draw conclusions about pathogenicity from the many published laboratory-based PCR prevalence studies. The classical criteria for disease causality, Koch's postulates, cannot be tested in the absence of an *in vitro*

culture system and an animal model. Instead, the alternative causality criteria developed for molecular diagnostics by Fredricks and Relman (1996) can be applied. Recent studies provide support for that HBoV fulfils many of these criteria, while some criteria have not yet been tested, e.g. presence of HBoV in the lower respiratory epithelium.

Four studies have reported that HBoV is more frequent in patients with respiratory tract symptoms than in asymptomatic individuals (Kesebir et al., 2006; Allander et al., 2007b; Fry et al., 2007; Maggi et al., 2007). In fact, HBoV is very rarely detected in asymptomatics, with no positives in three of the four studies. The study of Fry et al. (2007) on pneumonia in rural Thailand stands out for its large study material, population based approach, and well matched controls. Nevertheless, a problem with studies including asymptomatic controls is that respiratory tract samples obtained during an acute infection can be very different from those of asymptomatics, e.g. by volume and cell counts. Since HBoV is often co-detected with other pathogens and, in particular, since it is often detected at low vial loads, the sampling effect may be especially important for HBoV, and studies of this type must be interpreted with some caution. However, recent data suggest that acute HBoV infection appears associated with presence of HBoV DNA in the blood (Allander et al., 2007b). Studies comparing blood HBoV DNA in cases and controls may therefore help to settle that issue.

There is also statistical evidence not depending on asymptomatic controls for that HBoV is associated with respiratory tract disease. In a study of patients hospitalized for acute wheezing in Turku, Finland (Allander et al., 2007b), we found that HBoV DNA was much more prevalent in the patients' blood during the acute symptoms than after recovery. We also found that HBoV was significantly more prevalent in the respiratory tract in the absence of other detected viral agents, and that only the cases with high viral loads showed this association. Thus, in two ways, internal symptomatic controls could be used to support a link between HBoV and disease. Interestingly, results were highly statistically significant and at the same time 76% of HBoV-cases were co-detections with other viruses. This illustrates that frequent co-detections are not a strong argument against disease association. The Turku study suggested that high load and viremic HBoV infection is associated with respiratory tract symptoms, while detection of a low viral load in the nasopharynx alone has uncertain relevance. We hypothesized that these two entities represent primary infection and asymptomatic persistence, respectively. The duration of viral shedding in the respiratory tract has not been investigated, and this will require longitudinal studies. However, HBoV prevalence falls with increasing age, and HBoV is only rarely detected in adults (Bastien et al., 2006; Kupfer et al., 2006; Manning et al., 2006; Fry et al., 2007). Presumed primary HBoV infections were found in 11% of the Turku material. The high number of HBoV infections observed in this particular study population suggests that wheezing could be a main manifestation of HBoV infection. Notably, in the Thailand study of Fry et al. (2007), low HBoV loads were frequent among both cases and controls, but high viral loads were only seen in the pneumonia group and not among controls, consistent with our hypothesis. Among the Thailand cases there was also a statistical association between HBoV detection and reporting wheezing.

5. Human bocavirus and gastroenteritis

The animal bocaviruses have been associated not only with respiratory symptoms but also with gastroenteritis in calves and puppies (Durham et al., 1985; Carmichael et al., 1994). Arnold et al. (2006) early found that diarrhea was reported for 16% of HBoV-positive patients in a children's hospital. Vicente et al. (2007) investigated fecal samples and found 9.1% of the samples from children with gastroenteritis positive for HBoV by PCR. Neske et al. (2007) investigated 31 fecal samples of children positive for HBoV in the respiratory tract, and found 14 (45%) of the fecal samples positive. HBoV is thus quite prevalent in stool samples, but the role for HBoV in gastroenteritis remains unclear. It should also be kept in mind that diarrhea is a common symptom associated with a systemic infection response (Reisinger et al., 2005). Thus, the presence of diarrhea in association with HBoV infection does not automatically make HBoV a gastroenteritis agent.

6. HBoV in the immunosuppressed

The prevalence and potential pathogenicity of HBoV in the immunosuppressed remains largely unknown. Although the high prevalence and co-infection rates in children suggest some degree of viral persistence, HBoV has only rarely been detected in adult patients (Bastien et al., 2006; Kupfer et al., 2006; Manning et al., 2006; Fry et al., 2007). However, many of the HBoV findings in adults have indeed been made in immunosuppressed individuals (Kupfer et al., 2006; Manning et al., 2006; Maggi et al., 2007). Kupfer et al. (2006) reported a case of severe pneumonia where HBoV was the only detected microorganism. The finding of HBoV does not at all warrant that HBoV caused the symptoms, since unexplained pulmonary disease is frequent in this patient group. However, the presence of HBoV in immunosuppressed adults must probably be explained in terms of reinfection, persistence or reactivation. This is an important area for future studies, including longitudinal studies for confirming persistence. However, unlike parvovirus B19 and human parvovirus 4, HBoV could not be detected in post mortem blood, brain or lymphoid tissue samples of HIV infected individuals, or in plasma pools from healthy blood donors (Fryer et al., 2007; Manning et al., 2007). Notably, respiratory organ tissues were not examined in these studies.

7. Conclusions

HBoV has just only started to be investigated, and most published reports are prevalence studies not designed to investigate disease association. The only available diagnostic tool so far has been PCR. HBoV is prevalent world-wide in children with respiratory tract infections. More recently, HBoV has been identified in stool samples of children with gastroenteritis. There is gathering evidence for that HBoV is indeed a causative agent for respiratory tract disease, but HBoV is also frequently detected when it is less likely acting as a pathogen. A model is emerging in which primary HBoV infection occurs early in life and is a systemic infection associated with respiratory symptoms. The infection seems to be frequently followed by asymptomatic low level virus shedding in the respiratory tract. Therefore, PCR diagnostics on respiratory tract secretions may not be a suitable diagnostic strategy for HBoV. Application of serology and PCR detection in blood will probably be better strategies for future studies of HBoV association with disease. The high prevalence and apparently complex biology makes HBoV a challenging study object and a virus with many potential clinical consequences. HBoV is not just another respiratory virus. It is first and foremost a yet poorly investigated human parvovirus.

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