Diagnosis of respiratory tract infections caused by human bocavirus 1

Human bocavirus 1 (HBoV1) causes respiratory tract infections in infants and children. Diagnosis of acute HBoV1 infections is challenging as viral DNA is frequently detected in asymptomatic controls and as co-finding with other viruses. Recently developed novel HBoV1 mRNA and antigen tests may improve the diagnosis of acute HBoV1 infections.

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Background and molecular characteristics of HBoV1

Human bocavirus 1 (HBoV1), a small single-stranded DNA (ssDNA) virus belonging to the *Parvoviridae* family, was described for the first time in 2005 [1]. Its genome replication is dependent on the formation of double-stranded DNA (dsDNA) intermediates in the nucleus of the host cells. The dsDNA serves as template for transcription of messenger-RNA (mRNA) by the host replication machinery. The mRNA is further translated into viral proteins, such as structural VP2 protein. Structural proteins assemble as empty capsids into which genomic ssDNA is inserted. Thus, during acute infection, the replicating virus produces mRNA transcripts from the viral dsDNA which are translated into viral proteins. Formation of viral proteins and particles are essential for the multiplication and spread of viable viruses.

Epidemiology and clinical outcomes of HBoV1 infections

HBoV1 was originally discovered in hospitalized children with a respiratory tract infection (RTI) [1]. However, HBoV1 can cause RTI illnesses in varying severities. Mainly children at age 6–24 months are affected. By 6 years old almost all children are seropositive for HBoV1. Data on the disease pressure in adults are very scarce but apparently immunity lasts long and acute infections are rare. HBoV1 DNA is detected by PCR in 2–19% of patients with RTI worldwide. The most common symptoms of acute HBoV1 infection are common cold-like complaints, wheezing, bronchiolitis and pneumonia. HBoV1 is associated with asthma exacerbations [2]. Diagnostic positivity rate for HBoV1 has been high in some studies in summer [3]. This would differ from other RTI viruses like influenza and respiratory syncytial virus. However, most cases of HBoV1 DNA detection are reported in winter and spring [2] which may also be linked to the higher frequency of diagnostic testing during the influenza season.

HBoV1 may infect lower airways down to the bronchioles [2]. There has been no difference in HBoV1 prevalence between immunocompetent and immunocompromised patients [2]. It seems that that particularly young children who were born prematurely may be at risk in developing severe RTIs caused by HBoV1 [4, 5].

HBoV1 DNA is often found in stool samples from children. However, detection rates are similar among subjects with or without acute gastroenteritis. Also co-findings with other known gastroenteritis viruses are common. Thus, the detection of HBoV1 from stool is most probably rather a sign of respiratory tract or systemic infection, prolonged viral shedding or persistent infection than acute gastroenteritis [6].

Diagnostic methods and challenges in diagnosis of HBoV1 infections

HBoV1 infection cannot be accurately diagnosed based on clinical symptoms alone. There are four techniques to aid in the diagnosis of HBoV1 infections. These include serology [7], PCR using viral DNA as target [8], reverse transcription (RT) PCR using viral mRNA as target [9], and most recently antigen detection [10]. Also electron microscopy has been used to detect the presence of viral particles [5], although this technique is not suitable for routine diagnostics.

Serology can provide information as to whether the infection is acute or past and it can be used to confirm the findings of other methods. IgM positivity, low IgG avidity, seroconversion or a diagnostic (≥4-fold) increase in the IgG level in paired sera are signs of acute HBoV1 infection [2, 7]. A major drawback of serology is that it takes the human body 1–2 weeks to produce the antibody.

A number of commercially available multiplex PCR tests have included the detection of HBoV1 DNA in their test panels and some of the tests may provide results also in stat labs. However, detection of viral DNA from nasal samples may have little clinical significance since HBoV1 DNA is frequently (10–40 %) detected in asymptomatic controls and

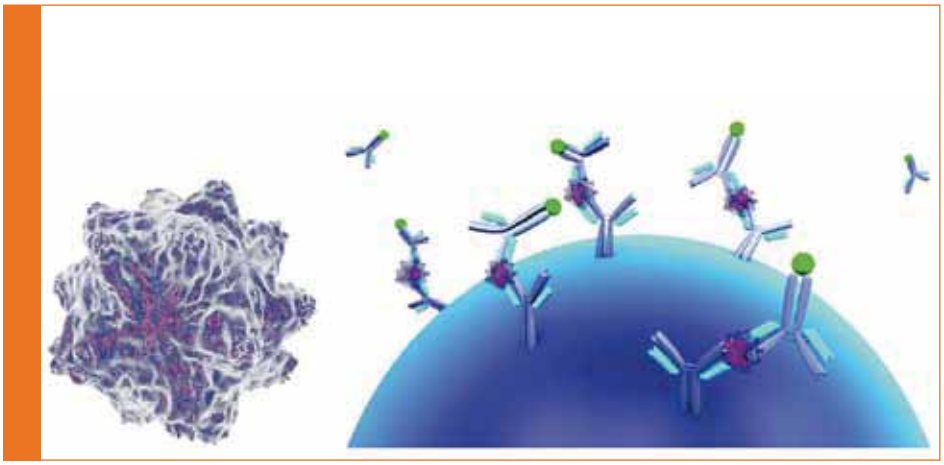

Figure 1. Schematic figure of HBoV1 (enlarged on left) binding to immunoassay (microparticle solid phase) reagents.

often found as co-findings (50–70 %) with other respiratory viruses. Prolonged shedding of the virus from infected shells, or long-term presence of virus or viral DNA in the airways may explain the high co-infection rate and prevalence in asymptomatic controls observed in almost every DNA PCR cohort study [11–14]. Currently, the mechanism for persistence is unknown but one possible explanation may be that the virus exists in a latent phase where the transcription of mRNA and protein translation is inhibited by the immune system.

Quantification of viral DNA by Ct-value gives a statistical correlation with severity but is not diagnostic in individual cases owing to, for example, the semi-quantitative nature of sampling. Thus, high viral DNA load and single findings are only indicative of the etiology [3, 8]. Extensive exclusion of the presence of other potential RTI pathogens together with high genome HBoV1 DNA load as single finding, viremia or the presence of the DNA in normally sterile body fluids has shown causality [4, 5]. Instead of extensive exclusion of other RTI viruses with high-cost multiplex PCRs, direct detection of actively replicating HBoV1 viruses by mRNA PCR or an antigen test could be a more straightforward, specific and cost-efficient approach.

mRNA RT-PCR methodology was developed to specifically detect the acute HBoV1 infections before the rise in antibody levels. mRNA RT-PCR is analytically as sensitive as DNA PCR. It provides the same clinical sensitivity but higher diagnostic specificity than DNA PCR. In one HBoV1 case, mRNA was detected up to 10 days from the onset of the symptoms while the DNA was detected at least up to 2 months although the patient was already fully recovered. The time span for positivity based on the mRNA RT-PCR correlated better with acute symptoms than DNA PCR [9].

Serology, mRNA RT-PCR and DNA PCR suffer from being slow, costly and/or labour intensive techniques, and they are only available in highly specialized diagnostic laboratories. Detection of viral antigens (e.g. structural VP2 protein) from nasal samples provides a rapid and specific alternative for testing of acute HBoV1 infections (Fig. 1). Recently the first HBoV1 antigen test, to our knowledge, was introduced into the automated and multianalyte mariPOC respi test (www.arcdia.com). The test provides most of the positive results in 20 minutes and low positives in 2 hours at the point-of-care. The new test has shown similar clinical specificity compared to mRNA RT-PCR test [15]. Antigen testing is feasible only during the acute phase of the infection (active viral replication phase) which seems to be approximately 5 days from the emergence of symptoms [10], as for most of the RTI viruses. The first days are often the most crucial when making clinical decisions and have impact, for example, for the decision on whether to prescribe antibiotics or not. The features of HBoV1 diagnostic methods are compared in Table 1.

Selected diagnostic cases

Case 1

A previously healthy full-term born Finnish girl developed symptoms of rhinorrhea, cough and high fever at 5 months of age. Upper RTI with no lower respiratory tract involvement or signs of otitis was diagnosed. HBoV1 secretion into nasopharyngeal samples was monitored by quantitative mariPOC antigen test up to day 5. Virus peak was at day 3 and viral levels were low at day 5, which coincided with the recovery of symptoms on day 6 [10]. The virus peak sample was estimated to contain 2×10^{10} viral particles per mL.

Case 2

A prematurely (week 27) born Turkish girl, at 5 months of age, after sepsis, developed high fever, wheezing and was treated for acute bronchiolitis before hospital discharge. The patient was found deceased the same night as the result of respiratory failure caused by pulmonary infection. HBoV was detected as single finding from nasopharyngeal swabs, stools and lung tissues [4].

Case 3

A prematurely (week 25) born Slovene child, at the age of 18 months, with chronic respiratory insufficiency was hospitalized. HBoV1 DNA was detected in tracheal aspirate (2.6×10^{10} copies/mL), in the nasopharyngeal swab (8.27×10^{6} copies/mL), and in plasma sample (7.42×10^{6} copies/mL). The presence of HBoV1 particles was confirmed by electron microscopy from tracheal aspirate and autologous plasma, which was taken the third day of illness [5].

Conclusions

As demonstrated above, clinical manifestations of HBoV1 range from simple common cold symptoms to fatal respiratory illnesses. Diagnosis of HBoV1 is now significantly more straightforward because of the recent advances in HBoV1 diagnostics. Rapid antigen testing and

Feature	Methodology			
	Antigen detection	Serology	mRNA PCR	DNA PCR
Cost	Low/moderate	Low/moderate	High	High
Rapid and POC compatible	Yes	No	No	No / yes
Differentiation of acute infection	Yes	Yes*	Yes	No
Differentiation of primary infection	Not known	Yes	Not known	No
Invasive	No	Yes	No	No
Bronchoalveolar lavage, sputum	Not studied	No	Yes	Yes

Table 1 . Pros and cons of HBoV1 diagnostic methods. *Serology is not suitable within the first week. POC, point of care.

mRNA RT-PCR provide accurate non-invasive diagnostics for acute HBoV1 infections. mRNA RT-PCR is so far only available in highly specialized diagnostic laboratories while rapid antigen test is applicable at point-of-care. DNA PCR may be most suitable for the detection of viral DNA from body parts, like cerebrospinal fluid during suspected systemic infection. The use of multiple diagnostic methods will provide a more accurate picture about the clinical significance and outcomes of the HBoV1 infections. The method of choice for accurate diagnosis of HBoV1 depends on the elapsed time since the onset of the symptoms, clinical signs and other clinical or research needs. There is no specific medication or vaccine for HBoV1 yet. However, the new diagnostic tests will increase our understanding about the clinical significance of HBoV1 and open new doors for therapy development.

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