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Chapter 15

Human Bocavirus

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CASE PRESENTATION

A twenty-month-old female child was hospitalized due to acute respiratory infection with signs of respiratory distress. She was diagnosed as having acute bronchiolitis. She was prematurely born after 27 weeks of gestation and her previous medical history was unremarkable.

After her admission she was treated with short-acting beta-2 agonist. Her medical condition failed to improve, so chest radiography was performed, and hyperinflation with infiltrates in the left lower lung field was observed. Parenteral steroid and amoxicillin-clavulanic acid were commenced. In the next 24 h, neck emphysema was observed and due to the imminent respiratory failure, the girl was intubated and transferred to the intensive care unit (ICU). The child was sedated, relaxed, and mechanically ventilated. The leukocyte count was 22.0×10^9/liter, the C-reactive protein level was 14 mg/liter, and hemoglobin concentration was 9.4 g/dl. Her medical condition failed to improve and her circulatory stability was supported by intermittent intravenous saline boluses with continuous infusion of dopamine. Repeated chest radiography revealed pneumothorax of the left and the right lung, which were immediately drained. Bronchoscopy performed through endotracheal tube showed edema and inflammation of the lower respiratory tract with a large amount of mucus. Carbon dioxide partial pressure increased, and after 18 h after ICU admission reached 19.6 kPa, with excessive respiratory acidosis (pH 6.92).

A nasopharyngeal swab (at admission), tracheal aspirate (after intubation), and blood sample (at admission) were tested by real-time PCR for the presence of 15 respiratory viruses. HBoV was the only respiratory pathogen that was detected in nasopharyngeal swab (8.6×10^9 copies/ml), tracheal aspirate (2.1×10^{10} copies/ml), and plasma sample (1.8×10^6copies/ml). At admission to the ICU, blood was collected for hemoculture; bronchoalveolar fluid (BAL), tracheal aspirate, and thoracal drainage fluid were collected

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and the routine cultures were set up for detection of bacterial and fungal pathogens and remained negative during hospitalization. Human bocavirus particles were visualized by electron microscopy and immunoelectron microscopy confirmed the immune response against virus in patient plasma.

Assisted controlled ventilation with positive inspiratory pressure was effective in lowering the $PaCO_2$. Subcutaneous emphysema occurred on the child's head, cheeks, neck, and chest, because of pronounced air leak. Pneumoperitoneum was also observed on the radiograph. Her clinical condition substantially stabilized after surgical incision in the neck, and after insertion of a new thoracic drain on the left side partially relieved pneumomediastinum. Air leak decreased steadily, whereas subcutaneous emphysema persisted. Chest drains were removed on the seventh day and the girl was extubated on the ninth day in the ICU and was discharged 4 days later. The HBoV viral load decreased slowly, and on the day of her discharge, the viral load in nasopharyngeal swab was 9.9×10^4 copies/ml and 3.6×10^4 copies/ml in the plasma sample.

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1. WHY THIS CASE WAS SIGNIFICANTLY IMPORTANT AS AN EMERGING INFECTION

Human bocavirus 1 (HBoV1) is a recently described respiratory virus and is detected worldwide.[2,3] It is recognized as one of the most frequently detected respiratory viruses in children with upper and lower respiratory infections. The severe clinical course of HBoV1 infection can be seen in prematurely born children or children, but rarely adults, with other underlying conditions.[1,4–7]

2. WHAT IS THE CAUSATIVE AGENT?

Human bocavirus 1 belongs to the family *Parvoviridae*, subfamily *Parvovirinae*, and genus *Bocavirus*.[2] The HBoV1 genome phylogenetic analysis showed that it is most closely related to bovine parvovirus (BPV1) and minute virus of canines (MVC), after which it was named.[2] The HBoV1 virions are icosahedral, non-enveloped and small, approximately 18−26 nm in diameter and their linear single-stranded DNA genome is 5543 bp in length[1,2,8–10] (accession no. JQ923422).

Figure 15.1 shows human bocavirus 1 virions examined with an electron microscope.

The HBoV1 genome, like all members of the *Bocavirus* genus, contains three open reading frames (ORF).[9] The left- and the middle-hand ORFs at the 5′ end encode for NS1 and NP1, two non-structural proteins, whereas the right-hand ORF, at the 3′ end, encodes for the two structural capsid viral proteins, VP1 and VP2.[2,8,9] The capsid of HBoV is assembled from 60 copies of VP2 protein and about five copies of VP1 protein and is most similar to that of parvovirus B19.[9,11]

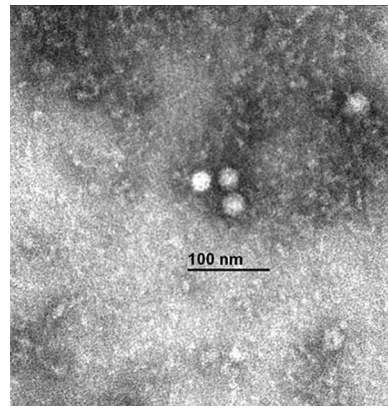

FIGURE 15.1 EM image of HBoV1 in a direct preparation from nasopharyngeal swab suspension, negatively stained with 2% phosphotungistic acid (PTA; pH 4.5) and examined with a transmission electron microscope (JEM 1200 EX II; Jeol, Japan) at a magnification of ×200,000.[1]

Three additional enteric HBoV-like viruses, HBoV2, HBoV3, and HBoV4, were identified recently in stool samples of children with gastroenteritis, but their clinical significance in symptomatic infections remains uncertain.[3] The HBoV2, HBoV3, and HBoV4 possess the same genome and capsid organization as HBoV1.[11–14]

3. WHAT IS THE FREQUENCY OF THE DISEASE?

Human bocavirus 1 was first described in 2005 in nasopharyngeal aspirates of Swedish children with acute respiratory tract infections[2] (ARTI). Since then, by using polymerase chain reaction (PCR), the HBoV1 has been found in approximately 10% of respiratory samples of children with upper or lower respiratory tract infections (URTI, LRTI) worldwide,[3,15] and in 1–9% of children with respiratory infection with or without gastrointestinal symptoms.[12,16,17]

Figure 15.2 shows the geographic distribution of HBoV1 in Europe and reported frequency of infection in children.[18–36]

The HBoV1 is one of the most frequently detected respiratory viruses in children with ARTI below 5 years of age, and is mainly detected in children between 6 and 24 months.[3,15,32,34,37–40] The reports on HBoV1 infection in adults and elderly are rare and the prevalence of HBoV1 in those age groups is rather low;[3,41] however, the infections caused by HBoV1 in immunocompromised individuals could be severe and may be even lethal.[3,42,43]

4. HOW IS THE VIRUS TRANSMITTED?

The transmission routes of HBoV1 are unknown. The HBoV1 virus is ubiquitous. It is likely that HBoV1 is transmitted similarly, as other parvoviruses, by

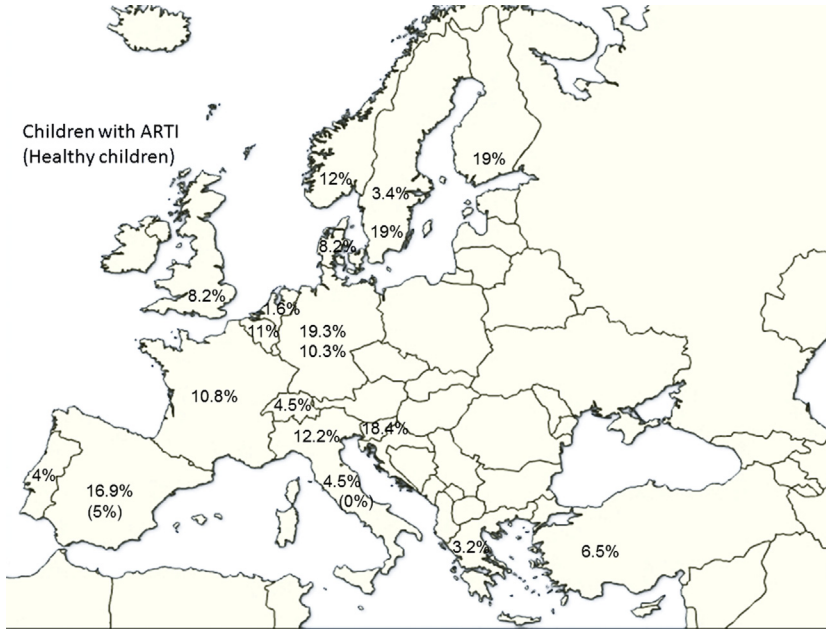

FIGURE 15.2 Distribution and frequencies of HBoV1 infection in Europe in children with ARTI (healthy children).

inhalation and by contact with infectious respiratory secretions. Respiratory infections due to HBoV1 are systemic as its DNA is frequently detected in many human secretions.[3,44] The seroepidemiological studies consistently show that most of the children are infected with HBoV1 by the age of 6[44,45] and that the IgG antibodies remain present for life.

Since the prevalence of IgG antibodies against HBoV1 is high among pregnant woman, the intrauterine infections are unlikely.[3,46]

5. WHICH FACTORS ARE INVOLVED IN DISEASE PATHOGENESIS? WHAT ARE THE PATHOGENIC MECHANISMS?

Until recently, the replication and pathogenesis of HBoV1 was not known, since no *in vitro* or animal models were available. However, in 2009 the HBoV1 was successfully cultured in primary airway epithelial cells differentiated into pseudo-stratified human airway epithelium.[47] It has been demonstrated that the transcription profile of HBoV1 displays features similar to bovine parvovirus 1 (BPV1) and minute virus of canines (MVC), which also belong to genus *Bocavirus*.[47] In 2012 the establishment of a reverse genetic system for studying HBoV1 in human airway epithelia (HAE) has enabled better understanding of HBoV1 replication and pathogenesis.[10]

The full-length genome of HBoV1 was sequenced for the first time, and cloned into a plasmid. It was demonstrated that the infectious clone was capable of replicating and producing progeny virions in the absence of a helper virus in polarized differentiated human embrionic kidney cells (HEK 293). The infectious virions are able to infect polarized differentiated human airway epithelia from both apical and basolateral surfaces.[10,48,49] HBoV1 infection disrupts the integrity of HAE by disruption of the tight barrier junction and leads to loss of cilia and airway epithelial cell hypertrophy. The permissiveness of the HBoV1 infection is dependent on various steps including virus attachment, entry, intracellular trafficking, and DNA replication, and all of these steps need to be investigated.[10] It has been shown that infection caused by MVC, which is most closely related to HBoV1, triggers the intra-S-phase arrest to slow down the host cellular DNA replication and, by activation of host DNA damage response, recruit the cellular DNA replication system for viral DNA replication.[50] It was demonstrated that HBoV1 NP1 induce apoptosis and cell cycle arrest in Hela cells, suggesting that amino acids at the N-terminal domain of NP1 protein may be critical for the cell cycle arrest and apoptosis induction in mammalian cells. The apoptotic cell death is mediated by mitochondrion pathway independent of viral genome replication or viral protein expression.[49]

In general, parvoviruses replicate their genomes via a "rolling hairpin" mechanism, a variant of a "rolling circle" replication.[11] Whether the HBoV replicates by the rolling circle or alternative rolling hairpin mechanism still needs to be answered.[11] In HBoV1, head to tail concatemeric intermediates were identified instead of head to head or tail to tail, by PCR and sequencing, suggesting that HBoV1 is replicating via a "rolling circle" mechanism.[51] Whether the persistence of HBoV1 up to several months is due to persistent replication and shedding, passive persistence after primary infection or recurrent mucosal contamination, remains unknown.[3] However, the HBoV1 persistence can be explained by the long-lasting apical and basolateral shedding of the virus, as it has been shown on *in vitro* HAE cultures. It is also possible that the HBoV1 genome can be presented as an episome with prolonged gene expression and replication.[10,51,52]

Only recently has it been demonstrated that HBoV1 productively infects two commercially available cell cultures, MucilAirway HAE (MatTek Co., Ashland, MA, USA) and EpiAirway HAE (Epithelix SaRL, Geneva, Switzerland). The HBoV1 infection resulted in destruction of the epithelial tight junction, loss of cillia and enlargement of the nucleus in the infected cells, HBoV1 persistence, indicating that those two *in vitro* cell culture systems are suitable for further HBoV1 replication and pathogenesis investigation.[53]

Although HBoV1 is respiratory virus, its DNA can be found in blood of patients with severe acute respiratory infection. After infection, the IgG and IgM against HBoV1 VP2 protein can be detected in serum, showing that HBoV1 causes systemic infections. It has been shown that HBoV1 VP2 virus-like particles (VLPs) can elicit typical virus-induced immune response

involving Th1 and Th2 cell cytokines.[54,55] In immunocompromised individuals HBoV1 dissemination can occur and the HBoV1 DNA could be detected in respiratory secretions, blood, feces, and urine.[42,56]

6. WHAT ARE THE CLINICAL MANIFESTATIONS?

Since the discovery of HBoV1 in 2005, the virus was most often detected in children with acute wheezing, bronchiolitis, and pneumonia as well as in children with common cold, asthma, upper respiratory infections, and acute otitis media.[3,15,20,34,40,57–59] Prematurely born children or children with other underlying diseases exposed to HBoV1 infection often have a severe clinical course of disease, which requires treatment in an ICU with the possibility of respiratory complications and even respiratory failure.[1,6,7]

Taken together, the prevalence of respiratory manifestations in HBoV1 PCR-positive children with respiratory infections included cough 79%, fever 67%, rhinorrhea 66%, hypoxia 40%, tachypnea 35%, and wheezing 27%. Conjunctivitis, vomiting, diarrhea, and rash are present less frequently.[3,15]

In one study, the HBoV1 and HBoV2 DNA have been detected in the cerebrospinal fluid (CSF) of four out of 67 children with encephalitis.[60]

7. HOW DO YOU DIAGNOSE?

The routine laboratory diagnostics of HBoV1 infections is almost exclusively based on detection of HBoV1 DNA in respiratory samples by PCR.[3,15] Since the HBoV1 DNA in respiratory secretions can persist for a long time after primary infection, diagnostics of HBoV1 primary infection should be supplemented with the detection of HBoV1 DNA in plasma and HBoV1 serodiagnosis.[3]

7.1 Molecular Detection: PCR

Real-time PCR is a highly sensitive method and is routinely used for the detection of HBoV1 DNA in respiratory secretions and plasma. Different primers and probes have been developed for the detection of the HBoV1 genome, including the primer/probe sets for the detection of the NS1, NP1, VP1 and/or VP2 gene region, which appears to be highly sensitive and specific.[2,61–63]

Due to frequent detection of HBoV1 in samples from healthy children and the high co-detection rate of HBoV1 with other respiratory viruses in respiratory samples by PCR, the accurate HBoV1 diagnosis requires detection of HBoV1 DNA or specific IgM response in plasma samples.[19,22,26]

7.2 Serological Diagnosis: ELISA

The serological diagnosis of HBoV1 infection is based on the detection of specific IgM and IgG antibodies against HBoV1 capsid protein VP2, which is

the major component of the HBoV capsid and is recognized as the predominant antigen. Detection of IgM antibodies and/or four-fold increase in IgG titer between acute and convalescent serum indicates recent infection. It has been shown that primary infections diagnosed serologically or by the presence of HBoV1 DNA in serum have been linked to respiratory symptoms.[3,22,64] It has been shown that HBoV1 mono-infection, high HBoV1 viral load determined by PCR, and viremia are associated with respiratory tract infection.[64] In one study, 94% of wheezing children with serologically verified HBoV1 infection were viremic.[22] In wheezing children with high HBoV1 DNA load in the nasopharynx, the HBoV1 IgM or an increase of IgG was detected in 96%, compared with 38% of those with low HBoV1 DNA load.[22]

Recently, IgG avidity EIA has been set up. Since the IgG avidity increases along the maturation of B cells, one can distinguish between acute and past infections or between primary and secondary infections.[3,65] However, no commercial kits for detection of HBoV specific antibodies are available at the time of this writing.

8. HOW DO YOU DIFFERENTIATE THE DISEASE FROM SIMILAR ENTITIES?

Like other respiratory viruses, HBoV1 is reported in the context of acute respiratory illnesses, including common cold, acute otitis media, exacerbation of asthma or wheezing, bronchiolitis, and pneumonia. Although some respiratory viruses are more strongly associated with specific signs and symptoms, there is usually an overlap in the clinical presentation and it is therefore not possible to distinguish between viral pathogens.[66] When the etiology of respiratory disease needs to be resolved, the differential diagnosis list should include all well-known respiratory viruses, including respiratory syncytial virus, human metapneumovirus, rhinoviruses, influenza virus A and B, adenovirus, parainfluenza virus 1−4, enteroviruses, and human coronaviruses.[66]

9. WHAT IS THE THERAPEUTIC APPROACH?

Most HBoV infections are probably self-limiting and uncomplicated. In children with severe clinical course of the HBoV infection, supportive care is the treatment of choice. The only randomized controlled study on wheezing children with serologically confirmed HBoV1 infection found prednisolone not to be effective.[3,67]

10. WHAT ARE THE PREVENTIVE AND INFECTION CONTROL MEASURES?

The preventive and infection control measures for HBoV1 are the same as for other respiratory viruses. As these viruses infect the respiratory tract, the

viruses are disseminated into the air by coughing, and although the major mode of transmission of respiratory viruses is through large droplets, the transmission through contact and infectious respiratory aerosols of various sizes may also occur. However, adequate hand hygiene, use of medical masks and gloves, and isolation precautions are general infection control measures for all respiratory viral infections.[68]

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