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Human Metapneumovirus and Human Bocavirus in Children

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ABSTRACT: Several new viruses have recently been described in children, including human metapneumovirus (hMPV) and human bocavirus (HBoV). hMPV has been established as a common cause of upper and lower respiratory tract infections in children, often second only to respiratory syncytial virus as a cause of bronchiolitis in infants. Diagnostic tools have been developed for the clinician and effective treatment and prevention strategies are being investigated. HBoV was more recently identified. Although it was initially identified in the airway of children, high rates of codetection of other viral pathogens and detection of the virus in the stool have raised questions about the true role of HBoV as a cause of respiratory infections. A focus on epidemiology, pathogenesis, clinical features, and diagnostic techniques for hMPV and HBoV is presented. **(*Pediatr Res* 65: 78R–83R, 2009)**

Until recently, the ability to discover new viruses or even to diagnose those that had been well described has been limited. Viruses have traditionally been isolated through the inoculation of clinical specimens into cell cultures, which then develop cytopathologic effects (CPE). Cell culture is time consuming, costly, and is limited by the fact that not all viruses grow in standard cell lines. Viral antigen detection from clinical samples, such as nasal washings, is rapid but lacks sensitivity. The ability to amplify nucleic acid has revolutionized virus detection and was important in the discovery and classification of the recently described viruses, human metapneumovirus (hMPV), and human bocavirus (HBoV). Although nucleic acid amplification has enabled clinicians to diagnose a specific pathogen where testing did not previously reveal one, it has also led to the dilemma of differentiating the pathogen from an innocent bystander. The presence of nucleic acid in the airway of a child with a respiratory tract infection does not prove causation. As will be described below, the discovery of a new potential pathogen now leads to a series of studies to attempt to describe its true role as a pathogen.

Human Metapneumovirus

Discovery. In 2001, van den Hoogen *et al.* described the discovery of a virus which caused CPE in cell culture of nasopharyngeal aspirates from children with respiratory infections, but from which no known virus could be identified. Extensive investigation using techniques, such as electron microscopy and random polymerase chain reaction (PCR) amplification, led investigators to identify a new pathogen, which they named hMPV (1). hMPV is an RNA virus of the Paramyxoviridae family, Pneumovirinae subfamily, Metapneumovirus genus, and is distantly related to respiratory syncytial virus (RSV), parainfluenza viruses, measles (rubeola) virus, and mumps virus. Through serologic testing, the authors demonstrated that this was a newly discovered but not newly emerged virus.[1]

Epidemiology. The epidemiology of hMPV has been described using seroprevalence, PCR, and cell culture. The reported prevalence of hMPV varies between 2 and 20%. Variable methods of detection as well as heterogeneous patient populations greatly affect the described prevalence. One of the most comprehensive studies to date was undertaken at Vanderbilt University (Nashville, TN) (2), where respiratory samples from children with respiratory tract infections were collected and stored over a 25-y period. Two hundred and forty-eight samples from children with lower respiratory tract symptoms, and from which no other virus could be identified, were tested for hMPV by culture and PCR. Forty-nine (20%) of the 248 samples were positive for hMPV (27 by PCR alone and 22 by cell culture) and although infections occurred year-around, there was a slight predominance of infection in the late winter and spring.

As with the Vanderbilt study, most populations have been made up of patients with respiratory symptoms and often samples are only taken during times of highest respiratory virus activity. Although such methods overestimate prevalence, it is clear from numerous studies that hMPV is a common cause of respiratory tract infections in children throughout the world (3–14) and is often second only to RSV as a cause of bronchiolitis in infants (2,15). The seasonal peaks and age distribution favoring young children is highlighted by the findings by one study group that MPV accounted for 6% of all positive respiratory samples but 82% of positive samples from children in day care (16).

Further evidence supporting the importance of hMPV as a cause of childhood illness is provided by seroprevalence studies.

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Abbreviations: CPE, cytopathologic effects; **DFA,** direct fluorescent antigen detection; **FDA,** food and drug administration; **HBoV,** human bocavirus; **hMPV,** human metapneumovirus; **IgG,** immunoglobulin G, **LRI,** lower respiratory tract infection; **LRT,** lower respiratory tract; **RSV,** respiratory syncytial virus; **URI,** upper respiratory tract infection; **VLP,** virus-like particle

The majority of children are born with hMPV specific IgG (IgG), which is presumably of maternal origin, and which wanes to around 25% by 6–12 mo of age. By 5 y of age, essentially 100% of children will have neutralizing antibody to hMPV (11).

Although many viruses are classified within serotypes or serogroups based on the ability of specific antibody to prevent CPE in cell culture, the ability to distinguish between the genetic groups of hMPV using serologic assays is unreliable (17). Two distinct genetic groups, (A and B) with four distinct genetic subgroups (A1, A2, B1, and B2) of hMPV have been designated based on the sequence of the fusion (F) protein gene (18). The four different subgroups can all circulate during the same season, however the predominance of each subgroup varies from year to year (17). Group A has been associated with more severe clinical illness (19). The pathogenesis of such differences has not been described.

Pathogenesis. Although hMPV is associated with both upper and lower airway symptoms, most of the morbidity is derived from lower respiratory tract illness. Supporting the clinical findings that hMPV is often associated with lower respiratory tract disease, hMPV can be isolated from the lungs of mice after nasal inoculation (20). Histopathologic examination in the rodent model reveals peak pulmonary inflammation on day 5, corresponding to the peak viral replication. Pulmonary findings include interstitial edema and inflammatory cell infiltrates of bronchioles and alveoli. Inflammatory changes can be observed for 21 d in some cases (20).

The fusion (F) protein is genetically stable and thought to play a major role in virus neutralization and provides some cross-protection between genetic groups (18). The attachment (G) protein is genetically more variable (18). Neutralizing antibodies to both F and G proteins from each genetic group are likely needed to protect against reinfection although immunity is often incomplete or wanes over time, as multiple hMPV infections can occur in the same individual (21,22).

The cytokine cascade that follows hMPV infection consists of elevation of both TH_1 and TH_2 cytokines. When specifically compared with RSV infection, however, the cytokine profile of hMPV is unique in some aspects. First, lower levels of TH_1 cytokines (particularly interferon-γ, tumor necrosis factor-α, and macrophage inflammatory protein 1α) are observed compared with RSV infections (20,23,24). Changes in IL-6, however, have been variably reported, with both higher (23) and lower (24) levels being reported after hMPV infections in humans.

Clinical spectrum. The spectrum of illness caused by hMPV infection is similar to RSV. In the large cohort of children from Vanderbilt, cough and coryza occurred in 90% of children and 52% had fever. Lower respiratory signs, including wheezing (52%), rhonchi (20%), and rales (8%) were common (2), although it should be mentioned that this cohort of samples was selected from a group of children diagnosed with lower respiratory tract infections. Vomiting occurred less frequently with hMPV (10%) compared with RSV (31%) (2).

Lower respiratory tract infection occurs more commonly in children less than 1 y of age, children with underlying lung disease, and infants born prematurely (2,25,26). Multiple studies have reported a male to female ratio of 2:1 or higher (2,9,14,27,28). Specific diagnoses attributable to hMPV LRI include bronchiolitis (59%), croup (18%), asthma exacerbation (14%), and pneumonia (8%) (2). As with other viral illnesses, many cases are treated at home, but approximately 2% require admission and some require admission to an intensive care unit (3). An area of ongoing debate is the role of coinfection in determining the severity of disease. Coinfection with RSV and hMPV has been reported to increase the severity of the illness compared with single infections (29,30), with as much as a 10-fold increase in the relative risk of requiring mechanical ventilation during coinfections (29).

hMPV also causes rhinorrhea and cough without clinically apparent pulmonary involvement, consistent with isolated upper respiratory infections (URI). URI may be more likely to occur in children more than 1 y of age, compared with RSV (31). hMPV has generally been found to cause between 6 and 15% of URI (2,16), although one prospective study in a day-care setting identified hMPV in 82% children with URI (16).

Nearly all children have serologic evidence of infection by 5 y of age (1,32). van den Hoogen *et al*. (1) originally found no hMPV in 400 samples obtained from asymptomatic children, whereas a group led by Williams tested 86 samples from asymptomatic children found only one positive sample for hMPV (1%) (2). That hMPV is commonly found in association with respiratory illness and uncommonly identified in asymptomatic children supports its role as a true pathogen.

Reports in solid organ and bone marrow transplant patients suggest that infection with hMPV can cause significant morbidity and mortality in immunocompromised hosts. Deaths attributed to hMPV infection have occurred in hematopoietic transplant recipients (33,34) and children receiving chemotherapy for acute lymphoblastic leukemia (35). A study of 100 lung transplant patients suggested that community-acquired respiratory viruses, including hMPV, may lead to acute rejection and a bronchiolitis obliterans syndrome (36).

An area of ongoing research is the link between hMPV and CNS symptoms or disease. A case report from Germany in 2005 described a 14-mo-old boy who died of encephalitis, and from whom hMPV was identified in brain tissue by PCR and cell culture (37). Subsequent reports by various groups have described patients with encephalitis from whom hMPV was identified in throat or nasal swabs, although infection of the CNS was not established in these cases (38–40).

Diagnosis. The gold standard for diagnosis of any virus is generally growth in cell culture. Cell culture techniques are expensive, labor-intensive, and slow, leading them to be infrequently used in the clinical setting. In addition, hMPV does not readily grow in the standard cell cultures. Special techniques such as the addition of low concentrations of trypsin, and "blind" passage of inoculated cells are used to enhance the growth of hMPV, but such measures are not practiced in most clinical virology laboratories. "Shell vial" culture is a more rapid method viral culture combining centrifugation, short incubation times, and fluorescent antibody staining for the pathogen of interest. Shell vial culture is commonly used for the detection of cytomegalovirus and has been described to be sensitive and specific for hMPV (41), but is not clinically available for hMPV.

Another common method of detection of respiratory viruses is direct fluorescent antigen detection (DFA). DFA generally applies virus-specific fluorescent antibodies directly to clinical specimens (such as nasal washings), followed by fluorescent microscopy. DFA has been described to be 73% sensitive and 97% specific for the detection of hMPV (42). Although DFA is a rapid method of detection, it often lacks sensitivity, is observer dependant, and requires the presence of patient cells. Food and drug administration (FDA) approved DFA assays currently exist for RSV, influenza viruses, parainfluenza viruses, and adenoviruses. An MPV-specific DFA is currently available in Canada (Diagnostic Hybrids; Athens, OH) and FDA approval for use in the United States is expected in 2009.

The diagnosis of hMPV is most often done using PCR. As with most PCR assays for respiratory viruses there is great variability between laboratories in both extraction techniques and PCR protocols. Because of the genetic variability of RNA viruses in general, and hMPV specifically, care must be taken to select regions that are more stable for PCR primers. One such region is the N gene, for which a real-time PCR assay that detects all four subgroups was described in 2004 and is commonly used (43). Until recently, respiratory viral PCR was done only using "home-brew" assays in research or reference laboratories. The FDA recently approved a benchtop PCR assay for clinical use. Although some reference laboratories currently offer hMPV PCR, xTAG RVP (Luminex Corporation; Austin, TX) is a PCR platform that can be used in a standard clinical laboratory and detects adenoviruses, RSV, rhinoviruses, hMPV, influenza viruses A and B, and parainfluenza viruses 1, 2, and 3.

Serologic detection of respiratory viruses is rarely useful in the clinical setting, although 4-fold changes in the concentrations of virus-specific antibody over 4–8 wk can confirm an infection in retrospect. Serologic assays using traditional cell-culture–based indirect fluorescent antibody (11), baculovirus-insect cell expression systems (44), and enzyme-linked immunosorbent assays (45) have all been described, but are not routinely available.

Treatment. There are currently no approved treatments for hMPV. Typical treatment for hMPV, as with most viral respiratory tract infections, is supportive. Many children are treated with over-the-counter medications, which are generally not effective. Aside from antipyretics, cold, and cough medications are not recommended for children under 2 y of age. Hospitalized patients known to be infected with hMPV should be cohorted to prevent nosocomial transmission, but with multiple viruses causing clinically indistinguishable illness and significant numbers of patients coinfected with more than one virus, such cohorting may not be practical.

Research is ongoing to discover potential therapies and develop vaccines. Potential treatment strategies include ribavirin, immunoglobulin administration (46), and fusion inhibitors (47). Virus-specific MAb are currently available for the prevention of RSV in high-risk infants and such preventive therapy may be available for hMPV in the future.

There is no vaccine available for the prevention of hMPV infection, although given the burden of hMPV in young infants, such a vaccine could be both cost effective and life saving.

Human Bocavirus

Discovery. HBoV was discovered using a high-throughput method of total nucleic acid amplification and screening (48). In a collaboration between researchers from Sweden and Singapore, libraries of amplified nucleic acid were generated from two separate pools of patients for whom specimens were submitted for routine testing. Specimens were included regardless of other testing results. The first pool consisted of 28 samples collected between November and December of 2003 and the second pool was 20 samples collected in March of 2004. The amplification and sequencing led to the development of 343 and 309 sequenced clones from pools one and two, respectively. The majority of the clone sequences were either human or bacterial in origin. Although most of the clones matched viruses that had been previously described, a parvovirus clone, which was not parvovirus B19 was identified in both pools. Further analysis based on deduced amino acid sequences revealed that the closest apparent relatives to the newly discovered parvovirus were bovine parvovirus and canine minute virus. The new member of the Parvoviridae family, Parvovirinae subfamily was thus named bocavirus (BO for BOvine and CA for CAnine).

Epidemiology. Following the original description of HBoV, a pool of heterogeneous data has followed which has shed some light on the frequency of infection, and those at risk. One of the biggest barriers to understanding the true prevalence of infection was that serologic studies were not initially available. The easiest way to develop a serologic assay is to use the cultivated virus as the source of antigen. Because HBoV is, as yet, noncultivable, recombinant technology was needed to engineer the antigens of interest. Several groups have recently developed such techniques, and reported on the seroprevalence of HBoV.

Lin *et al*. (49) manufactured virus-like particles (VLPs) based on the VP2 gene segment, and used these VLPs as the antigenic component for an ELISA-based serologic assay. Using this assay, they found VP2-specific antibodies were present in 13% of healthy children from 13–24 mo of age, with a gradual increase to a maximum of 48% of children at 4–9 y of age. A separate group used an *Escherichia coli* expressed VP1u and VP2 in an immunoblot assay for antibody detection. They, too, described rates of HBoV VP2-specific antibody in 20–40% of children between 1 and 5 y of age (50), and concluded that the VP2 antigen is superior for serologic diagnosis of HBoV. These data are consistent with a virus that circulates in childhood, but does not suggest a universal exposure or infection in young children.

By contrast, Endo *et al*. (51) expressed the VP1 protein in an insect cell line which was permissive to the vector. The intact cells were then fixed to slides and used for indirect fluorescent antibody detection (IFA). Using the indirect fluorescent antibody detection technique, 5.6% of samples were positive at 6–8 mo of age, with a gradual increase to 100% rate of positive samples by 6–9 y of age. Although this technique was potentially more prone to bias due to subjectivity of reading slides, the rates of positive serum are consistent with other common respiratory viral infections, such as hMPV. Although

there is general agreement that anti-HBoV–specific antibodies commonly develop during childhood, the best technique for serologic diagnosis of HBoV has not yet been established.

Amplification of HBoV in the airway is currently the most common method used to detect HBoV infections. Detection of HBoV DNA in the respiratory tract does not, however, prove causation as a respiratory pathogen. Because HBoV cannot be cultured and there are no readily available or reliable alternative methods of diagnosis, data regarding the true frequency of infection based on PCR must be interpreted with caution. Although there seems to be little genetic variation within this DNA virus (52,53), no single PCR assay has been accepted as the gold standard assay, leading to more difficulty in comparing data from different groups.

Because HBoV was first identified from the respiratory tract, it was presumed that HBoV was a respiratory infection leading to a large number of studies describing the rates at which HBoV is identified in the upper airway. Although some variability exists among the studies, between 5 and 10% of children with respiratory tract infections have HBoV DNA in the upper airway. Groups from around the world have verified the worldwide distribution of HBoV (27,54–66). The majority of children in these studies have a combination of fever, with upper and/or lower respiratory tract symptoms. Although these data suggest that HBoV is a respiratory pathogen, it may also reflect the fact that having respiratory symptoms was an entry criterion for most studies.

There are few studies that elucidate the role that HBoV plays in respiratory tract infections by comparing symptomatic and asymptomatic subjects. Fry *et al.* (67) compared a cohort of children and adults that had pneumonia and compared them with age-matched asymptomatic controls. From the cohort of children less than 5 y of age, HBoV DNA was identified in 44 of 369 (12%) of symptomatic children and 2 of 85 (2%) of asymptomatic children. The suggestion that HBoV occurs rarely in asymptomatic children was strengthened by Kesebir *et al.,* (68) who compared a cohort of children from whom clinical respiratory viral testing was ordered by the treating physician to a cohort of asymptomatic controls. HBoV DNA was identified in 5% of symptomatic children and in none of the asymptomatic controls. Together, these studies strongly suggest that HBoV plays a role in at least some respiratory infections in children.

Because of our increasing array of molecular diagnostic tests for a variety of respiratory pathogens, the presence of multiple pathogens has been increasingly observed. The original description of HBoV by Allander *et al.* (48) described coinfections with other viruses in 3 of 17 samples which tested positive for HBoV. Depending on how many other assays for viral agents are applied to the study samples, codetection has been described in as many as 78% of samples testing positive for HBoV (69), and is commonly described in 50–60% of samples (61,70,71). It is unknown whether HBoV exacerbates other viral illnesses, requires other viral pathogens to cause symptoms, or is simply not a pathogen at all. The latter possibility is unlikely given the other supporting evidence.

Pathogenesis. Because there are no permissive cell lines or animal models available, the pathogenesis of HBoV has not been well described. Based on the multitude of descriptions involving patients with URI, HBoV is presumed to be a respiratory pathogen. In addition, the association with lower respiratory tract symptoms, and in particular, wheezing, has led to speculation of its role in lower respiratory tract illness. One case of an HIV-infected patient with HBoV DNA detected in a bronchoalveolar lavage specimen has been described, supporting its role in lower respiratory tract illness (72).

Detection of HBoV in fecal samples has led to speculation of fecal-oral transmission (73,74). DNAemia (*vs.* viremia, which would be defined by a positive culture) has been described, and suggests dissemination (75), although unlike human parvovirus B19, HBoV DNA could not be recovered from lymphoid, brain, or bone marrow on autopsy specimens from 24 HIV-infected and 8 HIV-uninfected individuals (76). By contrast, PCR from lymphocytes isolated from tonsillar or adenoid lymphoid tissue demonstrated the presence of HBoV in 32% of children undergoing tonsillectomy or adenoidectomy for clinical reasons (77), suggesting the possibility of persistent or latent infection.

A single publication addresses the cytokine response to HBoV infections. Nasal aspirates from patients identified with HBoV were found to have elevated TH_1 and TH_2 cytokines. Compared with children infected with RSV, those infected with HBoV had higher levels of interferon-γ ($p = 0.21$), but lower levels of tumor necrosis factor-α and IL-10 ($p = 0.006$ and 0.04, respectively) (78).

Clinical spectrum. The clinical spectrum of patients infected with HBoV is potentially biased by two findings, which are discussed above. First, many of the studies recruit patients because they have respiratory illnesses, and therefore would potentially exaggerate the true role of HBoV in respiratory illness. Second, high levels of coinfections have been described, and therefore the role that HBoV alone plays in illness can be questioned. The fact, however, remains that in the majority of studies there is a group of children who have respiratory illness in whom no other pathogen has been identified. General trends among the case reports suggest that HBoV is most often associated with upper and lower respiratory tract infections. Of the groups of symptomatic children studied, about one-quarter have upper respiratory tract infections, and a variable range from 0 to 76% have had lower respiratory tract involvement (55,57,59,68,75,76,79–86). The high occurrence of lower respiratory tract symptoms, and in particular, wheezing, has led to speculation that HBoV may be a significant cause of asthma exacerbations (75).

One early description of HBoV included a small group of patients who had diarrhea (79). More recent reports describe HBoV DNA in 2% (73,74) and 9% (70,87) of stool samples obtained from patients with diarrhea. Once again, a high rate of detection of copathogens in the stool (56–58%) was found (70,73,74,87). There are no data available on how often HBoV DNA is found in the stool of asymptomatic individuals.

Cases of HBoV identified in immunocompromised patients have been described. The symptoms occurring in such cases include fever (88), upper and lower respiratory tract symptoms (89), seizures (89), gastrointestinal symptoms (89), and hep-

atitis (90). The role of HBoV as a sole pathogen in such cases is difficult to prove beyond doubt.

Diagnosis. Currently, the most common technique for diagnosis of HBoV is PCR, which is only available in research laboratories. There are no standardized or FDA-approved assays for HBoV and the best primer set and methodology has not been definitively established. Allander *et al.* (75) were able to correlate the quantitative viral load in the airway with viremia and detection as a sole pathogen. Serologic assays have been difficult to establish, but as described above, some progress has been made. It is likely that HBoV PCR will be available to the clinician through reference laboratories in the near future, so awareness of the potential pitfalls of HBoV detection will be important for the clinician. Because the mere presence of HBoV in the airway may not predict its role as a pathogen, the best method to establish HBoV as the pathogen in patients may involve quantitative PCR, serologic testing, or both.

Treatment. There are currently no treatments available for HBoV. Given the lack of cultivability, high frequency of detection as a copathogen, and relatively benign course in most patients, specific treatments will not likely be available soon.

Conclusion

hMPV and HBoV are newly described viruses associated with respiratory illness. The discovery of new viral pathogens will continue to occur as molecular methods become more sophisticated. With the discovery of each new virus, establishing its role as a true pathogen will need to follow. Although hMPV has been well described as a respiratory pathogen, the challenge of developing treatments and preventive measures remains. Models for pathogenesis have been difficult to develop for HBoV and will be needed to elucidate its true role as a pathogen in the respiratory and gastrointestinal tracts.

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