2.1 HUMAN METAPNEUMOVIRUS

Kim et al. (2011) collected paired nasopharyngeal (NP) and oropharyngeal (OP) swabs separately from pediatric and adult patients with influenza-like illness or severe acute respiratory illness in Kenya. The specimens were tested for eight respiratory viruses by real-time reverse transcription-PCR. Overall, OP swabs were significantly more sensitive than NP swabs for adenovirus (72.4% vs. 57.6%) and 2009 pandemic influenza A (H1N1) virus (91.2% vs. 70.4%). NP specimens were more sensitive for influenza B virus (83.3% vs. 61.5%), parainfluenza virus (PIV) 2 (85.7%, vs. 39.3%), and parainfluenza virus 3 (83.9% vs. 67.4%). The two methods did not differ significantly for hMPV, influenza A (H3N2) virus, parainfluenza virus 1, or RSV.

Mizuta et al. (2013) collected nasopharyngeal specimens from patients with ARIs and isolated the virus using a microplate method involving 7 cell lines. They isolated hMPV, along with other viruses which showed clear yearly seasonal pattern. RSV, hMPV, and hPIV3 were commonly isolated in 12.0-13.1% of specimens from children aged less than 4 years, whereas FluA was isolated in 7.3-8.2% of specimens from school-aged children.

Jain et al. (2014) identified the epidemiology and prevalence of hMPV and RSV in patients presented as severe acute respiratory illness (SARI) in a tertiary care hospital. Overall positivity of hMPV was 3.63% (5.1% in <5 years and 5.08% in 6-12 years) and genotype B (68.8%) was predominating. Adults having underlying chronic obstructive pulmonary disease were more prone to acquire RSV and hMPV infections. RSV and hMPV positivity was restricted to winter season. hMPV has emerged as an important cause of SARI in children <12 years of age. Alternative predominance of RSV and hMPV was an important observation.

Uyar et al (2014) determined the frequency of a broad panel of respiratory viruses in a group of children less than 24 months of age using multiplex PCR and DNA microarray. Including the co-infections, RSV was the most commonly identified virus (33.9%), followed by influenza A [H1N1] (29%), RV (29%), hMPV (21%), PIV
(16.1%), AdV (8%), HBoV (4.8%) and EV (1.6%). Of the samples from healthy children, at least one virus was detected in 63.6% and viral co-infections were detected in 21.2% samples. Including the co-infections, the most commonly detected virus was RV (30.3%), followed by influenza A [H1N1] (18.1%), AdV (1%), RSV (12.1%) and PIV (9%), however HBoV and hMPV were not detected in the control group.

Cai et al (2014) determined the prevalence and clinical characteristics of children with viral-induced acute respiratory tract infection, in Southern China. Samples from healthy subjects were negative for any virus. Co-infection with at least two of the viral pathogens under study was observed in 6.4% of the total number of cases.

The secretions of nasopharyngeal aspirates were analyzed in children younger than 21 years with acute respiratory infections with cancer and acute respiratory tract infection (ARI) and/or fever. The rapid test was used for detection of influenza virus and real-time multiplex PCR for detection respiratory viruses. Co-detection occurred in 19% of cases with 2 viruses and in 3% of those with 3 viruses, and was more frequent between rhinovirus and coronavirus (Benites et al. 2014).

In a recent study by Serin et al. (2014) in Turkey, rhinovirus was reported as most common viral agent (20%) among hospitalized community acquired pneumonia cases. Recently identified viruses, human coronavirus HKU1 and human bocavirus were not detected except one case of human metapneumovirus. Liu et al (2014) analyzed the characteristics of 17 common respiratory pathogens in children (≤14 years old) with ARI in Guangzhou, southern China over a 3-year period using real-time polymerase chain reaction. Pathogens were identified in 55.7% patients, and the positivity rate varied seasonally. When ranked according to frequency of occurrence, the pattern of co-pathogens was similar to that of the primary pathogens (RSV, Influenza), with the exception of HBoV, HCoV and hMPV.

Williams et al. (2010) conducted a 2 year population-based prospective surveillance of hMPV infection among hospitalized children <5 years old with ARI or fever. Nasal and throat specimens obtained with swabs were tested for hMPV by real-time reverse-transcription polymerase chain reaction and genotyped. An overall 3.8% of the
children tested were positive for hMPV and the annual rate of hMPV-associated hospitalizations per 1000 children <5 years old was 1.2. This rate was highest among infants 0-5 months old followed by children 6-11 months old. Bronchiolitis, pneumonia, and asthma were the most common diagnoses among children with hMPV infection. All four hMPV subgroups were detected.

Chen et al. (2010) described a hospital-based epidemiological study of hMPV in patients presenting to a children's hospital and showed the demographic and clinical characteristics associated with hMPV infection. RT-PCR assay was carried out on samples collected from hospitalized children with acute lower respiratory tract infections (ALRTI). The presence of hMPV was detected in 25.9% of the children studied and it circulated year-round in the area, peaking during the winter-spring season. Younger children (aged less than 6 months) had the highest positive rate; showed similar epidemiology and clinical manifestations as for RSV and were found in high co-infections with RSV. Subgroup A2 hMPV was the most predominant genotype identified. The study indicated that hMPV was one of the major respiratory pathogens in children.

Pilger et al. (2011) studied the occurrence of hMPV and HBoV, evaluating children with suspected lower respiratory tract infection. An RT-PCR was used for amplification and detection of hMPV and HBoV and to evaluate co-infections with respiratory syncytial virus (RSV), influenza A and B, parainfluenza 1, 2 and 3, human rhinovirus and human adenovirus. Of the total nasopharyngeal aspirates tested, hMPV was detected in 14.5% of samples and HBoV in 13.2%. A unique causative viral agent was identified in 46.2% samples and the co-infection rate was 43.7%. A total of 98.3% of HBoV and 84.8% were from patients with mixed infections. The study provided evidence of frequent involvement of both viruses in children with clinical signs of acute viral respiratory tract infection, although they mainly appeared as co-infection agents.

Mammas et al. (2011) determined the incidence and clinical features of hMPV infection in infants (< 12 months), from nasal swabs collected, presenting with ARTI at a tertiary children's hospital in Greece during two winter seasons. The specimens were tested for the presence of hMPV and RSV antigens using validated ELISA. hMPV was detected in 6.3% children, while RSV was detected in 30.7% children.
Li et al. (2012) evaluated the prevalence and clinical manifestations of hMPV in immunocompetent adults with acute respiratory tract infections. An RT-PCR assay targeting the P gene was developed and used to detect hMPV in nasal and throat swabs with ARTIs. Among the total patients studied, 1.7% were positive for hMPV, of whom 28.6% were positive for hMPV A2b, 38.8% for hMPV B1, and 32.6% for hMPV B2. hMPV A1 was not detected. The hMPV prevalence was higher in the sampled elderly (3.2%) than in middle aged adults (2.0%) and teenagers (0.9%).

Yahia et al. (2012) estimated the prevalence of hPMV, its epidemiological and clinical features in infants and children with respiratory infections by RT-PCR for hMPV. The overall prevalence of hMPV infection among studied patients was 8%. The rate was significantly higher among children aged 2-24 months compared to other age groups. The prevalence of hMPV was also significantly higher among females than males (12.6% vs. 6.6%). Cough, wheezing, rhinorrhea, fever and chest wall retraction were the most frequent presentations.

Papenburg et al. (2012) comparatively assessed the environmental, host, and viral determinants for severe hMPV and RSV infections in nasopharyngeal secretions from children aged <3 years with acute respiratory infection. hMPV was identified in 19.0% of outpatient children and 9.4% of hospitalized children, second only to RSV 48.2%. Among hospitalized patients, risk factors for severe hMPV disease were female sex, prematurity, and genotype B infection. Age <6 months, comorbidities, and household crowding were risk factors for RSV hospitalization; breast-feeding and viral coinfection were protective. Age <6 months and prematurity were associated with severe RSV cases among hospitalized children.

Edwards et al. (2013) carried out a prospective, population-based surveillance for acute respiratory illness or fever among inpatient and outpatient children less than 5 years of age. hMPV was detected by means of a RT-PCR. hMPV was detected in 6% of the hospitalized children, 7% children in outpatient clinics, and 7% children in the emergency department 1% asymptomatic controls. Overall annual rates of hospitalization associated with hMPV infection were 1 per 1000 children less than 5 years of age, 3 per 1000 infants less than 6 months of age, and 2 per 1000 children 6 to 11 months of age. Children hospitalized with hMPV infection were older when
compared with those hospitalized without hMPV infection and was more likely to receive a diagnosis of pneumonia or asthma.

Hamada et al. (2014) detected, an outbreak of human metapneumovirus (hMPV) infection in a long-term care facility within 2 days after the onset of symptoms in a putative index case using a newly developed rapid test. The outbreak was almost under control with the exception of two cases of hMPV. After the immunological diagnosis and the rapid test, it was proven that 18 patients were reported positive for hMPV infections. Horthongkham et al (2014) determined the prevalence of hMPV infection in all age groups in Thailand during 2011 using real-time PCR amplifying the partial F gene. The prevalence of hMPV for all age groups was 6.3% and the highest was in children aged <2 years. Of 71 hMPV-positive patients, three (4.2%) were coinfected with respiratory syncytial virus (RSV), two with rhinovirus (2.8%), one with coronavirus (1.4%), and one with RSV and adenovirus (1.4%). Phylogenetic analysis of F gene revealed that 96.8% of hMPV detected was subgenotype B1, 1.6% was sublineage A2a, and 1.6% was A2b. Genetic variation of F gene was found to be much conserved.

Embarek Mohamed et al. (2014) estimated the prevalence of hMPV in patients with community-acquired lower respiratory infection in Egypt. The overall infection rate was 4%, while 57% of the patients were children. Sequence analysis demonstrated circulation of subgroup B viruses with predominance of lineage B2. Nucleotide sequence identity within lineage B1 was 98.8%-99.7% and higher than that in lineage B2 (94.3%-100%). Three new amino acid substitutions (T223N, R229K, and D280N) of lineage B2 were observed. McCraken et al (2014) conducted surveillance among hospitalized cases of hMPV- and RSV-ARI in Guatemala by testing nasopharyngeal and oropharyngeal swab specimens for hMPV and RSV using real-time reverse transcription-polymerase chain reaction. They observed a seasonal pattern of RSV but not hMPV. The proportion hMPV-positive was low (3%) and RSV-positive high (41%) for age <1 month, whereas these proportions were similar (~20%) by age 2 years. The annual incidence of hospitalized hMPV-ARI was 102/100 000 children aged <5 years. hMPV was reported to be a substantial contributor to ARI hospitalization in Guatemala, but hMPV hospitalizations were less frequent than RSV and, in young children, less severe than other etiologies.
Kolli et al (2014) examined BALB/c mice depleted of alveolar macrophages (AM) for disease, lung inflammation and viral replication, following infection with hMPV or RSV. hMPV infected mice lacking AM exhibited improved disease compared to AM-competent mice. In addition, AM depletion was associated with significantly reduced hMPV titers in the lungs, suggesting that hMPV required AM for early entry and replication in the lung. The study demonstrated that AM have distinct roles in the context of human infections caused by members of the Paramyxoviridae family. Le Nouen et al. (2014) used gene-deletion viruses to evaluate the role of the attachment G and small hydrophobic SH glycoproteins. They found that hMPV attachment G and small hydrophobic SH glycoproteins reduce the ability of hMPV to be internalized by macro pinocytosis into human dendritic cells (DC). This results in a reduced ability of the hMPV-stimulated DC to activate Th1-polarized CD4 (+) T cells.

The study by Nascimento-Carvalho et al. (2011) described hMPV infection among children <5 years with CAP investigating bacterial and viral co-infections. hMPV RNA was detected in nasopharyngeal aspirates (NPA) by RT-PCR. Other bacteria and viruses were detected by a panel of tests. hMPV RNA was detected in NPAs of 4.1% children, of which 36% had only hMPV infection. The disease was significantly shorter among patients with sole hMPV infection in comparison with patients with mixed. The study showed that hMPV had a crucial a role in the childhood CAP burden.

Nokso-Koivisto et al. (2012) studied the role of hMPV in acute otitis media complicating upper respiratory tract infection (URI). 7% were positive for hMPV and 3.6% were with hMPV as the only virus. Overall, 24% of URI episodes with hMPV only were complicated by acute otitis media, and was the lowest rate compared with other respiratory viruses. hMPV viral load was significantly higher in children with fever, but there was no difference in viral load in children with hMPV-positive URI with or without acute otitis media complication.

Spaeder et al. (2013) conducted a retrospective cohort study to investigate the impact of hMPV on morbidity and mortality outcomes in children with severe viral respiratory infection. The median length of hospital stay was 7 days, ICU stay was 4 days and 9% of the hMPV positive patients did not survive to discharge. Predisposing
factors associated with increased mortality included female gender, presence of a chronic medical condition, and hospital acquisition of hMPV infection. Ali et al (2013) determined the role of hMPV, influenza A virus and respiratory syncytial virus (RSV) in children, aged 6 weeks to 2 years, hospitalized with WHO defined severe pneumonia (tachypnea plus any general danger sign or chest in-drawing) in Karachi, Pakistan. Throat swabs were obtained to detect respiratory viruses using real time RT-PCR and hMPV was detected in 14.2%, influenza A virus in 5.3% and RSV in 17.8% and was found to be the common cause of WHO-defined severe pneumonia in hospitalized children.

Roussy et al (2014) evaluated the importance of viral load along with other factors in hMPV disease severity from nasopharyngeal aspirates among children with lower respiratory tract infections, aged <3 years old. Of the 118 hMPV cases 60 belonged to genotype A and 58 to genotype B. Baseline characteristics were similar in hMPV-A and hMPV-B mono-infected patients. In multivariate analysis, hMPV hospitalization was found associated with viral load ≥1000copies/10^4 cells, age <6 and presence of ≥3 children in the household. A high hMPV viral load was also found to be associated with pulmonary rales, use of bronchodilators and inhaled corticosteroids.

Jokela et al. (2010) designed a sensitive real-time RT-PCR assay for the detection of RSV and hMPV in comparison with direct fluorescent assay (DFA) and to determine the incidence of hMPV and RSV as causative agents of respiratory infections. The duplex real-time RT-PCR assay achieved a sensitivity of 10^3 copies/mL of specimen for RSV and hMPV type A viruses and 10^4 copies/mL for type B hMPV. The detection rate of the RT-PCR assay was compared with those for DFA detection of hMPV and RSV. Of the samples analyzed, 12.3% were positive for RSV by DFA and an additional 13 specimens 3.7% were positive for RSV by RT-PCR. Only 1.1 % were found to be positive for hMPV RNA by RT-PCR, with two of them also positive by DFA. The duplex real-time RT-PCR assay described in the study was found to be sensitive for the identification of hMPV and RSV in clinical specimens.

Matsuzaki et al. (2010) compared the results of virus isolation using Vero E6 cells with real-time RT-PCR for the detection of hMPV from the nasopharyngeal swabs specimens obtained from patients with acute respiratory infection. hMPV was found
to be positive in 19.2% by cell culture and 27.7% by real-time RT-PCR. The sensitivity and specificity of the culture was 67.7% and 99.4%, respectively. The sensitivity of the cell culture was 76.2-87.5% when specimens were collected within 3 days after the onset of symptoms, and the sensitivity decreased to 50% or less thereafter. Among specimens collected within 3 days after symptom onset, all of the real-time RT-PCR positive specimens having a viral load of more than $1.25 \times 10^5$ copies/ml were found positive by cell culture.

The study by Pancer et al. (2011) established the usefulness of ELISA test in detecting hMPV antigen. Nasopharyngeal swabs from children and adults with respiratory tract infections were examined by ELISA method. hMPV antigen was detected in 24.5% of all swabs. Infection due to hMPV was found in 26.5% of children and 24.0% of adults with recognized pneumonia, respectively in 28.4 and 17.6% of patients with bronchitis. Bronchiolitis was diagnosed in two children with hMPV. Co-infections were confirmed in 8% of pneumonia, 11% of bronchitis and 24.2% of the rest concomitant diagnoses. The study found that hMPV infection was a significant agent of pneumonia not only in children but also in adults. The study concluded that ELISA hMPV antigen test can be used in diagnosis of etiological agent of respiratory infections in children and adults and in co-infections as well.

Klemenc et al. (2012) developed a broadly reactive real-time RT-PCR assay for rapid, sensitive, and specific detection of hMPV. Three previously described hMPV assays were modified based on analysis of multiple hMPV sequences obtained from GenBank. Original and modified assays were tested against prototype hMPV strains from each genetic sublineage, multiple isolates of hMPV from different years, a collection of clinical specimens, and commercial validation panels. The modified assay was found to be more effective in the detection of hMPV. Wang et al. (2012) developed a visual RT-LAMP assay for the detection of N gene of hMPV. A total of 13.1% were positive for hMPV by RT-LAMP, but 10.2% were positive by RT-PCR. The detection limit of the RT-LAMP assay was approximately lower (10 viral RNA copies) than traditional RT-PCR (100 RNA copies). There was no cross reactivity observed with other respiratory viruses.
Zhou et al. (2013) established a rapid neutralization assay based on a recombinant virus expressing Renilla luciferase (Rluc). A recombinant hMPV expressing both Rluc and green fluorescent protein (GFP) was created by reverse genetics method. The novel assay was completed within 24 h and eliminated multistep replication in cultured cells and laborious processes including the plaque assay with immunostaining. Neutralization titers correlated well with those determined by other methods. Song et al (2014) developed an RT-LAMP assay targeting the M gene of hMPV for detecting and identifying hMPV genotypes A and B. The detection limit of the genotype-specific hMPV RT-LAMP assay was 10 times greater than that of conventional RT-PCR. Clinical specimens (n=115) were detected for hMPV genotypes A and B with RT-LAMP, RT-PCR and real-time SYBR PCR. Compared with RT-PCR and real-time SYBR PCR, the genotype-specific RT-LAMP showed better specificity, sensitivity and was more convenient to perform with reduced turn-around time.

Brotons et al (2014) evaluated the diagnostic performance of the 2-photon excitation-based mariPOC© Assay (ArcDia Laboratories, Turku, Finland) for antigen detection of respiratory viruses versus real-time PCR on nasopharyngeal samples from pediatric patients with suspicion of acute respiratory infection. Sensitivities and specificities of the mariPOC Assay were for RSV, 78.4% and 99.2%; influenza virus A, 66.7% and 99.6%; influenza virus B, 63.6% and 100.0%; hMPV, 60.0% and 100.0%; adenovirus (ADV), 12.5% and 100.0%, respectively. The mariPOC Assay was reported to be highly specific method for simultaneous detection of 8 respiratory viruses but has sensitivities that range from moderately high for RSV to moderate for influenza and hMPV and low for ADV.

Pitoiset et al. (2010) analyzed the distribution of the A and B genotypes over 7 years and investigated a possible association between hMPV genotypes and disease severity. hMPV isolates were genotyped from children <3 years old. Phylogenetic analysis indicated a change in the distribution of hMPV genotype over the years. The hospitalization rate was greater when genotype B was involved 72.5% versus 53.3%. The study showed that clinical severity was not clearly associated with hMPV genotype. Moattari et al. (2010) studied the relationship between asthma and wheezing with hMPV in hospitalized children. Nasal pharyngeal swabs obtained from
children aged 1-60 months, hospitalized during a one year period, were tested for the hMPV by RT-PCR. hMPV was detected in 16.6% of patients suffering from wheezing. In addition to wheezing, 10.8% of the patients had asthma.

The study by Banerjee et al. (2011) identified and characterized hMPV in children with acute respiratory infection in India. By RT-PCR, hMPV was detected in 3% of the nasopharyngeal samples from children with acute respiratory infection. Most hMPV detections were during the winter and spring seasons. The majority (67%) of children positive for hMPV were within 24 months of age. Phylogenetic analysis of partial F and N gene and the full G gene sequences showed three sub-lineages of hMPV circulating during the study period, B1, B2, and the novel sub-lineage A2b. The circulation pattern of hMPV genotypes varied by season. Sequence analysis also revealed the F gene was relatively conserved whereas the G gene was more variable between the A and B lineages. The study demonstrated that hMPV as an important contributor to acute respiratory infection in children in India, resulting in both outpatient visits and hospitalizations.

Nasopharyngeal aspirate samples negative for common respiratory viruses were tested for the presence of hMPV RNA by RT-PCR (Apostoli et al. (2012)). Among the total samples, 9.3% were positive for hMPV. All four hMPV subtypes were identified, including the proposed subtype A2 sublineages "A" and "B". Qaisy et al. (2012) diagnosed nasopharyngeal aspirates from children ≤ 13 years old hospitalized with lower respiratory tract infection. hMPV was detected by RT-PCR, cloned and the positive samples were sequenced. hMPV was detected in 12.7%. hMPV A and B were detected in 93% and 28.6% respectively. Co-infection with hMPV A and B was detected in 21.4%. The main symptoms in patients infected with hMPV were cough 92.9%, fever 82.1%, and wheezing 78.6% and hMPV was seasonal in distribution. Most infections with hMPV were reported in the late winter and early spring.

Nakamura et al. (2013) investigated the molecular epidemiology of hMPV infections in acute respiratory infections (ARI), and performed genetic analysis of the F gene in hMPV these. hMPV was detected in 7.2% and subgroups A2, B1 and B2 were detected on subsequent phylogenetic analysis. The prevalence of hMPV was peaked between January and June. A high degree of nucleotide identity was seen among
subgroup A2 strains (95.6-100%) and subgroup B2 strains (97.5-100%). In addition, no positively selected sites (substitutions) were found in the F gene in these hMPV strains. Velez Rueda et al. (2013) tested nasopharyngeal aspirates from pediatric patients with moderate and severe acute lower respiratory tract infections by real time RT-PCR for hMPV. Results showed that 14.65% were positive. The phylogenetic analysis of the sequences of the G and F genes showed that genotypes A2 and B2 co-circulated during 2009 and 2010 and that only genotype A2 circulated in 2011 in Argentina. The selection pressure analysis of the F protein showed that although this protein has regions with polymorphisms, it has vast structural and functional constraints.

In the study by Matsuda et al. (2013) hMPV was detected from the nasal (and some pharyngeal) swabs from hospitalized patients. Based on phylogenetic analysis of the viral genomes, the virus was grouped into subgroups A2 and B2. There were decreased lymphocytes and increased monocyte counts in the blood in 59% of the patients and 65% had elevated C reactive protein levels and fever. Hamada et al. (2014) detected an outbreak of human metapneumovirus (hMPV) infection in a long-term care facility within 2 days after the onset of symptoms. The outbreak was almost under control with the exception of two cases of hMPV. According to an immunological diagnosis as well as the rapid test, it was eventually proven that 18 patients had hMPV infections.

In the study by Reiche et al. (2014) hMPV was detected in 3% of samples from outpatients with influenza-like illness and 11.9% from children below the age group of 4 years using a real-time RT-PCR assay. Investigation of outpatients revealed that hMPV infections occurred in individuals of all ages but were most prevalent in children (0-4 years) and the elderly (>60 years). The most present clinical features of hMPV infections were cough, bronchitis, fever/shivers and pneumonia. Molecular characterization of hMPV revealed a complex cyclic pattern of group dominance where hMPV subgroup A and B viruses predominated in general for three consecutive seasons. German hMPV represented all genetic lineages including A1, A2, B1, B2, sub-clusters A2a and A2b Two newly emerging amino acid substitutions (positions 223 and 280) of lineage B2 were detected in seven German hMPV sequences.
Legrand et al. (2011) performed an epidemiological and phylogenetic study on children admitted to hospital with an acute lower respiratory tract infection (LRI). Nasal swabs were tested by a direct immunofluorescence assay (DFA) and RT-PCR common respiratory viruses. An RT-PCR designed for the M gene was performed on negative samples for hMPV detection and phylogenetic analyses. For the three consecutive winters, hMPV was detected in 10%, 22.6%, and 8.8% of virus-negative samples, respectively. In most cases, clinical symptoms indicated a LRI with a final diagnosis of bronchiolitis. Dokos et al. (2013) reported the occurrence of fatal lower respiratory tract disease associated with hMPV infection in a 10-year-old girl with chronic graft-versus-host disease following allogeneic hematopoietic stem cell transplantation (HSCT) for secondary chronic myeloid leukemia.

Souza et al. (2013) investigated the infections caused by hMPV in two groups of patients admitted to hospital: Immunocompromised patients with a potential risk of severe outcomes and immunocompetent patients with severe acute respiratory syndrome. hMPV was detected in 14.2% of all samples: 17% of immunocompetent patients with suspected H1N1 infection and 10.6% among hematopoietic stem cell transplant recipients. hMPV accounted for 12.1% of immunocompetent adults patients with severe respiratory infections. Two hMPV subtypes were identified, A2 (26.9%) and B2 (73.1%) but no difference was observed between the patient groups in terms of age or immunosuppression level. Hahn et al. (2013) carried out a retrospective cohort study among children aged 0–15 years hospitalized over a 3-year period. Pulmonary diseases were associated with all outcomes of care, while congenital heart disease (CHD) and neuromuscular disorders were associated with longer LOS, and CHD and trisomy 21 were associated with worse severity scores independent of other co variables. Fever, retractions, use of steroids and albuterol were also associated with enhanced disease severity.

Ali et al. (2013) retrospectively examined children diagnosed with cancer whose nasopharyngeal swabs were positive for hMPV by direct fluorescent testing over a 5-year period. Infection was confined to the upper respiratory tract in 53.3% of the children, whereas lower respiratory tract infection occurred in 46.7%. Renaud et al. (2013) carried out a retrospective cohort analysis in hematopoietic cell transplantation (HCT) patients with hMPV or respiratory syncytial virus detected in bronchoalveolar
lavage samples by reverse transcription PCR to determine disease characteristics and factors associated with outcome. Mortality rates at 100 days were 43% for both hMPV and respiratory syncytial virus lower respiratory tract disease.

Eggleston et al. (2013) evaluated the risk factors, clinical courses and outcomes of severe hMPV disease relative to severe RSV in children ≤18 years admitted to the PICU with acute respiratory tract infection. They concluded that the children admitted to the PICU with hMPV were significantly older and more likely to have congenital heart disease than those with RSV; the course of illness was similar between the 2 groups. Ryder et al. (2010) generated a recombinant form of G ectodomain (GDeltaTM) from mammalian cells, purified and tested its immunogenicity in cotton rats. Animals were immunized with PBS, GDeltaTM alone or adjuvanted, or were infected once with hMPV, and challenged with live hMPV Animals vaccinated with adjuvanted and non-adjuvanted GDeltaTM developed high levels of serum antibodies to both recombinant and native G protein but failed to develop neutralizing antibodies and were not protected against virus challenge.

Scagnolari et al. (2011) determined whether in vitro hMPV is sensitive to the antiviral activity of IFN-β, leukocyte IFN-α, and several IFN-α subtypes in a human Hep-2 cell line. The results showed that 50% inhibitory concentration values against hMPV for the various type I IFN preparations were significantly higher than those against the IFN-sensitive vesicular stomatitis virus, and some IFN-α subtypes appeared to be more active against hMPV than others, with IFN-α subtypes 5, 6, 8, and 10 being the most potent, and IFN-α2, 17, and 21 the least potent. The results showed that hMPV grown in Hep-2 is partially resistant to the antiviral activity of type I IFNs.

Talaat et al. (2013) characterized the infectivity and immunogenicity of rhMPV-SHs a recombinant hMPV (rhMPV) in healthy adults based on a biologically derived wild-type hMPV strain to determine whether it would be suitable for use as the parent virus for the development of live attenuated rhMPV vaccines. Healthy adults were inoculated intranasally with plaque-forming units of rhMPV-SHs. Neutralizing antibody responses, serum immunoglobulin G and A, and nasal wash specimen immunoglobulin A antibody responses to the hMPV F protein were measured. Induction of nasal cytokines was assessed with electrochemiluminescence assays. A
total of 43% were infected with challenge virus as determined by virus detection and/or ≥4-fold rise in serum antibody titers. Based on the results they concluded that rhMPV-SHs virus is infectious and is a suitable parent virus for development of live-attenuated hMPV vaccine candidates.

Palavecino et al. (2014) identified that immunization with rBCG strains expressing the phosphoprotein from hMPV can induce protective Th1 immunity. Mice immunized with rBCG were protected against weight loss, airway inflammation, and viral replication in the lungs after hMPV infection. The rBCG vaccine also found to induce the activation of hMPV-specific T cells producing IFN-γ and IL-2, which could protect from hMPV infection when transferred to recipient mice. In a study reported by Wei et al. (2014), (i) α5β1 and αv integrins were found essential for cell-cell fusion and viral replication, (ii) the first two residues in the RGD motif were found essential for fusion activity, and (iii) inhibition of the interaction of the integrin-RGD motif was proposed as a new target to rationally attenuate hMPV for the development of live attenuated vaccines.

Ren et al. (2014) suggested that the M2-2 receptor inhibits innate immunity in human dendritic cells. They also identified the domains of M2-2 responsible for the immune inhibitory function. hMPV contributes to immune evasion by inhibiting MyD88-dependent cellular responses in human dendritic cells. Cox et al. (2014) generated hMPV virus like particles (VLPs) by expressing the viral matrix (M) and fusion (F) proteins in mammalian cells. They found that mice immunized with VLPs induced a neutralizing-antibody response that was enhanced by the addition of adjuvant. Two doses of VLPs conferred complete protection against hMPV replication in the lungs of mice. These results suggested that non replicating VLPs are a promising vaccine candidate for hMPV.
2.2 HUMAN BOCAVIRUS

Pedrosa-Corral et al. (2011) analyzed the impact of HBoV in outpatients and in patients hospitalisation or emergency attention for acute respiratory infections. Respiratory viruses were investigated by real-time PCR, direct antigen detection and/or viral culture by shell-vial assay. Nasopharyngeal aspirates, BAL and/or sputum samples and nasal/throat swabs from outpatients were used. Respiratory viruses were detected in 47% of the samples. HBoV detection rate was 12.6%, only preceded by RSV (25%). Co-detections were observed in 12.9% of samples, and HBoV was present in 81% of them. Higher HBoV loads were observed in children, and in patients. Pilger et al. (2011) studied the occurrence of hMPV and HBoV in Brazil, among children with suspected LRTI. Among the nasopharyngeal aspirates tested, hMPV was detected in 14.5% of samples and HBoV in 13.2%. For HBoV, 98.3% of all positive samples were from patients with mixed infections. Similarly, 84.8% of all hMPV-positive results were also observed in mixed infections. Both HBoV and hMPV appeared with RSV. The study demonstrated that there was frequent involvement of both hMPV and HBoV viruses in children with acute viral respiratory tract infection, and they mainly appeared as coinfection agents.

Uršič et al. (2012) assessed the prevalence of HBoV, and other respiratory viral pathogens, in a 2-year retrospective study of children and investigated whether viral loads of HBoV DNA were associated with severity of infection. Respiratory samples were tested for the presence of respiratory viruses by real-time PCR or direct immunofluorescence testing. The virus detected most frequently was rhinovirus, followed by respiratory syncytial virus, HBoV, and hMPV. HBoV DNA was detected in 18.4% of children. HBoV was the only viral pathogen detected in 40.2% of HBoV DNA-positive children and in 7.4% of the total samples. 97% of children with an HBoV single infection were diagnosed as having lower respiratory tract infection. HBoV DNA viral load was higher in children when HBoV was detected as a single pathogen. Higher HBoV DNA viral loads were associated with prematurity and age.

Nunes et al. (2014) determined the prevalence and clinical characteristics of HBoV, humanrhinovirus (hRV), polyomavirus-WU (WUPyV) and -KI (KIPyV) and human coronaviruses (CoV)-OC43, -NL63, -HKU1 and -229E by multiplex RT-PCR from
HIV-infected and –uninfected children (<2 years age) hospitalized with LRTI. At least one of the viruses were identified in 53.0% and 54.0% of HIV-infected and -uninfected children, respectively. Human rhinovirus was the most prevalent in HIV-infected 31.7% and-uninfected children 32.0%, followed by CoV-OC43 12.2% and HBoV 9.5% in HIV-infected; and by HBoV 13.3% and WUPyV 11.9% in HIV-uninfected children. Respiratory viruses were identified in 60.9% of HIV-infected and 78.3% of HIV-uninfected children. The study established that at least one respiratory virus was identified in the majority of HIV-infected and HIV-uninfected children hospitalized for LRTI.

The study by Song et al. (2010) detected HBoV1 and HBoV2, in 7.6% and 4.3% of the total nasopharyngeal aspirates, respectively, from children hospitalized with acute respiratory tract infection. The results suggested that, like HBoV, HBoV2 was distributed worldwide and may be associated with respiratory and enteric diseases. The epidemiological and virological characteristics of HBoV infection in children with acute respiratory tract infection were studied by Zheng et al. (2010). HBoV was detected 7.1% of the study subjects. Among the children infected with HBoV, 55.2% were coinfected with other respiratory viruses, most commonly respiratory syncytial virus (RSV). The most common clinical features were cough and acute upper respiratory infection, and acute bronchopneumonia. The NP-1 gene of HBoV showed minimal sequence variation.

Nadji et al. (2010) analyzed the frequency and phylogeny of HBoV in the respiratory and stool samples of children with acute respiratory tract illnesses and gastroenteritis in Tehran, Iran. Respiratory and stool samples were screened for HBoV by nested PCR amplifying the NS-1 gene. Among the total respiratory samples 6.8% and 12.8% of the stool were positive for HBoV. Martin et al. (2010) performed HBoV testing on nasal swab samples from a prospective, longitudinal study of respiratory illness. HBoV was detected in 59% of children and in 33% of illness. Another virus was detected in 72% of HBoV-positive cases. HBoV was detected in 44% of asymptomatic samples, and HBoV prevalence and viral load did not differ significantly between children with and children without symptoms. HBoV-positive illnesses were longer than HBoV-negative illnesses.
Salmón-Mulanovich et al. (2011) estimated the frequency of HBoV infection among pediatric populations. A cross-sectional study was performed using stored samples of an influenza-like illness surveillance program. Nasopharyngeal or nasal swab specimens were randomly selected and tested using real-time PCR from patients younger than 6 years old. The prevalence of HBoV was 10.8% in Argentina, 33.3% in Nicaragua and 25.1% in Peru. The findings demonstrated the circulation of HBoV in Argentina, Nicaragua and Peru as a respiratory pathogen among children.

Kim et al. (2011) investigated the prevalence of HBoV infection and the association between viral load and clinical features of the infection from nasopharyngeal aspirates of patients in all age-groups using real time PCR. Detection rate of HBoV was 4.8% with peak incidence of infection being observed in patients aged 6-12 months. HBoV was co-detected with other respiratory viruses in 18.3% of the HBoV-positive patients. Patients infected with HBoV alone showed a higher viral load than those patients in whom HBoV was co-detected with other respiratory viruses. Kantola et al. (2011) investigated HBoV1-4 seroepidemiology among adults and wheezing children, by enzyme immunoassays with recombinant virus like particles (VLPs). The sera were also tested for HBoV1-4 DNA by quantitative PCR. The seroprevalences of HBoV2-4 in adults were 34%, 15%, and 2% and in children aged 1-2 years 25%, 10%, and 5%, respectively.

Liu et al. (2011) analyzed the characteristics of HBoV-positive samples from ARTI patients with a wide age distribution from southern China. Throat swabs were collected and analyzed from children and adults with ARTI. HBoV DNA was detected in 2.3% samples. Seasonal peaks of 4.8% and 7.7% were detected in May and June, respectively. 43.1% HBoV-positive samples were co-detected with other potential pathogens. In the serological survey performed by Guido et al. (2012) the prevalence of antibodies against HBoV was determined. Anti-HBoV IgG antibodies were analysed using a standardized ELISA test based on the use of recombinant HBoV VP2 virus-like particles. Among the total number of cases 89.1% displayed anti-HBoV-IgG. The seroprevalence was significantly increased in children from 2-4 years (64.2%) to 5-9 years (96.4%).
Ghietto et al. (2012) investigated the prevalence of HBoV in children with lower acute respiratory infection from nasopharyngeal aspirates. The general prevalence of HBoV was 21.5% and the positive cases (HBoV+) were more frequent during winter and spring. Among the HBoV positive cases the prevalence of HBoV was 26.3% in patients < 3 months of age, 22.1% in 3 to 6 months, 25.3% in 6 to 9 months, and 18.8% in 9 to 12. The phylogenetic analysis of the NP1 region confirmed that the isolates were HBoV1. In the study by Koseki et al. (2012), HBoV 1, 2, 3, and 4 were detected in 15.5%, 0.6%, 0.4%, and 0.6% of total nasopharyngeal swab samples collected from children with respiratory tract infections, respectively.

Arnott et al. (2012) investigated the prevalence and genetic diversity of HBoV amongst hospitalized patients with ALRI by multiplex RT-PCR/PCR for 18 respiratory viruses. Of the samples tested, 1.5% were positive for HBoV and HBoV infection was detected year round. The incidence of HBoV infection was highest in patients aged < 2 years. Pneumonia or bronchopneumonia was the most common clinical diagnosis, regardless of age. All isolates were classified as HBoV type 1. A total of 44% were co-infected with other respiratory pathogen. In the study Guo et al. (2012), the prevalence of specific anti-VP2 IgG antibodies against HBoV1-4 was determined in different age groups of healthy individuals aged 0-70 years old in China, using a competition ELISA assay based on virus-like particles of HBoV1-4. The seroprevalence of HBoV1-4 was 50%, 36.9%, 28.7%, and 0.8%, respectively, in children aged 0-14 years; whereas the seroprevalence of HBoV1-4 was 66.9%, 49.3%, 38.7% and 1.4%, respectively, in healthy adults (≥ 15 years old). The data indicated that HBoV1 was more prevalent than HBoV2, HBoV3, and HBoV4 in the study population.

Xu et al. (2012) investigated the epidemiological and genetic variation of HBoV in South China by screening throat swab samples from children and adults with acute respiratory infection. The specimens were screened for HBoV by real-time PCR and other 6 common respiratory viruses by RT-PCR or PCR. HBoV was detected in 1.68% samples, mostly from pediatric patients and inpatient children. Of these HBoV positive cases, 32.76% had co-pathogens. Phylogenetic analysis showed that the HBoV strains were HBoV1, and were most genetically close to ST2.
Zhao et al. (2013) conducted a 3-year prospective study on the prevalence of HBoV infection in patients with LRTI and its correlation with disease severity. Nasopharyngeal aspirates were tested by PCR for common respiratory viruses and by real time PCR for HBoV subtypes 1-4. Nasopharyngeal swabs from healthy controls and serum samples and stools from inpatients were also tested for HBoV1-4 by real time PCR. Viral loads were determined by quantitative real time PCR in all HBoV positive samples. HBoV1 was detected in 7.0% of inpatients, with annual rates of 5.1%, 8.0% and 4.8% in 2010, 2011 and 2012, respectively. High HBoV viral loads (>10^6 copies/ml) were significantly more frequent in inpatients and outpatients than in healthy controls. do Amaral de Leon et al. (2013) studied the frequency, seasonality, and clinical behavior of HBoV detection in a series of episodes from children aged 1-24 months presenting LRTI. A total of 13.2% was positive for HBoV by virus-specific PCR. Diarrhoea was present in 18% and co-detection was a frequent finding occurring in 95% of cases. The distribution of HBoV was clearly seasonal and was influenced by temperature, relative humidity, and precipitation.

The study by Misigo et al. (2014) was to primarily determine if the emerging HBoV infections exist in Kenya as coinfections with other respiratory viruses and to describe the genotype of the virus in circulation. HBoV DNA was amplified from 1.8% of screened specimens. Coinfection with, parainfluenza virus, adenovirus, and enterovirus was 2.5%, 2%, and 1.4%, respectively. Multiple coinfections consisting of HBoV plus two other viruses were found in 3% of specimens. Chen et al. (2014) studied the frequency, season, and clinical characteristics of hospitalized children <14 years of age with HBoV infection. Multi-pathogens were detected in nasopharyngeal aspirate samples. The average incidence of HBoV infection was 6.6% and the rate was highest among the 7-12 months groups (12.9%). They observed that HBoV infection occurs throughout the year with a peak during the summer.

Sun et al. (2013) showed that the NP1 of HBoV1 induced apoptotic cell death in HeLa cells in the absence of viral genome replication and expression of other viral proteins. They also demonstrated that the cell cycle of NP1-transfected HeLa cells was transiently arrested at G2/M phase followed by rapid appearance of apoptosis and that the N terminal domain of NP1 was critical to its nuclear localization and function in apoptosis induction in HeLa cells.
Wang et al. (2010) studied the clinical and virological prevalence in HBoV-positive outpatient children, and also determined the genetic and serologic characteristics of HBoV in China. Nasal/throat swabs and sera were obtained from children with respiratory tract infection and examined the presence of HBoV and its co-infection. The seroepidemiology of HBoV was studied by ELISA and Western blot. HBoV was identified in 11.8% of the total number of cases. The co-infection rate with other respiratory viruses was 51%. IgM was detected in 55.7% of HBoV RT-PCR-positive patients, and in 72.7% of those who had high viral genome load.

Zaghoul (2011) detected HBoV in NPA of infants by qualitative PCR and also estimated antibodies in serum by ELISA. Among the total number of patients 22% were positive for HBoV by qualitative PCR, while ELISA HBoV IgM antibodies were present in 18% patients who were also positive by PCR. None of the controls were positive by both techniques. The study demonstrated that ELISA was less sensitive than RT-PCR but can be a useful serological tool for the diagnosis of acute HBoV infection by estimation of IgM antibodies in serum. Nascimento-Carvalho et al. (2012) described acute HBoV infections among children hospitalized for community-acquired pneumonia. Paired serum samples were tested by IgG, IgM, and IgG-avidity enzyme immunoassays (EIAs) using recombinant HBoV VP2. HBoV DNA was detected in nasopharyngeal aspirates of 23% children and 57% were seropositive.

In the cross-sectional study conducted by Hustedt et al. (2012), blood samples were collected from pediatric patients and tested for the presence of HBoV-specific antibody using a virus-like-particle based ELISA. HBoV-specific antibodies were found to be present in 76.7% of samples collected from the pediatric population. Seroprevalence of HBoV was highest in those ≥2 years old. The seroepidemiological profile suggested that most children are exposed to HBoV during the first two years of life in the study population. Christensen et al. (2013) developed a qualitative RT-PCR to detect spliced mRNA from HBoV1 and to determine whether HBoV1 mRNA correlated better with RTIs than did HBoV1 DNA, using samples from HBoV1 DNA-positive children, with and without RTIs. A real-time RT-PCR, targeting 2 alternatively spliced mRNAs, was developed. HBoV1 mRNA was detected in 25% children with RTIs but in none of 28 controls.
Yu et al. (2013) determined the presence of HBoVs and corresponding HBoV-specific immunoglobulins (Igs) in CSF from children with suspected viral encephalitis. Among the total 13.4% were HBoV1 DNA-positive, while 15% was HBoV2 DNA-positive. HBoV-specific IgM and IgG antibodies were detected in the CSF samples from three children; two samples were HBoV1 DNA-positive and one sample was negative.

Zhou et al. (2014) used genomic and immunologic methods to identify the reactive epitopes of HBoV species and their potential as a novel diagnostic test for HBoVs. They mapped four epitope cultures of HBoVs by generating HBoV 1-3 VP2 gene fragment phage. Using this method they identified two immunodominant peptides P1 and P2 conserved among HBoV1-4 and the epitope immunogenicity was confirmed. These peptides were used for the evaluation of epitope-based peptide-IgM ELISA and they found that the P1+P2-IgM ELISA showed a higher sensitivity and specificity in HBoVs IgM detection than the assays using a single peptide.

Chen et al. (2014) developed a novel integrated strategy for human bocavirus detection: hemi nested PCR assay combined with boiling lysis method of samples. The detection limit of the hemi nested PCR assay was 1.2 copies of a recombinant DNA plasmid, and no cross-reaction with other respiratory viruses or bacteria was observed. The results suggested that the novel integrated strategy was a convenient, sensitive, cost-effective and reliable detective method for HBoV detection and will have broad application prospects in clinical diagnosis.

In the study by Haidopoulou et al. (2010) throat swab samples were collected from previously healthy children, aged 14 days to 13 years, admitted in paediatric wards because of respiratory tract infection. HBoV was detected in 3.2% children by PCR amplifying a part of the NS1 gene. The most common symptoms were fever, cough and various degrees of respiratory distress. All children were clinically diagnosed to have LRTI, mainly pneumonia and acute laryngotracheobronchitis.

The report by Zappa et al. (2011) described the molecular epidemiology of hMPV and HBoV infections among children hospitalized for acute respiratory tract infections. Pharyngeal swabs were collected from children ≤3 years of age and tested for
respiratory viruses, including hMPV and HBoV, by molecular methods. hMPV-RNA and HBoV-DNA positive samples were characterized molecularly and a phylogenetical analysis was performed. PCR analysis identified 54.6% samples positive for at least one virus. The frequencies of hMPV and HBoV infections were similar (8.3% and 12.1%, respectively). Both infections were associated with lower respiratory tract infections: hMPV was present as a single infectious agent in 7.2% of children with bronchiolitis, HBoV was associated with 18.5% of pediatric pneumonias and was identified frequently as a single etiological agent. Genetically distinct hMPV and HBoV strains were identified in children examined with respiratory tract infections. Phylogenetic analysis showed an increased prevalence of hMPV genotype A (A2b sublineage) compared to genotype B (80% vs. 20%, respectively) and of the HBoV genotype St2 compared to genotype St1 (71.4% vs. 28.6%, respectively).

Chuang et al (2011) conducted a prospective clinical and molecular study of HBoV from throat swabs of pediatric patients with symptoms and signs of respiratory tract infections. Among the total HBoV positive patients 76% were less than 5 years; their common symptoms were cough, rhinorrhea, and fever; the most common diagnoses were bronchitis (34%) and sinusitis (31%) followed by pharyngitis (29%) and asthma exacerbation (26%). Their study concluded that HBoV is an important pathogen associated with respiratory tract infection in children.

The study by Fu et al. (2011) performed the phylogenetic and recombination analysis of bocavirus over the complete genomes available in GenBank. The results confirmed that recombination existed among bocavirus, including the likely inter-genotype recombination between HBoV1 and HBoV4, and intra-genotype recombination among HBoV2 variants.

The study by Al-Rousan et al. (2011) investigated the prevalence of HBoV among children hospitalised with LRTI as well as the clinical feature associated with HBoV infection, the seasonal distribution of HBoV and the DNA sequencing of HBoV positive samples. HBoV was detected in 9.1% of patients and children under the age of 12 months were more susceptible to HBoV infection. The main clinical diagnoses of patients infected with HBoV were bronchopneumonia (35%) and bronchiolitis.
(30%). Coughing (100%), wheezing (82.7%) and fever (68.2%) were the most prominent symptoms in infected patients and the HBoV infections were seasonal; increasing in cooler months, diminishing in the summer. On sequencing 155 of the strains were found to be identical to Stockholm 1 and 2 isolates.

In the case study by Körner et al. (2011) reported a confirmed HBoV infection in an 8-month-old girl with hypoxia, respiratory distress, wheezing, cough, and fever. It demonstrated that lower respiratory tract infection caused by HBoV can lead to severe and life-threatening disease. Hao et al. (2013) detected 13.2% of HBoV1 positive cases by real time quantitative PCR from children hospitalized with respiratory tract infection. The number of monoinfections was 40.2% and the rest were viral coinfections. Genotyping of HBoV1-positive cases yielded 27 full HBoV1 sequences, as well as two NS1 gene sequences, 15 NP1 gene sequences and 10 VP1/VP2 gene sequences harbouring 24, 10, but it had no relationship on the clinical manifestations.

Shen et al. (2013) studied the clinical and molecular epidemiology of human bocavirus (HBoV) 1-4 in hospitalized children in Shanghai suffering from acute lower respiratory tract infection with symptoms of wheezing. Only HBoV1 was detected in 5.45% by nested PCR, was second only to RSV and was co-detected with other potential pathogens in most of the samples. The phylogenetic analysis of the VP1/VP2, NP1 and NS1 was carried and it demonstrated that the epidemic strains were clustered in one independent branch. The results demonstrated that HBoV1 as one of the common pathogens responsible for the hospitalization of children with acute lower respiratory tract infection and symptoms of wheezing.

Deerojanawong et al. (2013) evaluated the incidence and factors associated with recurrent wheezing during 1 year after HBoV LRI. Nasopharyngeal aspirates from children less than 5 years of age with LRI were subjected to PCR for the detection of respiratory viruses. 47% of the HBoV positive cases had co-infection with other respiratory viruses. Generalized wheezing was the most common lung sign detected in 73% of cases. However 27% of HBoV infected patients developed recurrent wheezing associated with respiratory tract infections. Mori et al. (2013) identified HBoV DNA by PCR in cerebrospinal fluid from adults and children with encephalitis in Sri Lanka. HBoV types 1, 2, and 3 were identified among these cases. Phylogenetic
analysis of HBoV1 strain sequences found no subclustering with strains previously identified among encephalitis cases in Bangladesh.

The study by Tran et al. (2014) described the epidemiological, clinical, and molecular characteristics of HBoV infections in hospitalized pediatric patients. Nasopharyngeal swab samples were obtained from patients with acute respiratory infections and screened for HBoV by PCR and sequenced. HBoV was found in 7.2% children and co-infection with other viruses was observed in 66.7%. Children 12-24 months old were the most affected age and HBoV was identified to be a cause of severe infection considering the higher rate of clinical symptoms. The phylogenetic analysis of partial VP1 gene showed that all HBoV sequences belonged to species 1 (HBoV1).

Genetic analysis of representative strains of hMPV and HBoV from paediatric patients with influenza-like illness or other respiratory tract infections was carried out by Pogka et al. (2014) over three consecutive winter seasons of the years 2005-2008. Representative positive samples for both the viruses were sequenced, G gene for hMPV and VP1/VP2 for HBoV. Phylogenetic analysis revealed circulation of one single lineage (B2) for hMPV viruses and predominance of ST2 genotype for HBoV viruses. Higher levels of heterogeneity were observed between hMPV compared to HBoV strains. A possible recombination between ST1 genotype strains of HBoV was observed.

Zeng et al. (2010) studied the prevalence and clinical features of HBoV in children with ARTI and explored the causative implication of HBoV in ARTI. Nasopharyngeal aspirate samples were collected and screened for seven common respiratory viruses by immunofluorescence and further tested for HBoV by PCR. HBoV was detected in 4.6% of the samples, and it was the second most commonly detected virus after respiratory syncytial virus. 19% HBoV-positive samples were dual infection with respiratory syncytial virus or parainfluenza virus type 3. Of the total children with community-acquired ARTI, HBoV was identified to be positive in 3.4%. Out of the children with nosocomial ARTI, 19.2% had bronchitis which was found to be HBoV positive without co-detection of other viruses. The major clinical features were cough (100%), wheezing (68.8%) and fever (62.5%). Thus HBoV was found to be
associated with community-acquired ARTI and plays a pathogenic role in nosocomial ARTI.

Bharaj et al. (2010) collected nasopharyngeal aspirates from children <5 years of age with acute respiratory tract and screened for the presence of HBoV by two separate sets of a PCR. A total of 7.2% children who had acute respiratory infection were found to be positive for HBoV by both sets of PCR. The main clinical symptoms were cough (95%), runny nose (64%), and fever (59%). In two samples, HBoV was identified together with respiratory syncytial virus in one sample and influenza A virus in another. HBoV appeared to have no seasonal distribution and was associated with both upper and lower respiratory tract disease in young children in India. Moriyama et al. (2010) investigated distinctive clinical features of HBoV-positive children LRTI. Hospitalized children younger than 2 years with LRTI prospectively examined virus genomes in nasopharyngeal swabs for HBoV, RSV, rhinovirus, metapneumovirus, parainfluenzavirus, and adenovirus. The HBoV genomes were identified in 8.5% of patients. The minimum age of HBoV-positive patients was 5 months. The main clinical features were respiratory distress and hypoxia.

In the study of clinical specimens from young children by Kaida et al. (2010), real-time PCR was undertaken to examine whether HBoV infection is associated with RTI. Specimens were collected from patients with RTI during. Analyses revealed HBoV in 15.7%. Of HBoV-positive patients, children under the age of 3 years comprised 94.9%. Of the HBoV-positive samples, 47.5% were codetected with other respiratory viruses. The viral load was greater in subjects with infection with HBoV alone than in subjects with mixed respiratory viral infections. These results together, suggested that HBoV is probably associated with RTI in young children.

Christensen et al. (2010) investigated the presence of HBoV1 in children with URTI, LRTI and a control group of children without RTI. NPA and blood samples were collected and PCR was carried out to detect 17 infectious agents including HBoV1 from children and from children admitted for elective surgery who had no RTI. Blood samples were tested with HBoV1-PCR only. HBoV1 was detected in NPAs from 10% of patients and 17% of controls. In the HBoV1-positive NPAs, at least one other virus
was detected in 75% and the virus appeared alone in 25%. The study showed that HBoV1 was associated with RTI and LRTI.

Durigon et al. (2010) screened nasopharyngeal aspirates for hospital-acquired HBoV from infants hospitalised with respiratory infection. Among 55 children with HBoV infection, 10 cases were hospital-acquired. Compared with the community-acquired cases, coinfection with other respiratory viruses in these patients was uncommon. The study determined that HBoV should be considered for inclusion in screening protocols for nosocomial childhood respiratory infections, especially in intensive care units. Ursic et al (2011) described a case of life-threatening human bocavirus infection of a previously healthy pediatric patient. There was an initial clinical presentation of acute bronchiolitis which developed into an extremely severe course of disease characterized by pneumothorax, pneumomediastinum, and acute respiratory failure.

Human bocavirus DNA was detected by means of a quantitative, real-time polymerase PCR at low levels in the 5.51% of sera obtained from healthy blood donors by Bonvicini et al. (2011). The study suggested that viral detection in blood was not necessarily associated with disease status. Lehtoranta et al. (2012) examined the prevalence and persistence of HBoV in consecutive NPS of otitis-prone children, and whether an association exists between HBoV and the child's characteristics, respiratory symptoms, and AOM pathogens, and whether probiotics reduce the occurrence of HBoV. A high load (>10,000 copies/ml) of HBoV DNA was detected in 17.1% and 10.5% showed a prolonged presence of HBoV for at least 3 months. None had DNA of HBoV2-4. Prevalence or persistence of HBoV was not significantly associated with other characteristics, respiratory symptoms, or AOM pathogens.

Ghietto et al. (2012) detected HBoV1 in children and adults hospitalized with acute disease of the lower respiratory tract. HBoV genome was detected by PCR in nasopharyngeal swabs. HBoV was found in 22.7% patients, 64.7% of them infants younger than 1 year old and 29.4% adults older than 30 years. Of all HBoV positive cases, 35.3% were co-infected; all co-infections occurred in children (≤13 years old)
and 83.3% of them were HBoV- RSV co-infections. The rate of co-infection in infants was significantly higher when compared to the adults. The results of the study suggested that HBoV1 was involved in acute respiratory disease.

Deng et al. (2012) studied the impact of HBoV viral load on clinical characteristics in HBoV positive children who suffered severe LRTI. HBoVs were detected by real-time PCR and other 10 infectious agents were examined using PCR and/or direct fluorescent assay. Fifteen samples had a high viral load (>10^4 copies/mL) and the other sixteen samples had a low viral load (<10^4 copies/mL). The duration of presented wheezing and hospitalization was longer in children with high viral load of HBoV than that in children with low viral load.

Norja et al. (2012) determined whether the DNAs of HBoV1-4 and PARV4 persist in human tissues long after primary infection. Biopsies of tonsillar tissue, skin, and synovia were examined for HBoV1-4 DNA and PARV4 DNA by PCR. Serum samples from the tissue donors were assayed for HBoV1 and PARV4 IgG and IgM antibodies. To obtain species-specific seroprevalences for HBoV1 and for HBoV2/3 combined, the sera were analyzed after virus-like particle (VLP) competition. DNA was detected exclusively in the tonsillar tissues of 3.7% individuals, all of them ≤8 years of age. HBoV2-4 and PARV4 DNAs were absent from all tissue types. HBoV1 IgG seroprevalence was 94.9%. No subject had HBoV1 or PARV4 IgM, nor did they have PARV4 IgG. The results indicated that HBoV1 DNA occurred in a small proportion of tonsils of young children after recent primary HBoV1 infection, but did not persist long in the other tissue types studied, unlike parvovirus B19 DNA.

Sousa et al. (2012) tested faecal samples by PCR targeting the NS1 gene of HBoV DNA. Of the samples tested, 5.8% were positive for HBoV-1 or HBoV-3 and co-infection was observed in 31.8% of the 44 HBoV-positive samples. The genomic sequencing of the NS1 partial sequence HBoV-samples showed that all the samples were characterised as HBoV-1 except one which was characterised as HBoV-3. The phylogenetic analysis showed that the HBoV-1 samples had a high sequence homology to others previously identified in China, Sweden and Brazil. This was the first study conducted in the Central-West Region of Brazil to detect HBoV-1 and HBoV-3 in faecal samples from children with acute gastroenteritis.
The study by Eyigor et al. (2013) investigated whether children with middle ear effusions (MEE), adenoid and tonsil tissues are associated with HBoV using PCR. HBoV was detected in only 6 (4.8%) adenoid tissue samples each belonging to a different patient. Their results were consistent with the results of other studies, reporting approximately 5 - 10% of the samples being positive for HBoV.

In the study by Abdel-Moneim et al. (2013), swabs from children with respiratory tract infections were examined for the presence of HBoV by RT-PCR. Direct gene sequencing was used to determine the genotype of the detected virus isolates. HBoV was detected in 22.5% of the examined patients. The NP1 partial gene sequence from all patients showed that the circulated strains were related to HBoV-1 genotype. Most of HBoV infected patients showed evidence of mixed coinfection with other viral pathogens. Ricart et al. (2013) analyzed the viral load (VL) of human hMPV and HBoV in infants <12 months admitted for bronchiolitis. VL correlated with length of hospital stay in both viruses, hMPV VL with the duration oxygen therapy and human bocavirus VL inversely with days of respiratory effort before admission. Infants coinfected by other viruses were younger, but no differences were seen regarding VL.

Caccia et al. (2012) investigated the etiology of HBoV among adults and children in different groups at risk of presenting complications arising from acute respiratory infection. The HBoV DNA was detected by PCR from community and hospitalized patients. Of the 598 tested samples, 2.44% (8/328) of children, including five children with heart disease, and 0.4% (1/270) of adult bone-marrow-transplant were HBoV positive. Schildgen et al. (2013) analyzed the role of HBoV in lung and colorectal cancers. Tumor samples were screened for HBoV DNA by PCR, Southern blotting, and sequencing. Positive tissues were further subjected to fluorescence in situ hybridization analysis to specifically detect HBoV DNA in the infected cells. In total, 18.3% lung and 20.5% colorectal tumours tested positive for human bocavirus DNA by PCR and were confirmed by sequencing and fluorescence in situ hybridization analysis. The finding suggested that this virus may indirectly contribute to the development of some colorectal and lung cancers.

Feng et al. (2014) collected respiratory specimens, blood or serum collected from hospitalized ALRI patients for diagnostic testing for RSV, human influenza virus,
ADV, human parainfluenza virus (PIV), hMPV, human coronavirus (hCoV) and HBoV. RSV was most commonly identified (9.9%), followed by influenza (6.6%), PIV (4.8%), ADV (3.4%), HBoV (1.9), hMPV (1.5%) and hCoV (1.4%) and the co-infection rate was 7.2%.

In the study by Zhang et al. (2014) nasopharyngeal swabs from hospitalized patients were evaluated for the presence of respiratory viruses from children and adults with respiratory tract infections. Influenza virus, RSV, PIV, ADV, hMPV, hCoV and HBoV were detected by PCR/RT-PCR. Co-infection was also observed. In a study by Cantais et al. (2014) to detect the etiology of community acquired pneumonia ADV, RSV, hMPV, HBoV, rhinovirus/enterovirus, HCoV, influenza viruses A and B, PIV, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* were detected using biplex commercially-available RT-PCR assays. Infection with at least a single pathogen was observed in 95.3% and co-infection by at least two viral pathogens were observed in 43.5% of the study population.

Nasopharyngeal aspirate samples from pediatric patients with suspected acute respiratory tract infection and from healthy subject controls were collected by Cai et al. (2014) from children with viral-induced acute respiratory tract infection. The multiplex PCR assay was positive for at least one virus in 54.9% of the total samples and all the healthy controls were negative for any respiratory virus. Nasopharyngeal aspirate samples. The respiratory viruses identified were RSV, Flu A, HRV, HBoV, PIV3, PIV1, ADV, WU polyomavirus (WUPyV), hMPV, and Flu B. Co-infections were detected in 16.4% of the subjects. A multiplex PCR/RT-PCR assay was performed by Sentilhes et al. (2013) targeting 18 major common respiratory viruses from patients with ALRI. Viruses were detected in 55% of the samples. A single virus was detected in 48% of the total ALRI cases and co-infections were observed in 8% of the samples. The most frequent viruses were rhinovirus/enterovirus (35%), RSV (26%), and influenza viruses (13%). PIV were detected in 9%, ADV in 6%, hMPV in 4%, HCoV in 4%, and HBoV in 3% of ALRI specimens. Most viral infections occurred in patients below 5 years of age.

Bicer et al. (2013) studied the incidence and seasonal distribution of viral etiological agents using a multiplex reverse-transcription PCR from nasopharyngeal aspirate.
Viral pathogens were detected in 66.5% of patients which included RSV (32.0%), ADV (26.2%), PIV type 1-4 (19.4%), rhinovirus (18.4%), influenza A and B (12.6%), hMPV (12.6%), HCoV (2.9%), and HBoV (0.9%). Co-infections were also noticed.

da Silva et al. (2013) tested nasopharyngeal aspirates from hospitalized patients younger than 3 years of age for 13 different respiratory viruses by real-time PCR. The rate of viral detection was 85% from patients with LRTI and co-infection was observed in 65%. The viruses detected were RSV (54%), followed by hMPV (32%) and HRV (21%). They also identified HBoV in certain number of patients. Nasopharyngeal aspirates from children were subjected to RT-PCR/PCR by Xiao et al. (2013) to determine the prevalence and clinical characteristics of hMPV. Among the total samples 6.5% were positive for hMPV. The hMPV co-infection rate was 57.9% of which HBoV was the most commonly encountered. Kouni et al. (2013) identified 17 different viruses and its subtypes using a DNA/RNA microarray assay from rhinopharyngeal washes of children. The assay detected 70.1% of the viral infection, a single virus was found in 22.57% and two or more viruses in 42.5%.

Zhang et al. (2012) conducted a comparative study between VRDAL multiplex PCR for 10 common respiratory viruses and Seeplex® RV15 ACE detection kit for 15 respiratory viruses on nasopharyngeal swabs were collected from children below 3 years hospitalized due to acute respiratory infection. Viruses were detected in 51.2% patients by VRDAL multiplex PCR, and 4.9% of cases were mixed infections. Using the Seeplex® RV15 ACE detection kit, viruses were detected in 78.7% patients, 29.9% were co-infected cases. The Seeplex® RV15 ACE detection method was found to be a more reliable tool than VRDAL method to simultaneously detect multiple respiratory viruses. In the study conducted by Guerrier et al. (2013) among hospitalized children with acute lower respiratory tract infection 18 viral pathogens were detected. Among the total 55% were found to have viral infection, a single virus was detected in 89%.

Proenca-Modena et al. (2012) studied the role of respiratory viruses in chronic adenotonsillar diseases using TaqMan real time PCR (qPCR) in nasopharyngeal secretions, tonsillar tissues and peripheral blood of children. Respiratory viruses were detected in 97.5% of the patients and the co-infection rate was 69.5%. Esposito et al.
(2013) studied the clinical and virological data collected from children with radiographically confirmed CAP using Luminex xTAG Respiratory Virus Panel Fast assay. A total of 73.5% were positive for at least one virus.

Guido et al. (2011) studied the occurrence of HBoV, hMPV and Inf A-B from oropharyngeal swabs collected in an Apulian population with respiratory tract infections. They detected a high rate of HBoV infection in adult (18.9%) and elderly (26.4%) subjects. The co-infection was common in children and the rate was higher for hMPV (81.8%) compared to hBoV (49.1%), and InfA-B (61.0%). Pilger et al. (2011) studied the occurrence of hMPV and HBoV in children with suspected lower respiratory tract infection. A real-time PCR method was used for amplification and detection of hMPV and HBoV and to evaluate co-infections with RSV, influenza A and B, PIV 1, 2 and 3, human rhinovirus and human adenovirus. hMPV was detected in 14.5% of samples and HBoV in 13.2%. A unique causative viral agent was identified in 46.2% samples and the co-infection rate was 43.7%. For HBoV, 98.3% of all positive samples were from patients with mixed infections. Similarly, 84.8% of all hMPV-positive results were also observed in mixed infections. Both HBoV and hMPV usually appeared with RSV.

Fuenzalida et al. (2010) studied the usefulness of two new commercial techniques available for the detection of hMPV in clinical samples from children: an enzyme immunoassay, hMPV EIA (Biotrin International Ltd), and a molecular assay, real-time RT-PCR (Pro hMPV Real Time Assay Kit; Prodesse). Real-time RT-PCR for hMPV detected 8% and the EIA detected 9.3% positive cases. A relatively low co-infection rate (15%) was observed in our patients. Xiao et al. (2010) screened nasopharyngeal aspirates (NPA) specimens by reverse transcription-polymerase reaction for detection of hMPV in children. The rate of positivity was 6.80%. A high incidence of hMPV infection (84.4%) was observed during the winter-spring season of which 55.6% were co-infected with other respiratory viruses, and respiratory syncytial virus (RSV) was the most common additional respiratory virus.