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Newly discovered respiratory viruses: significance and implications

Jeffrey S Kahn

With the recent advances in molecular biology and the ability to amplify viral genomes in a non-sequence-dependent manner, several previously unidentified human respiratory viruses have been discovered. There are accumulating data that some of these new pathogens are responsible for a substantial proportion of respiratory tract diseases, particularly in children. This review will focus on several of these newly identified pathogens for which there are clinical data implicating a role of these viruses in respiratory tract disease specifically, human metapneumovirus, human coronaviruses NL63 and HKU1, and the human bocavirus. Antivirals and effective vaccines for these new agents may decrease the burden of respiratory tract diseases.

Addresses
Yale University School of Medicine, Department of Pediatrics, Division of Infectious Diseases, PO Box 208064, New Haven, CT 06520-8064, United States

Corresponding author: Kahn, Jeffrey S (Jeffrey.Kahn@yale.edu)

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Introduction

Respiratory tract infection is a leading cause of morbidity and mortality worldwide. While the clinical features of respiratory tract disease are easily recognized, the etiological agent responsible for disease is often not detected. In one study of community-acquired pneumonia, routine microbial investigation of sputum, blood, serology, and lower airway or pleural fluid (if available) lead to the identification of an etiological agent in only 46% of cases [1]. The etiology of a majority of lower respiratory tract infection is thought to be viral [2], yet in only 40% of cases can a viral agent be identified, even with use of state-of-the-art genomic amplification methods [3]. In children, respiratory syncytial virus (RSV), parainfluenza viruses, and influenza are known as the major causes of bronchiolitis and lower respiratory tract infection (LRTI). However, in up to a third of these cases, an infectious agent cannot be identified [4,5]. This suggested that previously unidentified viruses may be circulating. Since 2001, many new human viruses have been discovered. Data from many studies have demonstrated that some of these new viruses are responsible, at least partly, for the disease that previously could not be attributed to common respiratory viruses such as RSV, rhinoviruses, influenza viruses, parainfluenza viruses, and adenoviruses. This review will focus on four new respiratory viruses, human metapneumovirus, human coronaviruses NL63 and HKU1, and the human bocavirus

Human metapneumovirus

In 2001, van den Hoogen *et al.* reported the discovery of a new paramyxovirus identified in children with respiratory tract disease [6]. Paramyxoviruses are enveloped, single stranded RNA viruses of negative polarity meaning that their genomes must be transcribed to mRNA to be translated into protein. Serological studies of stored serum specimens suggested that hMPV had been circulating in the human population for more than 50 years [6]. By the age of 5 years, more than 90% of children have serological evidence of hMPV infection [6,7,8•,9]. There appear to be at least two major genotypes of hMPV, A and B [10]. There are conflicting data as to whether genotypes A and B represent distinct genotypes [10,11].

hMPV infections have been identified worldwide [6,12•,13–15] and has been shown to be the cause of both URI and LRTI in infants, young children [6,15–19], the elderly [20–22] and the immunocompromised populations [17,23,24]. Asymptomatic infection, at least in young children, appears to be uncommon [15]. In fact, in young children, hMPV is a major cause of bronchiolitis accounting for 5–15% of all cases [15–19,25]. The spectrum of disease caused by hMPV is similar to that of RSV. Both hMPV and RSV cause seasonal epidemics with the peak of hMPV activity typically overlapping or following the peak of RSV activity. Because of this, the potential for dual infection exist. One study reported that 70% of children with severe RSV bronchiolitis were co-infected with hMPV suggesting that the disease caused by RSV may be augmented by a concurrent hMPV infection [26]. However, population-based and case control studies of hospitalized children have found that hMPV and RSV co-infections are uncommon [19,27]. hMPV can cause an influenza-like illness in adults [28] and has been associated with wheezing and asthma in young children [29], though this also occurs with other common respiratory viruses. The pathogenesis of hMPV-associated lower respiratory tract infection may involve co-infection with pneumococcus in some instances, as vaccination with pneumococcal conjugate vaccine reduced the incidence

of hMPV-associated lower respiratory tract infection and clinical pneumonia in both HIV-infected and HIV-uninfected children [30••].

hMPV is associated with severe and fatal diseases in immunocompromised individuals. Clinical features associated with hMPV infection included respiratory failure, pulmonary hemorrhage, and culture-negative septic shock [31•]. However, this study did not sample asymptomatic patients, so a causal link between hMPV and disease could not be made. In fact, persistent symptomless hMPV infection has been reported in hematopoietic stem cell transplant recipients [32]. Recently, an outbreak of hMPV was reported in a long-term care facility [33••]. In that study, half of the individuals with severe lower respiratory tract infection tested positive for hMPV, and of those, 50% died. Histopathological studies of tissue from one of the fatalities revealed distribution of hMPV antigen in the bronchiolar epithelium of affected lobes indicating that the virus was present at the site of the disease. This is one of the strongest demonstrations yet that hMPV causes lower respiratory tract disease.

Several candidate hMPV vaccines are under development. These vaccine candidates fall into one of three categories: subunit (based on a single viral protein or peptide) [34,35], DNA (encoding a single viral protein) [35•], and live-attenuated, recombinant vaccines [11]. Both the subunit and DNA-based vaccines use the viral F protein (or gene) to elicit immunity. The F gene is the most highly conserved surface virion glycoprotein among the three virion glycoproteins and is relatively conserved between the two genotypes [10]. Each of the vaccine candidates has shown promising results in experimental animals [36] though to date no human studies have been performed.

Other than influenza, there are no respiratory viruses for which an effective and safe antiviral compound is available. Thus, the development of an antiviral for hMPV will be challenging. Ribavirin, an antiviral compound with *in vitro* activity against RSV, can also inhibit hMPV replication *in vitro* [37]. However, the effectiveness of ribavirin therapy for RSV is limited, at best, and remains a controversial issue. A humanized monoclonal antibody, palivizumab, has been approved for its use in high-risk infants (those with a history of prematurity, lung or cardiac diseases) to prevent serious disease due to RSV, though does not prevent infection and is not a therapeutic agent. An hMPV monoclonal antibody that neutralizes both genotype A and B viruses has been developed and has been shown to protect experimental animals from hMPV infection [38]. This molecule may have the potential as a prophylactic agent to protect against hMPV-related disease. Other compounds, such as NMSO3, a sulfated sialyl lipid has potent hMPV antiviral activity in cell culture assays [37].

Human coronaviruses

Before 2003, only two human coronaviruses were characterized. Human coronavirus (HCoV)-229E (a group I coronavirus) and HCoV-OC43 (a group II coronavirus) were discovered in the 1960s and subsequently have been found to cause mainly mild upper respiratory tract disease [39]. Since 2003, three new HCoVs have been identified. The first of these to be characterized was the severe acute respiratory syndrome (SARS) coronavirus. This virus probably entered the human population sometime in the late 2002, and the epidemic spread to 29 countries in North and South America, Europe, and Asia. Overall, more than 8000 individuals were infected, and the case fatality rate was ~10%. The last cases on SARS were in April 2004, and these were laboratory-acquired infections (http://www.cdc.gov/ncidod/sars/index.htm).

Unlike the SARS coronavirus, the other new human coronaviruses have been probably circulating in humans for a very long time and tend to cause mild, self-limiting infection. HCoV-NL63 was initially isolated from a seven-month-old girl with coryza, conjunctivitis, fever, and bronchiolitis [40]. Shortly thereafter, another Dutch group reported the identification of a coronavirus, HCoV-NL, which was initially isolated in April 1988 from a young boy with pneumonia [41]. These viruses are essentially identical. Genomic sequence and phylogenetic analyses indicate that HCoV-NL63 is a group I coronavirus. HCoV-NL63 has worldwide distribution and has been identified in 1–10% of respiratory tract infections in children [42•] though data for asymptomatic controls are lacking. HCoV-NL63 is often associated with mild upper respiratory tract infection, though severe lower respiratory tract infection has been observed. The virus is associated with respiratory tract disease in both children and adults. HCoV-NL63 may be a common cause of croup (laryngotracheitis). Of children <3 years of age who had relatively high viral loads and absence of another viral pathogen in nasopharyngeal secretions, 43% tested positive for HCoV-NL63 compared with 6% in the HCoV-NL63-negative group ($p < 0.0001$) [43].

Currently, diagnostic tests are limited to genomic amplification techniques as the virus is difficult to propagate in cell culture. The complete genome sequence for several HCoV-NL63 isolates has been completed, and there appear to be at least two genotypes [44].

In 2005, Woo *et al.* reported the discovery of a novel coronavirus, HCoV-HKU1, a group II coronavirus, initially in respiratory secretions from a 71-year-old man with chronic respiratory tract disease hospitalized with fever, cough, and infiltrate on chest radiograph [45••]. Western blot and ELISA assays using recombinant HCoV-HKU1 nucleocapsid protein revealed some reactivity in healthy donor serum suggesting wide spread infection with this virus. However, cross-reactivity between antibodies

specific for HCoV-HKU1 and HCoV-OC43, the other known human group II coronavirus, was not explored and therefore the serological data may be difficult to interpret.

HCoV-HKU1 has been detected in a small percentage of individuals with respiratory tract disease. In one study of community-acquired pneumonia in Hong Kong, 10 (~2.4%) of 418 adults tested positive for HCoV-HKU1 [46]. In another study from Hong Kong, 13 (0.3%) of 4181 individuals with acute respiratory tract infection tested positive for HCoV-HKU1. Most of the HCoV-HKU1-infected individuals were children [47]. In a study from the United States, HCoV-HKU1 was detected in 9 (1%) of 851 infants and young children (<5 years old) with respiratory tract disease [48•] though these studies did not include a group or asymptomatic controls. Infection with HCoV-HKU1 may not be limited to the respiratory tract as the virus has been detected in the stool. However, neither of the two children whose stool tested positive for HCoV-HKU1 RNA had evidence of gastrointestinal disease [49]. Attempts to propagate the virus have been unsuccessful, to date, and diagnosis relies on genome amplification techniques.

The need to develop effective vaccines and antiviral compounds specific for HCoV-NL63 and HCoV-HKU1, as well as HCoV-229E and HCoV-OC43, may be limited because these viruses are probably an infrequent cause of serious respiratory tract disease. Nonetheless, there has been much work on the development of a SARS vaccine that could be potentially applied to the prevention of infection due to common respiratory viruses [50]. The experience with the SARS virus is a reminder that trans-species transmission of viruses occurs and has the potential to lead to worldwide epidemics.

Human bocavirus

In 2005, Allander *et al.* described the discovery of a novel human parvovirus isolated from the respiratory tract of individuals with clinical features of respiratory tract disease [51••]. The new virus was discovered by non-specific amplification and cloning of viral genomic sequences. This novel virus was most closely related to the only two members of the Genus *Bocavirus* (Family *Parvoviridae*), a bovine and canine parvovirus (the term ''boca'' was derived from the first two letters — ''bo'' and ''ca'' — of bovine and canine) and was called human bocavirus (HBoV). To date, there have been more than 20 studies from Europe, North America, Middle East, Asia, Africa documenting the detection of HBoV in respiratory secretions of individuals with respiratory tract disease. Most studies have found HBoV in 1–8% of respiratory specimens; however, in some other studies the frequency of positives was more than 18%. The majority of HBoV-positive specimens have been obtained from children, though this may represent a sampling bias.

While many studies have reported the detection of HBoV in respiratory secretions, few have addressed the issue of whether HBoV is the cause of respiratory tract disease. In a study of children <2 years old, Kesebir *et al.* [52••] screened respiratory specimens from children who had a sample submitted to a Clinical Virology laboratory that tested negative for RSV, influenza, parainfluenza viruses (1–3), and adenoviruses as well as asymptomatic controls. Overall, 22 (5%) of 426 specimens submitted to the diagnostic laboratory and 0 (0%) of 96 asymptomatic controls tested positive for HBoV ($P < 0.013$). Several of the HBoV-infected children had diarrhea suggesting that the disease caused by HBoV may extend beyond the respiratory tract (see below). These are some of the most compelling data yet implicating HBoV as the etiological cause of respiratory tract infection and disease.

Co-infection of HBoV and other common respiratory viruses may be common. In a population-based surveillance for pneumonia in Thailand, 4.5% of individuals with pneumonia tested positive for HBoV (83% were <5 years old), and of those, 83% were co-infected with another virus; rhinoviruses, RSV, and parainfluenza viruses were the most frequently observed co-pathogens [53••]. Four of five HBoV-positive individuals had evidence of virus in the serum indicating that a viremia occurs during infection. In a study of asthma in children, HBoV was detected in 19% of children, though co-infection with other viral pathogens was frequently observed [54•]. In this study, a majority (53.5%) of HBoV-positive children who had HBoV detected in a nasopharyngeal aspirate also had HBoV (or at least HBoV DNA) in serum. Curiously, 7% of children whose nasopharyngeal aspirate tested negative for HBoV also had evidence of HBoV in serum. The significance of this finding remains unclear at this time.

Vicenti *et al.* report the detection of HBoV in the stool of children <3 years old with acute gastroenteritis *without* respiratory tract disease [55]. However, for 58% of these children, another enteric pathogen was detected. This study did not include a control group. The role of HBoV in enteric disease will require further investigation. The spectrum of disease caused by HBoV and its significance should be defined before the need for antivirals and potential vaccines can be considered.

Other newly identified viruses

As the technology to detect previously unidentified pathogens continues to improve, it is not surprising that several new viruses, in addition to those described above, have been identified. PARV4, a parvovirus phylogenetically distinct from HBoV has been detected in serum from HIV, hepatitis B virus infected, or hepatitis C virus infected individuals [56]. Two TT-(torqueteno) virus-like viruses have been identified in human sera [56]. Two new human polyomaviruses, KI and WU have been identified in secretions originating from the human respiratory tract

[57•,58•]. Polyomaviruses have oncogenic potential and therefore study of these new viruses is important. A novel virus, the mimivirus, is the largest (in size and genome) known virus, can be seen with light microscopy, and contains a genome that is larger than some bacteria [59]. Serological data suggest that the mimivirus may be a cause of pneumonia. However, the virus has not yet been isolated from individuals with pneumonia [60•]. Clinical studies of these new viruses are lacking. Whether these new microbes are human pathogens remain to be defined. However, it is clear that the landscape of respiratory viruses continues to change.

Conclusions

Several new human respiratory viruses have been discovered recently. It is now clear that hMPV is a significant human pathogen and can cause severe disease in many patient populations, specifically infants, the elderly, and the immunocompromised. There is a need for an effective vaccine and antiviral compounds to protect against and treat hMPV-related disease. Like the previously known human coronaviruses, HCoV-NL63 and HCoV-HKU1 are associated with both upper and lower respiratory tract diseases though the burden of disease caused by these viruses remains to be fully determined. The full spectrum of disease associated with HBoV remains to be defined. Presence of the virus in respiratory secretions, serum, and stool suggests that this virus may cause systemic illness.

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