

# Update on Human Rhinovirus and Coronavirus Infections

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## Abstract

Human rhinovirus (HRV) and coronavirus (HCoV) infections are associated with both upper respiratory tract illness (“the common cold”) and lower respiratory tract illness (pneumonia). New species of HRVs and HCoVs have been diagnosed in the past decade. More sensitive diagnostic tests such as reverse transcription-polymerase chain reaction have expanded our understanding of the role these viruses play in both immunocompetent and immunosuppressed hosts. Recent identification of severe acute respiratory syndrome and Middle East respiratory syndrome viruses causing serious respiratory illnesses has led to renewed efforts for vaccine development. The role these viruses play in patients with chronic lung disease such as asthma makes the search for antiviral agents of increased importance.

## Keywords

- ▶ human rhinovirus
- ▶ human coronavirus
- ▶ polymerase chain reaction

More than 50% of all common colds are caused by human rhinoviruses (HRVs) and coronaviruses (HCoVs).<sup>1–3</sup> The common cold includes rhinitis and pharyngitis, as well as sneezing, hoarseness, and cough.<sup>4,5</sup> The illness is usually self-limited in healthy people, but can be associated with complications in individuals who suffer from heart or lung disease, or who are immunosuppressed.<sup>6–11</sup> As a frequent cause of the common cold, these viruses are responsible for millions of lost work days, school absenteeism, and physician visits. In infants and young children, these viruses are associated with many cases of acute otitis media (AOM) and rhinosinusitis.<sup>12</sup> Newer more sensitive diagnostic tests have increased the percentage of respiratory illnesses attributable to these viruses.<sup>13–15</sup> Most HRV infections are symptomatic, but asymptomatic virus shedding has been reported with increasing frequency.

In the past 10 years, several new HRVs and HCoVs have been identified.<sup>16–18</sup> In addition, recent studies have provided a better understanding of the pathogenesis of these viral infections which could lead to more specific treatments. This review will update recent advances in our knowledge of these two virus groups and their importance in respiratory viral infections.

## Rhinoviruses

### Virology

HRVs are positive-sense, single-stranded ribonucleic acid (RNA) viruses with icosahedral symmetry. The capsid is composed of four proteins: VP1, VP2, VP3, and VP4.<sup>4,19</sup> VP4 is on the inside of the virus and anchors the RNA core to the viral capsid. The complete sequencing of all known HRV genomes has been reported.<sup>20</sup>

More than 150 serotypes of HRVs have been identified. Most of the HRV serotypes of the HRV-A and HRV-B families use intercellular adhesion molecule-1 (ICAM-1; major group) as the cell receptor for the virus entry. A few HRVs used heparin sulfate proteoglycan as an additional receptor and approximately 10 serotypes use low-density lipoprotein as the cell receptor (minor group). In 2002, a new clade of HRVs was reported and labeled HRV-C.<sup>19</sup> Although HRV-C strains have been characterized by genome sequencing, the receptor used to infect epithelial cells is currently unidentified.

During replication, the RNA genome serves as an mRNA, which encodes the capsid or structural proteins, as well as the nonstructural proteins.<sup>4</sup> After cell entry, the viral genome is translated into a polyprotein, which undergoes proteolytic cleavage to produce the structural and nonstructural gene

products. Serotypes of HRVs are based on amino acid differences in the four capsid proteins.

Enteroviruses (EVs) are not typically thought of as causing acute respiratory tract infections. However, EV68 has properties of both EVs and HRVs, and has been independently identified as HRV serotype 87.<sup>21</sup> EV68 and EV104 have been associated with respiratory illness in multiple countries.<sup>22–26</sup>

### Pathogenesis

HRV infections initially involve the upper respiratory tract. After deposition of HRV in the eye or nose, the virus attaches to host cell epithelium. Infection of nasal epithelial cells results in increased neutrophils detectable in the nasal mucosa and secretions with release of inflammatory mediators. Although HRVs replicate best at 33°C, they have been found in lower respiratory tract cells where the temperature is 37°C.

HRVs do not produce cytopathologic changes; however, HRVs appear to disrupt tight junctions between cells, leading to increased vascular leakage and mucus secretion. Using primary nasal epithelial cells, HRV was found to alter the function and the expression of cystic fibrosis transmembrane conductance regulator (CFTR),  $\alpha$ -epithelial sodium channel ( $\alpha$ -ENaC),  $\beta$ -epithelial sodium channel ( $\beta$ -ENaC), and  $\gamma$ -epithelial sodium channel ( $\gamma$ -ENaC).<sup>27</sup> These findings may help explain the mechanism by which altered mucociliary clearance is associated with HRV infections.

Most of the symptoms following HRV infection can be ascribed to the host response to the virus, including those mediated by the innate, mucosal, and cellular immune systems. Following epithelial cell attachment and internalization, HRV infection results in cytokine expression, including type I and type III interferons (IFNs), interleukin (IL)-6, IL-11, IL-12, IL-15, and IL-1B.<sup>28–32</sup> Neutrophil recruitment results from release of growth factors and chemokines.

VP-I binds to ICAM-1 and is the major target for the memory immune response, residing in IgG1 subclass and IgA class antibodies. The majority of VP-1-specific antibodies are directed against an N-terminal 20-mer peptide (Pla), which is located inside the capsid. The antibody response to HRV in humans appears to be misdirected against a non-neutralizing epitope that is exposed during uncoating.

In a group of children with asthma and upper respiratory infections (URIs), type III IFN- $\lambda$  levels were significantly higher in wheezing children infected with HRV compared with non-wheezing children.<sup>33</sup> Although type III IFN- $\lambda$  levels were lower at baseline in children with asthma compared with healthy children, they were found to be hyperexpressed during asthma exacerbation. In addition, a study reported that IFN- $\lambda$  has antiviral and anti-inflammatory properties in primary epithelial cells from asthmatic patients exposed to rhinovirus.<sup>34</sup>

In a recently published mouse model of HRV infection, adult mice had an enhanced type 2 immune response and attenuated type 1 response compared with neonatal mice.<sup>35</sup> The authors reported long-term mucosal metaplasia and airway responsiveness in HRV-infected neonatal mice. In addition, they found increased expression of epithelial

IL-25 with neonatal rhinovirus infection. IL-25 appears to mediate metaplasia and airway hyper-responsiveness in the HRV-infected neonatal mice. Induction of type 1 cytokines, IFN- $\gamma$ , IL-12p40, and tumor necrosis factor- $\alpha$  was diminished in neonates compared with adult mice. HRV infection elicits an increased ILC2 response in neonatal mice. These observations may help our understanding of the intersection of early HRV infections and subsequent development of asthma.

### Epidemiology

HRVs cause respiratory illnesses throughout the world, in all age groups, and throughout the year, although most prevalent in the fall and spring in temperate climates.<sup>36</sup> In one study, HRVs accounted for approximately 50% of common colds.<sup>37</sup> HRVs commonly infect children in early childhood and into adulthood. School-aged children frequently introduce HRV infections into the home. Secondary attack rates range from 25 to 70%. Day-care centers and schools are also important locations for the spread of HRV. HRV transmission can occur by close contact, autoinoculation, fomites, or aerosols.

Clinical outcomes appear to be similar between the HRV species. The recently identified HRV-C infections can have symptoms of the common cold, pharyngitis, croup, AOM, bronchiolitis, and/or pneumonia.<sup>38–40</sup> These infections have been reported in healthy children and adults, as well as in those with asthma, immunocompromised conditions, CF, or multiple sclerosis.<sup>41–45</sup>

HRV-Cs, more than HRV-As and -Bs, are major causes of wheezing in infants and of asthma exacerbations in older children.<sup>46</sup> Of all viruses detected from middle ear fluids in children with otitis media, HRV-Cs accounted for half of the documented infections.<sup>47</sup> Although reported infections have come mainly from respiratory tract specimens, HRV-Cs have been rarely reported in blood and pericardium.<sup>48,49</sup>

With more sensitive polymerase chain reaction (PCR) methods for HRV detection, several reports of long periods (>2–3 weeks) of HRV positivity have been published.<sup>50,51</sup> Where strain typing has been used, however, HRV shedding normally stops within 11 to 21 days. Therefore, persistence may represent serial or overlapping infections by multiple untyped strains.<sup>52</sup> In immunocompromised children, HRV-C strains were detected threefold longer (53 vs. 16 days) than in immunocompetent children.<sup>53</sup>

Recent studies have documented HRV species in all months of the year in tropical, subtropical, and semi-arid regions.<sup>54,55</sup> Many HRV-C strains have been found to circulate during a single year and may be detected in subsequent years.<sup>56</sup>

### Diagnosis

Standard tissue culture methods for isolation are useful for detecting HRV infection but are not very sensitive. With the development of PCR techniques, the ability to detect respiratory viruses has increased significantly. Detection of HRVs in respiratory specimens was enhanced by reverse transcription-PCR (RT-PCR), involving the use of hybridization probes or double-stranded DNA binding dye. Several studies have

found an increased sensitivity of RT-PCR compared with viral culture techniques.<sup>57-61</sup>

Antibody assays are reported for HRVs, but are not readily available or helpful clinically. Since there is no common antigen for HRVs, serotype-specific neutralizing antibody assays are necessary to detect rises in serum antibodies following acute infections, but the large number of HRV serotypes makes this approach impractical.

### Clinical Features

The incubation period for the common cold is 12 to 72 hours. Rhinorrhea and sneezing plus nasal congestion are usually the initial symptoms.<sup>62,63</sup> Sore throat is common and may be an early symptom. Fever is unusual. Headache and malaise are often mild. Resolution of symptoms occurs in almost all cases within 4 to 9 days.

With the use of PCR techniques, HRVs have been reported commonly in asymptomatic children.<sup>3</sup> This may be a result of prolonged shedding from a previous respiratory illness, a mild illness that went unrecognized, or a new virus infection during the incubation period. Asymptomatic HRV shedding is not as common in adults as it appears to be in children. In addition, coinfections with other respiratory viruses during respiratory illnesses are well described.<sup>64-71</sup>

With the global spread of influenza A (H1N1) in 2009, a series of surveillance protocols were activated to monitor and characterize the pandemic. Many countries have reported on their findings in patients with influenza-like illnesses (ILI) who had respiratory specimens tested for a wide range of respiratory viruses. Most of the reports used PCR techniques in children or adults (→ **Table 1**).<sup>70,72-86</sup> The percentage of specimens that were positive with HRV ranged from 6.3 to

40.6%. In all the studies, the influenza illnesses could not be distinguished from HRV-related illnesses by clinical characteristics. During influenza outbreaks, other respiratory viruses, and especially HRV, continue to cause significant cases of URIs and lower respiratory infections (LRIs).

### Asthma and HRV Infections

Asthma exacerbations in children and adults are frequently associated with respiratory virus infections, especially HRVs.<sup>87-90</sup> HRV infections lead to more severe and longer-lasting lower respiratory tract symptoms.<sup>90</sup>

Lower airway dysfunction following HRV infection may result from direct infection of the lower airway or by stimulating inflammatory, immunologic, or neurogenic mechanisms in the upper airway, thereby impacting the lower airways. HRV has been detected in the columnar and basal cell layers of the lower airways.<sup>91</sup> Using in situ hybridization, the replicative strand of HRV in the lower airways has been detected.<sup>92</sup>

Experimental HRV infections in asthmatic subjects have demonstrated airway narrowing, markers of eosinophil activation, bronchial infiltration with eosinophils, CD4 cells and CD8 cells, activation of prostaglandin and leukotriene pathways, and induction of nitric oxide.<sup>93-95</sup> Innate immune responses were found to be defective in bronchial epithelial cells obtained from asthmatic subjects. Impaired Th1 responses to HRV were found in peripheral blood mononuclear cells, as reflected by significantly lower levels of IFN- $\alpha$  and IL-12, and higher levels of IL-10 from asthmatic patients compared with normal healthy volunteers.<sup>96</sup>

Several studies have found deficient induction of IFN- $\lambda$  by HRV in bronchial epithelial cells from asthmatic patients.<sup>97</sup>

**Table 1** HRV isolates from patients with influenza-like illness

Reference	Location	Specimen	Age group	Positive for HRV (%)
Hombrouck et al <sup>72</sup>	Belgium	NP	Children	13
Schnepf et al <sup>73</sup>	France	NS	Children and adults	20
Memish et al <sup>74</sup>	Saudi Arabia	NP/TS	Adults (health care workers)	21
Pascalis et al <sup>75</sup>	Reunion Island	NS	Children and adults	13.4
Chang et al <sup>76</sup>	Texas	NP	Children	29.8
Thiberville et al <sup>77</sup>	Vietnam	NS	Children and adults	20.3
Yang et al <sup>78</sup>	China	NP/TS	Adults	6.3
Nisii et al <sup>79</sup>	Italy	NP	Adults	10
Kraft et al <sup>80</sup>	United States/CDC	?	Immunocompromised adults	12.6
Delangue et al <sup>81</sup>	Bolivia	NP	Children and adults	8
Dia et al <sup>82</sup>	Senegal	NP	Adults older than 50 years	17.2
Ju et al <sup>83</sup>	China	NP/TS	Children and adults	7.5
Marcone et al <sup>84</sup>	Argentina	NP	Children	40.6
Pariani et al <sup>85</sup>	Italy	NS/BAL	Children and adults	9
Gilca et al <sup>70</sup>	Canada	NS	Adults	7
Zimmerman et al <sup>86</sup>	United States	NS	Children and adults	15.4

Abbreviations: HRV, human rhinovirus; NP, nasopharyngeal swab; NS, nasal swab; TS, throat swab.

Induction of IFN- $\lambda$ 1 and IFN- $\lambda$ 2/3 mRNAs was significantly reduced in asthmatic compared with nonasthmatic subjects. Bronchoalveolar cells in asthma patients were deficient in IFN- $\lambda$  after HRV was added. These studies support the view that innate immune responses in asthmatic subjects have deficiencies in two IFN families, in several lung cell types, and in response to HRV infection.

HRV infections are a major cause of wheezing episodes in infants and children.<sup>98–100</sup> Wheezing episodes in infancy that are virus induced are often associated with asthma development in children. HRV infections that resulted in hospitalization during infancy were recently implicated as early predictors of subsequent development of asthma.<sup>101,102</sup> Almost 90% of wheezing children in year 3 of the study had asthma diagnosed by 6 years of age. Outpatient HRV wheezing illnesses during infancy were also found to be predictors of wheezing through 3 years of age.<sup>103–107</sup> The possible relationship between HRV wheezing illness and genetic risk of childhood-onset asthma was studied in a group of prospectively studied children.<sup>108</sup> The participants were children who had been studied since birth and cultured for HRV. In children who had HRV wheezing illnesses, there was significant association with 17q21 gene locus.

### COPD and HRV Infection

Multiple longitudinal studies have documented the importance of respiratory viral infections in acute exacerbations of chronic obstructive pulmonary disease (COPD).<sup>109–113</sup> Other studies, using PCR techniques for respiratory virus detection, have found that more than 40% of COPD exacerbations are associated with respiratory viruses, especially HRVs. Inflammatory mediators, especially IL-8, have been found in increased levels of respiratory secretions obtained from stable COPD patients.

Studies have demonstrated an increase in *Staphylococcus aureus* and *Streptococcus pneumoniae* adherence to respiratory epithelial cells infected with HRV. In an in vitro study using primary differentiated human cell culture lines, a strain of nontypeable *Haemophilus influenzae* was found to potentiate airway epithelial cell responses to HRV by increasing ICAM-1 and Toll-like receptor-3 expression.<sup>114</sup> However, it is unclear whether the interaction of respiratory virus with bacterial pathogens is a common cause of exacerbations of COPD or whether respiratory viruses, such as HRV, cause these pulmonary complications.

### AOM and HRV Infections

Viruses, especially HRVs, result in an inflammatory reaction that results in mucociliary damage, impaired middle ear function, and increased mucus in the Eustachian tube. This leads to superinfection of the middle ear by bacteria and fluid accumulation (effusion). Recent studies detected HRVs in 40% of children with otitis media with effusion.<sup>115–118</sup> HRVs were cultured in 24% of nasopharyngeal specimens. In a prospective study of 121 otitis-prone children, nasopharyngeal swabs were assayed by PCR for respiratory viruses and by culture for bacterial pathogens. HRVs were found at baseline in 30% of specimens. Positive PCR tests for HRV

correlated with culturing *Moraxella catarrhalis* and *S. pneumoniae* but not nontypeable *H. influenzae*. HRV and bacterial pathogens were found in otitis-prone children even in the absence of clinical symptoms. Most new otitis media episodes are coincident with a HRV URL.

In a recently published study in South African children with AOM, 74.2% of cases had respiratory viruses detected from middle ear fluid specimens.<sup>119</sup> HRVs were detected in 37.7% of children with AOM. Respiratory viruses were recovered in 72% of episodes which had negative bacterial cultures. HIV status did not alter the spectrum of respiratory viruses recovered.

### Rhinosinusitis and HRV Infections

Patients with the common cold syndrome have sinus abnormalities detectable by computed tomography.<sup>120,121</sup> Abnormalities are most frequently detected in the maxillary and ethmoid sinuses, and resolve without antibiotics in 80% of patients followed over several weeks. Less than 20% of cases of viral rhinosinusitis are complicated by bacterial infection.<sup>122</sup> In a study of 20 adults with acute rhinosinusitis, 15% had virus cultures positive for HRV, but 50% were positive using RT-PCR on maxillary sinus aspirates or nasal swabs.<sup>123</sup> Intranasal pressure increases following nose blowing, sneezing, and coughing, and is high enough to propel virus-infected nasal reactions into the sinuses.<sup>124</sup>

### Community-Acquired Pneumonia and Bronchiolitis in HRV Infections

Multiple studies using PCR assays have shown that HRVs do cause community-acquired pneumonia (CAP) in both children and adults.<sup>125–128</sup> Studies in children have reported a range of 0 to 52% positive specimens for HRV (►Table 2).<sup>129–148</sup> Bronchiolitis in children has been commonly reported in infants and young children. The most commonly reported virus recovered in acute cases has been RSV. As with CAP series, mixed infections with a second respiratory virus were common. In studies testing for respiratory viruses in adults with CAP, HRV was detected in 4 to 40% of cases (►Table 3).<sup>149–155</sup> As many cases of CAP had mixed infections, it is difficult to be certain if the HRV contributed to CAP.

### Cystic Fibrosis and HIV Infections

A few studies have examined the role of respiratory viral infections in CF patients.<sup>156</sup> Picornavirus was detected in more than 40% of URIs in children with underlying CF.<sup>157</sup> There was no difference in pulmonary function in those children with proven HRV infection versus other respiratory viruses. Smyth et al followed up 108 patients with CF for 1 year, and detected HRV in 16% of exacerbations.<sup>158</sup> Those patients with proven HRV infection did not show deterioration in clinical activity, but did receive more days of intravenous antibiotics. Goffard et al reported on 64 samples from 46 adult patients with CF.<sup>159</sup> HRVs were most frequently identified in CF patients with clinical exacerbations and identified in 14%. Ramirez et al found similar HRV detection in CF patients with exacerbations and measured increased

**Table 2** HRV isolates in childhood community-acquired pneumonia and/or bronchiolitis

Reference	Country	Design	Clinical	Specimen	% HRV ⊕
Choi et al <sup>129</sup>	Korea	Retrospective	Pn	BAL	23.8
Launes et al <sup>130</sup>	Spain	Prospective	Pn	NPA	52
García-García et al <sup>131</sup>	Spain	Prospective	Pn	NPA	19
Miller et al <sup>132</sup>	Argentina	Prospective	Br	NS	40
Esposito et al <sup>133</sup>	Italy	Prospective	Pn	NS	29
Homaira et al <sup>134</sup>	Bangladesh	Prospective	Pn	NW	12
Guerrier et al <sup>135</sup>	Cambodia	Prospective	Pn/Br	NPS	34
Cho et al <sup>136</sup>	Korea	Prospective	Pn	NS	18.5
Suzuki et al <sup>137</sup>	Philippines	Prospective	Pn	NPS	30.5
Uršič et al <sup>138</sup>	Slovenia	Prospective	Pn/Br	NPS/TS	33.1
Pretorius et al <sup>139</sup>	South Africa	Prospective	SARI	NPS	25
Lu et al <sup>140</sup>	China	Prospective	Pn	NPA	30.9
Chidlow et al <sup>141</sup>	New Guinea	Prospective	Pn	NS	63
Ghani et al <sup>142</sup>	South Africa	Retrospective	Pn/Br	NPS/TS/BAL	39
Chen et al <sup>143</sup>	Taiwan	Prospective	Br	NPS	12.4
Antunes et al <sup>144</sup>	Portugal	Prospective	Br	?	0
Mansbach et al <sup>145</sup>	United States	Prospective	Br	NPA	25.6
Zeng et al <sup>146</sup>	China	Prospective	Pn/Br	NPA	23.2
Ouédraogo et al <sup>147</sup>	Burkina Faso	Prospective	Pn/Br	NPA	40
Gooskens et al <sup>148</sup>	The Netherlands	Retrospective	LRTI	NPA/TS/S	30

Abbreviations: BAL, bronchoalveolar lavage; Br, bronchiolitis; HRV, human rhinovirus; NPA, nasopharyngeal aspirate; NS, nasal swab; NW, nasal wash; Pn, pneumonia; TS, throat swab.

chemokine expression in sputum samples of HRV-positive patients.<sup>160</sup>

### HRV Infections in Immunocompromised Hosts

Respiratory virus infections are common causes of acute respiratory illness in patients after solid-organ transplantation or following bone marrow transplantation.<sup>161–166</sup> In these immunocompromised patients, HRV was the number one detected respiratory virus by PCR assays. Ison et al found an 83% (5/6) fatality rate in hematopoietic stem cell transplantation patients with bronchoalveolar lavage (BAL)-posi-

tive samples for HRV.<sup>162</sup> In a study of 215 patients with underlying HCT, 30% had infections at 100 days posttransplant.<sup>166</sup> The incidence for HRV was 22.3%. Median duration of virus shedding was 3 weeks. HRV infection was associated with URI symptoms.

In a prospective study of patients with malignancies and neutropenia, a virus was detected in 35% of patients.<sup>167</sup> HRVs were the most common virus detected by quantitative PCR in nasopharyngeal aspirates. In a prospective, multicenter study in children with cancer and fever and neutropenia, 57% had a virus detected by PCR-DNA. A third of the patients had mixed

**Table 3** HRV-associated CAP in adults

Reference	Country	Design	Clinical	% All virus + % HRV
Qu et al <sup>149</sup>	China	Prospective	CAP	15.2
Seo et al <sup>150</sup>	Korea	Prospective	LRTI	12.0
Walker and Ison <sup>151</sup>	United States	Retrospective	CAP	40
Drieghe et al <sup>152</sup>	Belgium	Prospective	LRTI	5.2
Fica et al <sup>153</sup>	Chile	Prospective	CAP	23.7
Zhan et al <sup>154</sup>	China	Prospective	CAP	4.0
Jain et al <sup>155</sup>	United States	Prospective	CAP	30.0

Abbreviations: CAP, community-acquired pneumonia; HRV, human rhinovirus; LRTI, lower respiratory tract infection.

HRV/bacterial infections.<sup>168</sup> HRVs were the second most detected virus; RSV was most frequently identified. Significant morbidity and mortality in posttransplant patients have been reported following HRV infections.<sup>169</sup> In bone marrow transplant recipients with fever and URI symptoms, HRV was the most commonly detected respiratory virus.<sup>170</sup>

### Nosocomial HRV Infections

Hospital outbreaks of HRV infections have been reported in neonatal intensive care units (ICUs) and long-term care facilities.<sup>171–179</sup> In one study, neonates acquired HRV infection during their hospital stay.<sup>179</sup> In long-term care facilities where HRV outbreaks have occurred, there were potential deaths associated with the infection. Guidelines for hospital isolation recommend droplet precautions for patients with known HRV infection.<sup>180</sup>

### Treatment

There are no approved antiviral medications for HRV respiratory tract infections. Anticholinergic medications can be used for rhinorrhea. Nasal congestion can be alleviated by nasal and systemic decongestants. Several studies have suggested that heated, humidified steam may reduce nasal congestion in common colds, but the data are not conclusive.<sup>181</sup> Cough is a common accompanying problem in respiratory viral infections and can be suppressed with nonprescription cough suppressants. Other symptoms such as sore throat, myalgia, fever, or headache can be controlled with nonsteroidal anti-inflammatory drugs. Antibiotics are inappropriate for treating these viral infections.

Compounds targeting cell susceptibility, virus attachment, receptor blockage, virus uncoating, RNA replication, and viral protein synthesis have been evaluated. Although several agents have demonstrated both in vitro and in vivo success, none have received U.S. Food and Drug Administration approval due to poor bioavailability, poor side-effect profile, or limited potency.<sup>182</sup> Viral capsid-binding compounds, such as pleconaril, block virus uncoating in vitro.<sup>183</sup> Clinical trials demonstrated significant reduction in duration of respiratory symptoms in individuals receiving pleconaril, but the drug has not been approved.

Alternative medications, such as *Echinacea angustifolia* or zinc lozenges, have been tested in several volunteer trials but are not currently thought to be clinically effective.<sup>184–187</sup> All of these studies suffer from inadequate control groups or incomplete virologic evaluation.

Quinolones have been used to treat exacerbation of COPD because of their broad antibacterial spectrum and anti-inflammatory properties. Recently, levofloxacin has been found to inhibit HRV infection in primary cell cultures employing human tracheal epithelial cells.<sup>188</sup> Levofloxacin pretreatment decreased mRNA levels of ICAM-1. Macrolides have also been reported to reduce ICAM-1 expression and HRV-induced cytokines in vitro and in vivo.<sup>189</sup>

A recent randomized controlled study evaluated the clinical benefit of oral prednisolone for treating a first episode of HRV-associated wheezing in young children younger than 23 months.<sup>190</sup> No long-term benefits were demonstrated in

those children receiving oral prednisolone with their first HRV-associated wheezing episode. Short-term benefits were found in those receiving the oral prednisolone. This included less cough, rhinitis, and night-time respiratory symptoms. In a subset of children who had high viral loads (>7,000 copies/mL), a long-term benefit of prednisolone therapy was detected. Future studies will have to confirm these findings before a general recommendation can be made for corticosteroid treatment in asthma-prone children with HRV-associated wheezing episodes.

### Prevention

HRV can be recovered from the hands of approximately 40% of adults with colds. Hand-to-hand transmission of HRV has led to the evaluation of disinfectants that will eliminate virus on human skin. Trials to reduce hand transmission have reported differing results.<sup>191,192</sup> A recently published study in Bangladesh demonstrated that nasal swab samples and paired hand rinse samples had HRV detected by rRT-PCR in 21 and 29% of samples, respectively.<sup>193</sup>

A study using 2% of aqueous iodine decreased transmission in family members who were exposed to HRV-infected individuals. An evaluation of virucidal hand treatments confirmed the prevention of HRV infections by organic acids but not ethanol.<sup>192</sup> Hand washing with soap and water effectively cleans HRV-contaminated hands better than single treatment with ethanol hand rub.<sup>192</sup>

Vaccines have not been thought to be useful against HRVs because of the serotype-specific neutralizing antibodies produced following infection and because of the numerous serotypes.<sup>194–197</sup> However, recent studies in a mouse model have reported development of cross-serotype reactive antibodies to VP1, suggesting a basis for successful antibody-mediated vaccine development. Future studies will need to confirm and expand these preliminary observations.

## Coronaviruses

### Virology

Coronaviruses are positive, single-stranded RNA viruses that replicate in the cytoplasm and bud into cytoplasmic vesicles from the endoplasmic reticulum. Coronaviruses are divided into three groups: alpha, beta, and gamma. CD13 (human aminopeptidase N) is the cellular receptor for HCoV-229E (►Table 4).<sup>198–200</sup> Recent studies have shown that the newly reported HCoV-NL63 does not use CD13 as the receptor cell entry. HCoV-NL63, along with severe acute respiratory syndrome (SARS)-CoV, uses angiotensin-converting enzyme 2 (ACE2) as the entry receptor.<sup>201</sup> ACE2 is found on the ciliated nasal and tracheobronchial epithelial cells. The receptor for HCoV-OC43 is not known. Carcinoembryonic antigen (CEA) is the receptor for mouse hepatitis virus.<sup>202</sup> Phylogenetic relationships between coronaviruses have been based on deduced amino acid sequences of the coronavirus replicase, ORF1b gene.

Middle East respiratory syndrome (MERS)-CoV belongs to the genus Betacoronavirus (BCoV) in the family Coronaviridae.<sup>17</sup> It is the first lineage C βCoV and the sixth coronavirus known to infect humans. MERS-CoV is an enveloped,

**Table 4** Coronavirus receptors

Virus	Group	Receptor
Human coronavirus 229E (HCoV-229E)	Alpha	APN
Bat coronaviruses (BCoVs-multiple species)	Alpha	Unknown
Human coronavirus NL63 (HCoV-NL63)	Alpha	ACE2
Severe acute respiratory syndrome coronavirus (SARS-CoV)	Beta	ACE2
Bat SARS-related coronavirus (Bat-SrCoV)	Beta	ACE2?
Middle East respiratory syndrome coronavirus (MERS-CoV)	Beta	DPP4
Human coronavirus OC43 (HCoV-OC43)	Beta	Unknown

Abbreviations: ACE2, angiotensin-converting enzyme 2; APN, aminopeptidase N; DPP4, dipeptidyl peptidase 4.

positive-sense, single-stranded RNA virus. The replication cycle is similar to other CoVs. The host cell receptor for the MERS-CoV S protein is dipeptidyl peptidase-4 (DPP4) or CD26. This receptor is expressed on endothelial and epithelial cells of the kidney, lung, small intestine, liver, as well as on immune cells. This ability to infect many different organ cells may explain the extrapulmonary clinical characteristics in patients. After uncoating, there is translation of ORF/a/b, followed by transcription and genome replication. Following translation on the rough ER, budding and assembly of virions occur in the cytoplasm with exocytosis and virion release. ACE2 is a SARS-CoV receptor. Upon SARS-CoV infection, ACE2 expression in lungs is markedly downregulated and therefore helps explain SARS pathogenesis and progression to acute respiratory distress syndrome (► **Table 5**).

### Pathogenesis

Coronaviruses attach to cellular receptors by the spike proteins on their surface.<sup>203,204</sup> Internalization into host cells occurs by direct fusion with the plasma membrane or by endocytosis. Posttranslational proteolytic processes are important regulatory mechanisms. Polyproteins are cleaved by viral proteases, facilitating assembly of subunit protein complexes that are responsible for replication and transcription.<sup>205</sup> There is little information on the host response to coronavirus replication. Humoral immune responses are detectable following natural infection, but the role of cell-mediated immunity is largely unknown.<sup>206,207</sup>

There are several in vitro and in vivo differences between SARS-CoV and MERS-CoV infections. There are radiographic differences, as well as differences in cytopathic effect. The

**Table 5** SARS and MERS coronavirus infections: virology, epidemiology, and diagnostic tests

	SARS	MERS
<b>Virology</b>		
Virus	SARS-CoV	MERS-CoV
Phylogeny	B $\beta$ CoV	C $\beta$ CoV
Host receptor	ACE2	DPP4 (CD26)
Cell entry pathway	Endosomal fusion	Cell membrane fusion
<b>Epidemiology</b>		
Origin	China	Middle East
Natural reservoir	Bats	Bats
Intermediate host	Civets, raccoon dogs	Camels
Seasonality	Winter	?
Mode of transmission	Person to person, droplet, contact, airborne	Animal to human, droplet, contact, airborne (?)
Incubation period	2–14 d (up to 21 d)	2–15 d
<b>Diagnosis</b>		
1° Specimens	Lower respiratory tract	Lower respiratory tract
RT-PCR test	+	+

Abbreviations: CoV, coronavirus; MERS, Middle East respiratory syndrome; RT-PCR, reverse transcription-polymerase chain reaction; SARS, severe acute respiratory syndrome.

**Table 6** Clinical findings, treatment, and prevention of SARS and MERS coronavirus infections

	SARS	MERS
Clinical findings		
Presenting syndrome	CAP or HAP	CAP or HAP
Extrapulmonary manifestations	Diarrhea	Acute renal failure, diarrhea
X-ray findings	Ground-glass opacities, ARDS	Focal to diffuse and/or consolidation, ARDS
Case-fatality rate	~10	>35
Treatment and prevention		
Antivirals used	Interferon and ribavirin	Ribavirin and IFN- $\alpha$ 2b
Passive immunization	Convalescent plasma therapy	Adaptive transfer of serum
Vaccine development	Yes—early phases	Yes—early phases

Abbreviations: ARDS, acute respiratory distress syndrome; CAP, community-acquired pneumonia; HAP, healthcare assisted pneumonia; IFN, interferon; MERS, Middle East respiratory syndrome; SARS, severe acute respiratory syndrome.

host response to infection appears to be different. For example, there are observed differences in sensitivity to type I IFN *in vitro*. MERS-CoV is more sensitive to type I IFN compared with SARS-CoV.<sup>208</sup> SARS pathogenesis is controlled in part by innate immune signaling. SARS-CoV encodes proteins that modulate innate immune signaling by antagonism of IFN induction and by avoiding IFN-stimulated gene effector functions (► **Table 6**).<sup>209</sup>

### Epidemiology

Coronaviruses were detected as agents of respiratory infections approximately 40 years ago.<sup>210–212</sup> They were later identified as coronaviruses, labeled “OC43” and “229E,” and accepted as a new genus in 1975. In epidemiologic studies in adults, coronaviruses were estimated to cause 15% of adult common colds. Coronaviruses were found to cause epidemics every 2 to 3 years with reinfections being common. All ages are susceptible. From epidemiologic studies, coronaviruses were found to be associated with respiratory illnesses, usually in the upper respiratory tract, but occasionally causing pneumonia. In temperate climates, HCoV-OC43 and HCoV-229E are transmitted primarily during the winter. They have been linked to asthma and COPD exacerbations in children and adults.<sup>125,213</sup>

In 2004 and 2005, three closely related coronavirus species were reported.<sup>214–219</sup> NL63 was isolated from a 7-month-old girl with coryza, conjunctivitis, fever, and bronchiolitis. From epidemiologic studies, patients with HCoV-NL63 have ranged in age from 1 month to 100 years, with the highest infection rate occurring before age 5 years. Using molecular probes that targeted conserved regions of the coronavirus genome, a related coronavirus (HCoV-NH) was found in 79 of 895 young children tested by RT-PCR on respiratory specimens.

In a prospective study in Hong Kong, coronaviruses were detected in 2.1% of patients admitted to the hospital with signs and symptoms of acute respiratory illness.<sup>220</sup> Of the 87 infected patients, 13 were positive for HCoV-HKU1, 17 were positive for HCoV-NL63, 53 were positive for HCoV-OC43, and 4 were positive for 229E. HCoV-HKU1 and HCoV-OC43 peak in the winter months. Upper respiratory tract illness was the

most common presentation for HCoV-HKU1 infections. HCoV-NL63 infections occurred in early summer and fall but not in the winter.

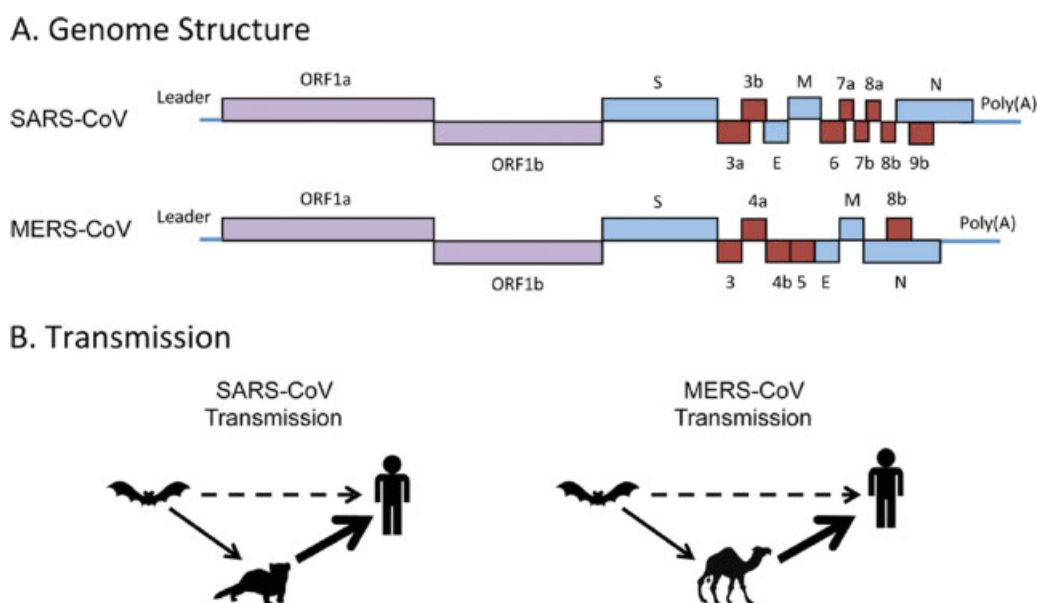
Using newer molecular assays, the group at Vanderbilt reassessed the role of the newly described coronaviruses in a large cohort of healthy children who had been followed up prospectively for 20 years.<sup>221</sup> Of the LRI samples available for screening, 8.4% had positive results for HCoV and all were younger than 2 years. AOM was found in half of the HCoV-infected children, but none of the children were hospitalized. Of the URI samples tested, 4.7% had detectable HCoV RNA. Fifty-one percent of these positive children were diagnosed with AOM. The burden of URI attributable to HCoV had significant year-to-year variation.

A recent study has provided evidence of genetic variability in OC43 strains.<sup>222</sup> The complete nucleotide sequence of two contemporary OC43 strains compared with the prototype strain (ATCC VR 759) demonstrated important amino acid substitutions in the potential cleavage site sequence of the spike protein.

In 2002, the first cases of SARS were reported in Guangdong Province of China.<sup>223</sup> Over the next few months, 29 countries reported cases in more than 8,000 patients with approximately 10% fatalities. A patient who acquired SARS in Guangdong traveled to Hong Kong and is thought to be the index case for over half of the cases. It was different from known human and animal coronaviruses by DNA sequencing. This new coronavirus was cultured from Himalayan palm civets, but it is now thought that bats are the primary reservoir (► **Fig. 1**). SARS infected more than 8,000 people resulting in more than 700 deaths.

MERS epidemiology originally implicated the dromedary camel as the source of human cases (► **Fig. 1**).<sup>224,225</sup> Of the first 500 confirmed cases, 62% required hospitalization. Comorbid medical conditions were common and males were more likely to be identified as infected. The median age was 56 years. Secondary cases were commonly identified in younger health care workers. The incubation period was 5.2 days (range: 1.9–14.7 days). Mortality rates were reported to be approximately 35%.<sup>226,227</sup>





**Fig. 1** (A) Genome structures of SARS-CoV and MERS-CoV. The single-stranded, positive-sense coronavirus genomes encode the structural proteins (blue), membrane (M), spike (S), envelope (E), and nucleocapsid (N), two replicase polyproteins (purple), ORF1a and ORF1b, and unique accessory proteins (red) that perform important functions in coronavirus replication and pathogenesis, such as blocking the innate immune signaling pathway. (B) Transmission routes. SARS-CoV transmission is thought to be from bats harboring SARS-like viruses to palm civet cats, which infected humans. SARS-CoV could also have been transmitted from bats to humans directly. MERS-CoV is thought to be transmitted from camels to humans, with the possibility that at some point bats infected camels. The dashed line identifies a low-level transmission event, the thin solid line identified a potential transmission event, and the thick solid line identifies a probable transmission event. CoV, coronavirus; MERS, Middle East respiratory syndrome; SARS, severe acute respiratory syndrome. (Reproduced from Coleman CM, Frieman MB. Coronaviruses: important emerging human pathogens. *J Virol* 2014;88:5209. doi: 10.1128/JVI.03488-13 with permission of the American Society for Microbiology.)

In May 2014, two cases of MERS-CoV infection in the United States were reported by the Centers for Disease Control and Prevention (CDC).<sup>228,229</sup> Both patients had acquired their infection while working in health care in Saudi Arabia. No contacts of these patients developed infections with MERS-CoV.

### Diagnosis

Coronaviruses require special cell lines or organ culture for detection by cultivation methods.<sup>230</sup> These cell or organ culture techniques are labor intensive, time consuming, and relatively insensitive. Coronaviruses have also been detected by RT-PCR with greater sensitivity than standard culture techniques.<sup>231</sup>

SARS-CoV was initially detected by RT-PCR and culture. Viral RNA is found in high levels in the feces of SARS patients. To confirm SARS-CoV infection, the WHO criteria require detection of viral RNA by PCR, increase in antibody titers in body fluids, or isolation of SARS-CoV from clinical isolates.<sup>232</sup> Laboratory diagnostic methods for SARS-CoV include (1) viral RNA detection using RT-PCR, (2) immunofluorescent antibody (IFA), and (3) ELISA. Virus isolation is less sensitive than these other methods and requires a biosafety level 3 (BSL-3) facility.

Antibody assays are reported for coronaviruses, but are not readily available or helpful clinically. Complement-fixing and ELISA antibody assays for coronaviruses 229E and OC43 have been published, but are not available in clinical laboratories.<sup>233,234</sup> Therefore, serologic tests for antibody-specific

responses are mainly reserved for research or epidemiologic studies.

Serological assays are a complement to nucleic acid detection assays for SARS-CoV and MERS-CoV.<sup>235-237</sup> They have been helpful in identifying the source of the infection, in transmission pattern analysis, patient contact studies, and diagnosing asymptomatic cases. Even with the number of assays available after the SARS and MERS outbreak, differentiating HCoVs by serologic means remains a problem. Viral neutralization tests remain the most specific assay available, but are limited to only a few laboratories.

Animal virologic data from surveillance studies support the theory that MERS originated from bats in Africa and crossed species barriers to infect camels many years ago. Infected camels were then transported to the Middle East where they transmitted MERS-CoV to humans. A recent report showed through complete genome sequencing that a virus isolated from a dromedary camel was identical to a human strain isolated from sick humans who had developed MERS following close contact with sick camels.<sup>238</sup> The mode of transmission from camels to humans is currently unknown, although droplet transmission seems likely.

Laboratory confirmation of MERS required nucleic acid amplification assays. The WHO criteria for confirmed cases includes (1) + RT-PCR for two different specific targets or (2) +RT-PCR from one specific target plus an additional different RT-PCR product sequenced and confirmed as MERS-CoV. A BSL3 is required for viral culture and neutralizing antibody detection assays.

### Clinical Features

In a prospective study of respiratory viral infections among hospitalized patients, 5.7% had coronaviruses identified.<sup>220</sup> The 47 coronavirus infections represent 10.5% of all the respiratory viral infections. In 14 patients, coronaviruses were associated with another respiratory virus. Lower respiratory tract infections (bronchitis, bronchiolitis, and pneumonia) were far more common than upper respiratory tract (rhinitis, pharyngitis, and laryngitis) infections, 75 versus 25%, respectively. Over half of the infections were due to OC43-like strains. Approximately 20% were due to 229E-like strains and approximately 20% were due to NL63-strains. Coronavirus infections in the first year of life were associated predominantly with OC43-like strains.

HCoV was identified in 5.4% of specimens from 279 hospitalized adult patients with lower respiratory tract infections.<sup>239,240</sup> The most frequently identified isolates were HCoV-OC43 in 12, followed by HCoV-229E in 7, HCoV-NL63 in 6, and HCoV-HKU1 in 4 specimens. Many patients had high-risk underlying conditions. In several recent studies evaluating multiplex PCR assays, HCoVs were detected in 3 to 8% of hospitalized children younger than 5 years with acute respiratory illnesses.<sup>241–243</sup>

Patients with SARS present with fever, cough, and/or myalgias. Dyspnea and hypoxemia were noted during the second week of illness. Rapidly progressive respiratory failure then occurred in a subset of patients. Patients appeared to be contagious after lower respiratory tract signs were observed.<sup>244,245</sup>

Nonspecific presenting symptoms are common in MERS-CoV-infected patients. Fever, chills, and sore throat are frequently reported.<sup>246</sup> Respiratory tract symptoms are also quite common. Within a few days of symptoms, respiratory failure can develop. Chest radiographic findings progress from a unilateral focal lesion to extensive multifocal or bilateral involvement, but are not specific to MERS-CoV infections.<sup>247,248</sup> The most common CT findings are bilateral, often basilar, airspace involvement. Cavitation has not been reported. Late CT findings in recovered patients may include fibrotic changes and organizing pneumonia.

Acute renal failure has often been observed during the second week of illness with MERS virus. This was also seen in a small percentage of SARS patients. Dialysis was often required. Other nonrespiratory tract involvement has been reported to include diarrhea (7–25% of patients), nausea, vomiting, pericarditis, and arrhythmias.<sup>249</sup>

Complications of MERS include superinfection or coinfection, septic shock, and delirium. ICU care is often needed within 5 days of admission. The case-fatality rates have ranged from 25 to 76%. As more cases were identified, there have been more asymptomatic or mild cases identified. These have predominantly been in young, healthy women without significant comorbidities.

### Treatment

There are no published randomized controlled trials of specific antiviral agents in the treatment of SARS or MERS. Supportive therapy is currently the only recommended plan.

When renal failure occurs, renal dialysis is necessary. Mechanical ventilation in an ICU is often required. Several candidate antiviral agents have been identified and include IFNs, ribavirin, and cyclophilin inhibitors.<sup>250–257</sup> Both in vitro cell culture experiments and animal studies have shown antiviral activity of these agents against MERS-CoV. However, very few human cases who received a combination of IFN- $\alpha$ 2b, ribavirin, and corticosteroids survived.

### Infection Control

Prompt isolation of suspected SARS or MERS cases and contact tracing of case contacts are the keys to preventing nosocomial transmission.<sup>258–260</sup> Standard, contact, and droplet precautions should be used in all cases. Airborne precautions should be employed for aerosol-generating procedures, bronchoscopy, tracheostomy, and airway insertion.

Vaccine design for SARS-CoV and MERS-CoV has focused on the development of chimeric spike glycoproteins containing neutralizing epitopes from multiple strains within or across subgroups.<sup>261–267</sup> Inclusion of nucleocapsid protein in chimeric vaccines could broaden the protective response. The S protein is the major determinant of protective immunity. Antibodies against S protein appeared to protect from SARS-CoV challenges in animal studies. N protein-specific immune response provides little protection and only cross-react within, but not between, subgroups. Thus, there are no approved vaccines for MERS or any of the CoVs. The use of convalescent-phase plasma or immune globulin with high titers of neutralizing antibody has not been evaluated in randomized controlled trials.<sup>268</sup>

### Conclusion

HRVs and HCoVs cause significant morbidity in immunocompetent people and in patients with underlying chronic or immunosuppressed medical conditions. Newer diagnostic tests have expanded our understanding of these respiratory viruses in clinical infections. These sensitive diagnostic tests have been used to detect new HRVs, such as HRV-C. Recent studies on the pathogenesis of HRVs and the host response to this group of viruses have provided insights into potential targets for therapeutic interventions. The recent outbreaks of SARS and MERS pose special problems in treatment and infection control.<sup>269–271</sup> Vaccine development for HCoVs and/or coronaviruses appears to be many years away.

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