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Emerging Respiratory Viruses Other than Influenza

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KEYWORDS

• Respiratory viruses • Middle East respiratory syndrome coronavirus • Adenovirus • Human rhinovirus • Human bocavirus

KEY POINTS

- Noninfluenza viral respiratory tract infections are a significant cause of morbidity and mortality worldwide.
- Early detection and characterization of novel and emerging viruses is important in limiting further transmission.
- Middle East respiratory syndrome coronavirus infection can cause severe respiratory illness with high mortality rates, and all cases to date have been epidemiologically linked to the Middle East region.
- Adenovirus 14 is associated with outbreaks of acute respiratory disease in military camps and the general population.
- Rhinovirus C and human bocavirus type 1 are commonly detected in infants and young children with respiratory tract illness, and are often associated with severe disease requiring hospitalization.

INTRODUCTION

Viral respiratory tract infections (VRTIs) are some of the most common infections worldwide, and represent a major public health concern. Noninfluenza respiratory viruses cause infections in all age groups and are a major contributing factor to morbidity and mortality. Disease severity can range from mild, common cold-like illness to severe, life-threatening respiratory tract infection. The burden of noninfluenza VRTIs is often more pronounced in individuals with chronic comorbidities or clinical risk factors. Moreover, it is estimated that 500 million noninfluenza VRTIs occur annually in the United States, resulting in combined direct and indirect costs of $40 billion.[1]

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In the past, a significant proportion of respiratory tract disease could not be attributed to a specific pathogen. With the advent of molecular detection and genotyping techniques, there has been a substantial increase in the recognition of several newly identified noninfluenza respiratory viruses involved in disease. These potential pathogens have included severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), adenovirus type 14 (Ad14), human rhinovirus species C (RV-C), and human bocaviruses. Diagnostic testing for these and other viruses is important because many of the signs and symptoms of infection overlap those of other viruses such as influenza, and would otherwise be ascribed to cases of influenza-like illness without an etiologic assessment.

Coronaviruses are ubiquitous worldwide and were associated with relatively mild respiratory disease (eg, the common cold) up to the emergence of the SARS-CoV in China in 2002. The SARS epidemic spread to 29 countries and infected more than 8000 people, with a case-fatality rate of approximately 10%. However, additional cases have not been documented since 2004. Nearly 10 years later, another virulent coronavirus, MERS-CoV, emerged. The index case of MERS-CoV occurred in Saudi Arabia in June 2012.[2] As of November 20, 2013, there have been 157 laboratory-confirmed cases and 66 deaths reported from 9 countries (**Fig. 1**).

Adenovirus-associated respiratory disease is most often associated with species B and C, with serotypes 3, 4, 7, and 21 being associated with outbreaks of acute febrile respiratory illness, particularly in military trainees. Vaccines against adenoviruses Ad4 and Ad7 were available for military recruits from 1971 to 1999, which decreased the burden of adenovirus-associated acute respiratory disease (ARD) in that population. Before vaccination efforts, adenovirus reportedly infected 10% to 20% of trainees and caused 90% of pneumonia cases at military training camps.[3,4] Ad14 (species B) has emerged as a new source of ARD in military trainees and the general public. Ad14 ARD was first described in 1955 in Dutch military recruits, but had been rarely reported since until 2 major outbreaks at military training centers in the United States occurred in 2007. Ad14 is now known to cause potentially severe acute respiratory illness in both military and civilian individuals.

RV-C and human bocavirus type 1 (HBoV1), recently identified by molecular methods, have been found to be prevalent, widely distributed geographically, and frequently associated with severe respiratory disease following primary infections, particularly in young children. RV-C is also an exacerbating factor in asthma and other chronic obstructive airway diseases. Serologic and quantitative polymerase chain reaction (PCR) analyses have provided compelling evidence for HBoV1 being the etiologic agent of several forms of respiratory tract disease.

This review details the virologic, epidemiologic, clinical, and diagnostic aspects of these viral species.

MICROBIOLOGY

MERS-CoV

Coronaviruses are enveloped, single-stranded, positive-sense RNA viruses with a relatively large genome (27–32 kb). The spike (S) glycoprotein protrudes from the virion, giving the virus its characteristic crown-like (ie, “corona”) appearance under electron microscopy. Only 6 coronaviruses have been described that infect humans, all belonging to the Alphacoronavirus and Betacoronavirus genera. CoV 229E and NL63 are alphacoronaviruses, whereas OC43, HKU1, SARS, and MERS are betacoronaviruses.[5] Both 229E and OC43 were identified by viral culture in the mid-1960s. NL63 and HKU1 were not described until after the SARS epidemic in

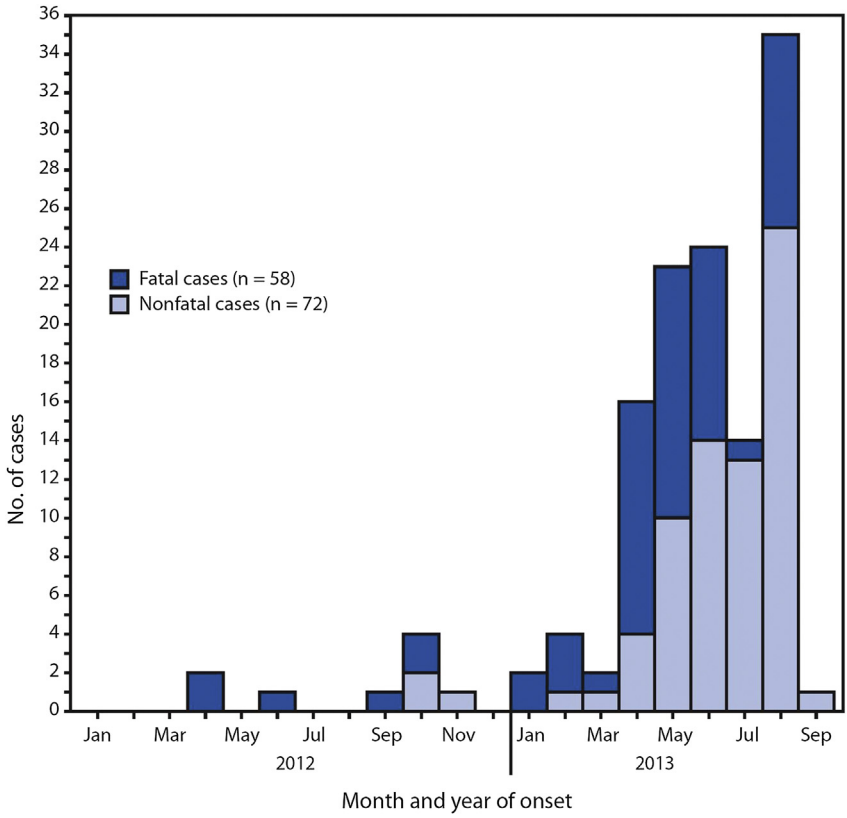

Fig. 1. Cumulative worldwide cases of Middle East respiratory syndrome coronavirus as of September 20, 2013 reported by month of illness onset. (*From* Centers for Disease Control and Prevention. Updated information on the epidemiology of Middle East respiratory syndrome coronavirus (MERS-CoV) infection and guidance for the public, clinicians, and public health authorities, 2012–2013. Morb Mortal Wkly Rep 2013;62:793–6.)

2003, and were detected by nucleic acid methods. However, studies have shown these viruses, unlike SARS-CoV and MERS-CoV, were not recently introduced to the human population. All human CoV have been cultured in vitro with the exception of HKU1.[5]

Ad14

Adenoviruses are large (70–90 nM) double-stranded DNA viruses. As a nonenveloped virus, adenovirus is stable in the environment, and is somewhat resistant to detergents and adverse environmental conditions. The linear DNA genome is associated with 2 core proteins and has a terminal protein covalently attached to each 5′ end. The adenovirus icosahedral capsid is formed by 7 structural proteins, including the hexon, penton base, and fiber.[6] The fiber protein is a spike-like projection with a terminal knob that interacts with host cell receptors along with the penton base. There are 7 species (A–G) of adenovirus that are grouped by oncogenic potential in rodents, hemagglutination properties, and DNA homology. Using serum neutralization and/or hexon gene sequencing, species are further characterized into serotypes. More than 60 serotypes have been described, which cause a variety of clinical syndromes (**Table 1**).

Table 1
Adenovirus species and serotypes, and associated clinical syndromes

Species	Serotypes	Clinical Syndromes
A	12, 18, 31, 61	Unknown; oncogenic in hamsters
B	3, 7, 11, 14, 16, 21, 34, 35, 50, 55	Respiratory infections (7, 14, 21 particularly in military recruits), conjunctivitis, hemorrhagic cystitis (7, 11, 21), myocarditis (7, 21), meningoencephalitis (7), disseminated disease (11, 34, 35)
C	1, 2, 5, 6, 57	Respiratory infections, intussusception, disseminated disease (1, 2, 5)
D	8–10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42–49, 51, 53, 54, 56, 58, 59, 60, 62–65	Respiratory infections, conjunctivitis
E	4	Respiratory infections (particularly in military recruits), conjunctivitis
F	40, 41	Gastroenteritis
G	52	Gastroenteritis

RV-C

A series of studies published in 2006 and 2007 described a novel clade of RVs detected in respiratory specimens from patients with acute respiratory illness, asthma, and influenza-like illness that were genetically distinct from existing rhinoviruses,[7–9] subsequently designated RV-C. RVs are now divided into 3 species based on phylogenetic analyses: 2 well-characterized species, A and B, and the novel RV-C species. Although newly described, there are indications that RV-C has been circulating for decades.[10] It seems that RV-C went undetected for years because it does not propagate using traditional virus-isolation methods.[9,11–13] Historically, RVs have been classified according to serotype, which is based on phenotypic characteristics. However, variants of RV-C are not currently assigned to serotypes, as methods for their in vitro culture have not been successful to date and their cross-neutralization properties remain unknown. RV-A currently comprises 77 different serotypes and RV-B 30 types, and, based on genotypic relatedness, RV-C can be separated into at least 60 (geno) types.[14] RVs are small, nonenveloped viruses of approximately 30 nm in diameter with a single-stranded, positive-sense RNA genome of approximately 7.1 kb, which belong to the genus Enterovirus within the family Picornaviridae. The genome organization of all RVs consists of a 5′ untranslated region (UTR), a single open reading frame encoding a single polyprotein, and a 3′ UTR before a polyadenylated tract (**Fig. 2**). RV-Cs are more genetically diverse than the other RV species, and the RV-C genome is the shortest among other reported RVs and human enteroviruses.[9,11] There are also several unique genetic elements that distinguish RV-C from the other species.[9,15]

HBoV1

HBoV1 was first identified in 2005 from respiratory secretions from patients who had pneumonia.[16] Although most commonly detected in the respiratory tract and sometimes in stool, it can also be found in blood, cerebrospinal fluid, and tonsillar tissues. HBoVs are members of the proposed genus Bocaparvovirus in the family Parvoviridae. These small, nonenveloped, icosahedral viruses are approximately 25 nm diameter with a 5.3-kb single-stranded DNA genome containing 3 open reading frames

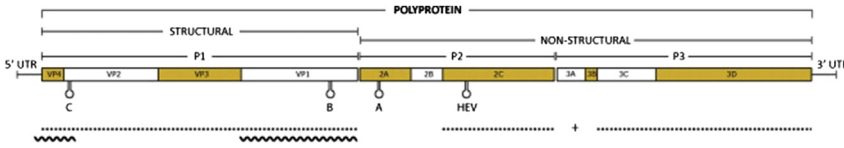

Fig. 2. The approximately 7200-nucleotide, positive-sense, single-stranded RNA genome of rhinovirus C. Regions sequenced for speciating are indicated by the dashed line, and those more commonly used by the wavy line. HEV, human enterovirus; UTR, untranslated region. (*Adapted from* McErlean P, Shackelton LA, Andrews E, et al. Distinguishing molecular features and clinical characteristics of a putative new rhinovirus species, human rhinovirus C (HRV C). PLoS One 2008;3(4):e1847.)

(ORFs). The first 2 sequential ORFs encode nonstructural proteins NS1 and NP1, and the third downstream ORF encodes 2 viral capsid proteins, VP1 and VP2.[16,17] Virus replication depends on the host cell machinery (eg, DNA polymerase). By 2010, 3 additional genotypes, HBoV2, 3, and 4, had been identified in human stool samples from children with gastrointestinal illness[18,19] and have since been rarely detected in respiratory specimens.[20,21] There are some data linking HBoV2 with gastroenteritis,[19] but HBoV3 and HBoV4 have not been conclusively associated with any clinical illness.

EPIDEMIOLOGY

MERS-CoV

To date, all human cases of MERS have had an epidemiologic link to the Middle East (Jordan, Saudi Arabia, United Arab Emirates, or Qatar) (**Fig. 3**). Both health care–associated and familial transmissions have been documented. MERS-CoV is closely related to other betacoronaviruses that have been recovered from bats in Hong Kong, Mexico, Europe, and Africa.[2,22] In addition, serologic studies have determined that dromedary camels in Oman have been exposed to MERS-CoV, possibly linking camels to the transmission cycle.[23]

Ad14

Adenovirus species B can be further subdivided into B1 and B2. Species B1 (3, 7, 16, 21, 50) are generally associated with respiratory disease, whereas members of species B2 (11, 14, 34, 35) tend to infect the genitourinary and/or respiratory tracts. Adenoviruses are transmitted by respiratory droplets and the fecal-oral route as well as by fomites. Military recruits are highly susceptible to adenovirus infections, including pneumonia and ARD likely attributable to crowding and numerous stressors.[3] Ad14 was first described in 1955 during an outbreak of ARD at a military training facility in the Netherlands. Rare outbreaks were described in the 1950s and 1960s in Great Britain, Uzbekistan, and Czechoslovakia, but there were no further described cases or outbreaks caused by Ad14 until sporadic cases were described in United States military recruits in 2006.[24] Outbreaks of Ad14 occurred in the United States at 3 military training facilities in 2007. In the landmark outbreak in San Antonio, approximately 550 trainees were infected with Ad14, although not all individuals had severe disease.[25] Subsequently, Ad14 was detected in 5 additional military bases and in civilians in Washington, Oregon, Alaska, Wisconsin, Pennsylvania, New York, and California.[26–28] From March to June 2007, 140 Ad14 cases were confirmed in 4 states, including 38% hospitalizations (17% intensive care) and 5% mortality.[26] The sequences of the Ad14 viruses from these outbreaks were identical but different from the 1955 virus from the Netherlands, suggesting the emergence of a new

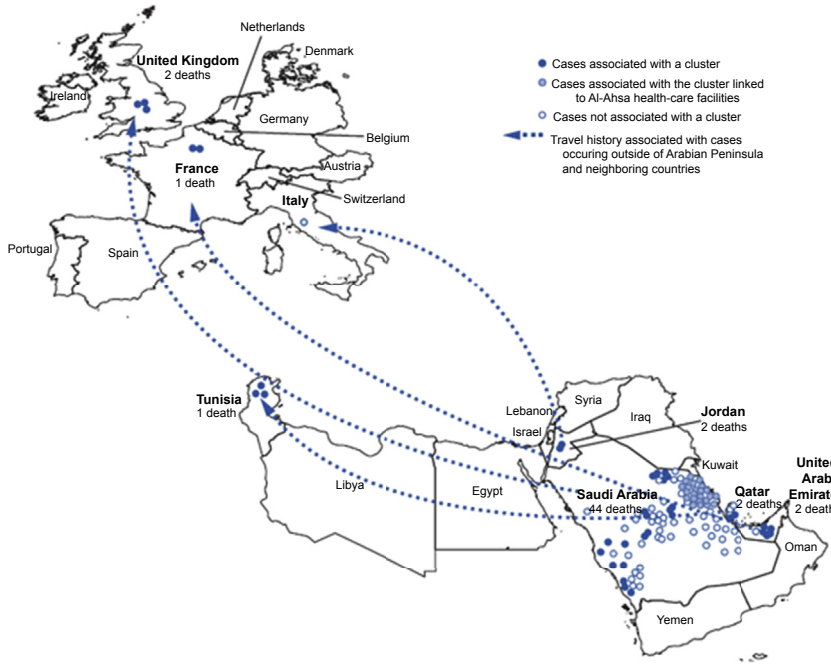

Fig. 3. The first 55 confirmed cases of Middle East respiratory syndrome coronavirus and associated travel history within 14 days of illness onset. (*From* Centers for Disease Control and Prevention. Updated information on the epidemiology of Middle East respiratory syndrome coronavirus (MERS-CoV) infection and guidance for the public, clinicians, and public health authorities, 2012–2013. Morb Mortal Wkly Rep 2013;62:793–6.)

variant.[26] It is likely that the cessation of adenovirus vaccination efforts in the US Military in 1999 likely contributed to the reemergence of adenovirus-associated ARD on military bases, including Ad14-associated disease.

RV-C

Similarly to other RVs, it is thought that RV-C can be transmitted person to person by both direct and indirect contact with aerosolized virus, and infection is efficiently initiated by intranasal and conjunctival inoculation.[29] RV-Cs have been detected on all continents throughout the world and often show seasonal patterns of infection similar to that identified for RVs in general, with high incidence peaks in early fall and late spring in most temperate or subtropical countries, and during the rainy season in the tropics.[9–11,30–34] In some areas, they may peak in winter or be evenly distributed year-round. RV-A and RV-C often cocirculate in near-equivalent proportions or may alternate as the most common RV species at different times of the year.[35,36] RV-B infections typically occur less frequently. The overall prevalence of RV-C in published studies of adults and children with respiratory symptoms ranges from 1.4% to 30.9%, with RV-C accounting for 14% to 81% of all RVs tested.[8,10,13,32,37–42] Differences among patient groups, specimen types, and detection methods may account for the broad range in reported prevalence.

The VP4/VP2 region has been most commonly used for genetic characterization of RVs in clinical samples,[43,44] and more recent phylogenetic analyses suggest that nearly full VP1 region sequencing can reliably separate the clades.[14] Use of the

5′ UTR sequences alone in species determination is sometimes problematic because of recombination events that affect the region.[43,45,46] Using molecular dating analysis of sequences of the VP4/VP2 coding regions, it is projected that RV-Cs have been circulating for at least 250 years, with an estimated evolutionary rate of 6.6×10^{-4} substitutions per site per year.[10]

HBoV1

The modes of transmission for HBoV1 are largely unknown, but may be similar to those of other parvoviruses that can be transmitted by various routes, including respiratory, urine, and fecal-oral.[47] Seroepidemiology studies indicate that HBoV1 is distributed worldwide and that more than 90% of individuals have been exposed, often early in life.[48,49] HBoV genotypes 2 to 4 may also be distributed globally, but their seroepidemiology has been complicated by cross-reactivity.[50] HBoV1 seropositivity is common in infants younger than 2 months owing to transfer of maternal antibody, after which it declines and then increases again because of primary infections, until 6 years of age when most children are seropositive.[48,51–55] The prevalence of HBoV1 in respiratory infections ranges from 1.6% to 21.5%, mainly in children younger than 3 years during the winter and spring months.[16,55–59] HBoV1 is detected infrequently in older age groups including adults,[58,60] although most have antibodies to HBoV1.[48,54,61] HBoV1 is estimated to be among the 4 most prevalent viruses along with respiratory syncytial virus (RSV), rhinoviruses, and adenoviruses in children hospitalized for respiratory disease.[54,62–64] However, serologic studies have shown that the mere presence of HBoV1 DNA in the respiratory tract is not proof of an acute primary infection.[48,50,65] Prolonged viral shedding could explain why some studies found HBoV1 DNA more frequently in asymptomatic than symptomatic cases,[58,66,67] and the high percentage of coinfections.[55,60] HBoV1 DNA has been detected in stool samples in 0% to 13% of patients with or without gastroenteritis[55] and in 0% to 44% of respiratory specimens from asymptomatic individuals.[55,58,68]

CLINICAL PRESENTATION

MERS-CoV

Individuals infected with MERS-CoV typically present with rapidly progressive pneumonia, with abnormal chest radiographs ranging from mild to extensive unilateral and bilateral opacities.[69] In addition, a significant number of cases also present with or develop acute renal failure. A case series of 47 patients indicated that 98% had fever, 83% cough, 72% dyspnea, and 32% myalgia. Gastrointestinal symptoms were also noted, with 26% having diarrhea, 21% vomiting, and 17% abdominal pain. Interestingly 96% of patients had comorbidities, including diabetes (68%), hypertension (34%), chronic heart disease (28%), and chronic renal disease (49%).[69] The mortality rate of MERS is approximately 50%, which is much higher than the 10% mortality reported for SARS, and the case-fatality rate appears to increase with age. Mild and asymptomatic cases have also been identified, bringing the full spectrum of disease caused by MERS-CoV into question.[70]

Ad14

Similar to Ad4, Ad7, and Ad21, Ad14 primarily causes febrile respiratory illness (FRI) in the outbreak setting. FRI is defined as fever ($\geq$38°C/100.5°F) plus one other sign of respiratory illness or diagnosis of pneumonia. The most common presentation reported is pneumonia.[24] Cases of pharyngoconjunctival fever caused by Ad14 have also been reported. One of the military-associated outbreaks found the most common

symptoms of Ad14 infection to be cough (89%), sputum production (78%), chills (56%), dyspnea (56%), and sore throat and nausea (44%).[3] Chest radiographs typically show patchy or interstitial infiltrates, with occasional cases of lobar consolidation.[3,27] The patient's white blood cell count is typically normal (89%), although a neutrophilic and monocytic predominance has been noted. The primary risk factor is military training, although one outbreak also demonstrated smoking, advanced age (≥65 years), and Alaskan Native heritage as risk factors.[27]

RV-C

Like other respiratory viruses, the clinical syndromes associated with RV-C range from mild or even asymptomatic infections to acute lower respiratory tract illnesses including pneumonia, recurrent wheezing, and bronchitis. RV-C has been detected in patients with acute illness of upper respiratory tract and lower respiratory tract,[9,11,12,71,72] wheezing,[9,37,39,45,73] bronchiolitis,[11,74,75] asthma exacerbations,[9,13,45,72,76,77] exacerbations of chronic obstructive pulmonary disease, pneumonia,[9,30,32,74,78–80] common cold and flu-like illness,[38] pharyngitis[74] and croup-like cough,[15] rhinitis, nasal congestion, bronchitis, and dyspnea.[74] Nonrespiratory symptoms in RV-C–positive patients include fever, febrile convulsion,[15] otitis media,[81] pericarditis,[80] poor appetite, and apparent life-threatening events.[75] Data suggest that RV-Cs are more frequently detected in older children than are RV-As.[13,72,82] RV-C has been detected in blood samples of children with severe respiratory infection, and viremia was associated with a significantly higher concentration of virus in the nasopharynx.[83,84] In fact, higher RV-C viral loads in respiratory specimens have been associated with severe upper and lower respiratory tract disease, and the median peak viral load in patients with RV-C infection has been shown to be higher than in those patients with RV-A or RV-B infection.[40,85] Stool specimens have also revealed the presence of RV-C and other RV species with viral loads comparable with those of enteroviruses, sometimes along with other gastrointestinal pathogens.[86–88] The clinical significance of RVs in fecal samples remains unclear. In patients hospitalized with disseminated disease, RV-C was detected in respiratory, stool, pericardial effusion, urine, and plasma specimens.[78,80] Health care–associated outbreaks of respiratory infections have been attributed to RV-C in a neonatal intensive care unit[89] and a long-term care facility.[90]

Several studies have demonstrated clinically significant differences among patients infected with RV-C in comparison with those infected with other RV species. Such differences prevail in acute upper and lower respiratory tract infections,[11,12,30,41,44,71,74,79,91–95] wheezing,[9,37,39,45] asthma,[9,13,32,45,72,76,77] and cystic fibrosis exacerbations.[96] On the other hand, some investigators report no significant differences between RV-C and RV-A with respect to disease manifestations.[33,35,42,92,97–99] Confounding this issue is the fact that RV-C may be found in coinfections with other respiratory viruses in up to 42% of cases.[32,35,79,92,94] Nevertheless, as evidenced by cases of RV-C monoinfection, this species contributes substantially to respiratory tract infections, many of which require hospitalization.[32,79,94]

HBoV1

The clinical manifestations of HBoV1 respiratory tract infection have ranged from mild upper respiratory disease to severe, life-threatening pneumonia. However, the direct impact of HBoV1 infection of the respiratory tract is often difficult to assess because of its frequent detection in asymptomatic children[58,66,67] and coinfection with other respiratory viruses in symptomatic children; a rate of up to 83% in respiratory samples.[55] Many studies have demonstrated a positive correlation between respiratory illness

and high copy numbers of HBoV1 DNA or the presence of HBoV1 monoinfection,[53,54,56,59,62,100,101] with monoinfection being associated with higher viral load than coinfection. Likewise, detection of HBoV1 viremia is distinctly associated with respiratory symptoms, but not in asymptomatic individuals.[48,62] Infections in patients with HBoV1 viremia, serologic evidence of acute infection, high ($>10^4$ copies/mL) viral load or mRNA in respiratory secretions, and/or monoinfection include pneumonia,[52,54,56,59,64,101–106] acute wheezing,[48,62,104,107,108] bronchitis/bronchiolitis,[48,52,54,56,64,109,110] asthma,[54,56,64] and acute otitis media (**Table 2**).[49,52,104,111,112] Reports of severe respiratory disease found a significant association between HBoV1 and otherwise unexplained lower respiratory tract infections.[101,113–115] In children, HBoV1 may cause encephalitis and life-threatening complications.[113,114,116,117] HBoV1 can be detected in tonsillar lymphocytes and adenoids of pediatric patients, suggesting a role in the pathogenesis of chronic tonsillar diseases.[118,119] Compared with other viruses known to cause respiratory illness in young children, patients with HBoV1 monoinfection may be older than those with RSV[48,56,64,103,109] or human metapneumovirus[56] infection. In some studies, HBoV1 monoinfection was associated with shorter duration of hospitalization,[56,110] higher C-reactive protein levels[64,109] and white blood cell counts,[64] lower clinical severity score,[110] and less frequent hypoxia compared with RSV-monoinfected patients (**Table 3**).[107]

PATHOGENESIS

MERS-CoV

Cell culture–based experiments have shown that the MERS-CoV receptor is different from the one used by SARS-CoV, and has been identified as DDP4 (dipeptidyl peptidase 4), which interacts with the viral S protein.[120] MERS-CoV also appears to have a broader host range than that of SARS-CoV, infecting human, primate, porcine, and bat cell lines.[120] Because there is not yet a small animal model for MERS-CoV infection, the pathogenesis has not been fully elucidated. However, viral antigen has been localized to the ciliated bronchial epithelium and unciliated cells of terminal bronchi. MERS-CoV replication has also been demonstrated in both type I and type II pneumocytes. Thus, it appears that MERS-CoV affects the alveoli and gas exchange as well as surfactant production and tissue repair.[121]

Ad14

The tissue tropism of adenovirus varies by species and serotype, and is thought to be mediated by specific fiber-receptor interactions. Species C and E, and some species B viruses infect the respiratory tract while other species B viruses infect the urinary tract; species A and F replicate in the gastrointestinal tract, and species D in the eyes.[122] Ad14 belongs to species B2 along with Ad11, Ad34, and Ad35, which have a predilection for the respiratory epithelium. Replication in the respiratory epithelium leads to cell death in the host; however, it is not clear whether the cytopathic effect of adenovirus is due to viral pathogenesis or the host immune response.[123] Adenoviruses also evade the host immune response by inhibiting interferon activity, apoptosis, and expression of major histocompatibility complex class I.[123] These immunomodulatory functions may also play a role in the development of latency or persistence for some of the adenovirus species (namely, species C).

RV-C

Early experiments have shown that RVs have a relatively low optimal temperature for growth (33°C), which may reflect their adaptation to the human nasopharynx and

Table 2
Number (%) of patients with indicated clinical and laboratory findings and human bocavirus type 1 (HBoV1) infection diagnosed serologically, as monoinfection, DNAemia, or mRNA in respiratory samples

N	CXR	Fever	Cough	Wheeze	Tachypnea	Dyspnea	AOM	Hosp.	Mean Age (y)	Serology	DNAemia	mRNA	$>10^4$ cop/mL	Monoinfection	Ref.
15	15 (100)	14 (93.3)	13 (86.7)	8 (53.3)	—	—	—	11 (73.3)	1.8	—	—	—	—	15	103
12	—	5 (41.7)	12 (100)	12 (100)	9 (75)	9 (75)	—	12 (100)	—	—	—	—	—	12	108
10	—	5 (50)	9 (90)	4 (40)	—	4 (40)	2 (20)	7 (70)	0.8	—	—	10	9	6	104
28	8 (28.6)	18 (64.3)	—	28 (100)	—	—	—	28 (100)	1.6	—	—	—	—	28	107
12	—	10 (83.3)	11 (91.7)	—	5 (41.7)	—	—	7 (58.3)	2.3	12	—	—	—	7	102
14	5 (35.7)	9 (64.3)	—	14 (100)	—	—	—	14 (100)	0.8	—	—	—	—	14	109
12	9 (75)	—	—	11 (91.7)	—	—	5 (41.7)	—	1.3	—	12	—	10	12	48,62
Occ.[a]	56.1%	67.0%	91.8%	84.6%	58.3%	59.1%	31.8%	86.8%	—	—	—	—	—	—	

Serology: detection of antibodies to HBoV1 consistent with primary infection.
DNAemia, detection of HBoV1 DNA in blood.
$>10^4$ cop/mL, greater than 10^4 copies/mL of HBoV1 DNA in respiratory samples.
Abbreviations: AOM, acute otitis media; CXR, abnormal chest radiograph; Hosp., hospitalized patients; N, number evaluated in each study.
[a] Percent occurrence of finding among all studies.

Table 3
Clinical and laboratory findings in patients with monoinfection with HBoV1, RSV, hMPV, and RV

Finding	HBoV1	RSV	hMPV	RV
Male	62.2%	55.9%	58.7%	63.1%
Fever	71.7%	77.9%	85.7%	60.9%
Abnormal chest radiograph	54.9%	50.9%	48.1%	40.5%
O_2 saturation <95%	55.5%	65.9%	56.2%	48.8%
Antibiotics administered	33.7%	34.0%	66.7%	42.3%
Range of mean age (mo)	4.9–21.6	2–18	5.9–33.6	3.1–46.8
Range of mean hospitalization (d)	1.3–5.6	1.6–7.2	3.5–6.2	0.8–6.9
Range of mean WBC ($\times 10^3$ cells/mm^3)	8.5–14.8	9.4–12.3	9.5–15.4	11.8–17.3
Range of mean CRP (mg/L)	7.5–50.1	8–35.8	15.3–56	18–77.6

Abbreviations: CRP, C-reactive protein level; HBoV1, human bocavirus type 1; hMPV, human metapneumovirus; RSV, respiratory syncytial virus; RV, rhinovirus; WBC, peripheral white blood cell count.

Data from Refs.[48,56,62,64,103,107,109,110]

association with upper respiratory tract infections. However, later experiments determined that there were minimal differences in replication capacities at 33°C and 37°C for several RVs.[124] RVs have been shown to replicate in the nasal epithelium and nasopharynx and, unlike influenza virus and RSV, do not cause destructive cytopathology of the upper respiratory tract. In the lower respiratory tract, RVs have been shown to cause changes in both interstitial and alveolar processes with inflammatory findings. RV-B and most RV-A variants use the intracellular adhesion molecule 1 receptor for cell entry, whereas a subset of RV-A types use the low-density lipoprotein receptor. The receptor used by RV-C types is currently unknown. The predicted capsid structure of RV-C differs significantly from those of RV-A and RV-B, particularly in regions relating to receptor and antiviral binding footprints.[11,15] RV-Cs do not grow in typical cell-culture lines but have recently been grown in vitro using sinus mucosal tissue or fully differentiated human airway epithelial cells, and the virus appears to use a cellular receptor distinct from that of RV-A and RV-B.[125,126] In general, shedding of RV-C does not extend beyond 3 weeks after resolution of symptoms,[45,82] but immunocompromised patients may shed virus for extended periods.[40]

HBoV1

HBoV1, like RV-C, is difficult to cultivate in vitro, and no definitive animal model of HBoV1 infection has been established. Thus, the mechanisms of virus replication and related host immune response remain largely unknown. Studies on children with pneumonia, acute wheezing, asthma, or bronchiolitis suggest that HBoV1 is able to infect the lower airways down to the bronchioles.[51,62,64,107] In some patients, HBoV1 infection may be systemic because viral DNA can be detected in blood and cerebrospinal fluid.[48,52,54,59,62,116] HBoV1 has recently been cultured in differentiated human airway epithelial cells, with documented productive infection causing cytopathogenesis.[127–129] Apical and basolateral infection of cultured epithelial cells results in disruption of the tight junction barrier, loss of cilia, and cell hypertrophy.[130] Recent studies have implied that HBoV DNA can exist episomally in infected human tissues and can likely establish persistent infection in the host.[131] It is not currently known whether this represents a latent state that can be reactivated, but HBoV1 has been detected frequently in tonsillar and adenoid tissues,[118,119,132] suggesting that

lymphatic tissue might represent a site of persistent infection, as well as in sinus mucosal tissues.[133] HBoV1 DNA has been detected for up to 6 months in serial nasopharyngeal specimens from otherwise healthy infants and young children,[68,134,135] and in respiratory samples for up to 5 months in immunocompromised pediatric patients.[136,137] Prolonged replication or passive persistence may account for the frequent presence of HBoV1 in both symptomatic and asymptomatic children.

DIAGNOSIS

For most VRTIs, diagnosis of the specific cause cannot be made based solely on clinical signs and symptoms. Establishing the viral etiology of infection is highly dependent on appropriate and accurate diagnostic methods. For many newly identified or emerging viruses, the prevalence of infections may have historically been underestimated owing to the lack of sensitive detection methods.

MERS-CoV can be cultured in LLC-MK2 and Vero E6 cell cultures,[2] although commercially available reagents are not available to identify the MERS-CoV virus in positive cultures. Furthermore, commercially available molecular tests (even those that detect other human CoVs) will not detect the MERS-CoV. However, the Centers for Disease Control and Prevention (CDC) have developed and validated laboratory-developed molecular tests for the detection of MERS-CoV.[138] The Food and Drug Administration (FDA) has issued an Emergency Use Authorization for the CDC real-time reverse transcriptase (RT)-PCR assay, and this test has been distributed to the Laboratory Response Network to aid in the identification of potential cases in the United States. The definition of a confirmed MERS case requires a positive RT-PCR test for at least 2 specific MERS-CoV targets, or a positive single target test with sequencing of a second target.

Respiratory infections caused by adenovirus are generally diagnosed using direct fluorescent antigen detection (DFA), viral culture, or nucleic acid amplification tests (NAATs). Several FDA-cleared NAAT-based tests that include adenovirus detection are currently available. DFA, culture, and NAATs all have a wide range of reported sensitivities because of the large number of serotypes and the genetic heterogeneity seen among adenoviruses. Ad14-specific NAATs have been described, but are generally used only for epidemiologic studies.[139]

RVs are best identified by RT-PCR targeting the 5′ UTR performed on respiratory secretions from infected individuals. Although relatively highly conserved in comparison with RV coding regions, sequence variation in the 5′ UTR between different RV types creates difficulties in designing primer pairs that can satisfactorily detect all RV types and species.[140] Specific identification of RVs using RT-PCR targeting the 5′ UTR is also complicated by the fact that enterovirus detection assays often cross-react with RVs because of sequence similarity in this region.[87] The high sensitivity of PCR can also be a limitation because the presence of viral nucleic acid in respiratory secretions of a patient with respiratory symptoms does not necessarily connote causality. RV-C, and other RVs, have been identified by RT-PCR in respiratory specimens from a high proportion of asymptomatic individuals,[55] possibly leading to an overestimation of the disease burden. However, one study that included asymptomatic controls identified RV-C more often in sick than in well patients, with RVs detected in only 3 of 93 asymptomatic individuals.[38] There is also some evidence that RV-C may interfere with infection by other viruses.[71,82] Co-detections of multiple viruses in respiratory infections have been seen more frequently as a result of the increased availability of multiplex and microarray detection assays.[141] The high co-detection rate also adds to the difficulty of differentiating RV-C as a true pathogen.

Some reports have indicated a correlation between higher viral loads and symptomatic disease.[85,142] However, quantitative RT-PCR testing is not readily available, and there is no standard for the quantification of all RV types. In general, accurate viral load testing of respiratory specimens can be challenging because of the various types of samples, collection techniques, and patient populations assessed.[143] Identification of the infecting species or serotype is of importance diagnostically for the detection of mixed infections or a reinfection with different RV serotypes, as well as in broader clinical investigations of the relationship between a serotype or species with disease severity and in epidemiologic investigations of the circulation and turnover of RVs.

HBoV1 can readily be detected by PCR assays targeting the NS, NP, or VP genes. However, as HBoV1 can be shed in respiratory secretions for weeks to months following primary infection or detected in asymptomatic individuals, detection of anti-HBoV1 antibodies in serum in addition to HBoV1 DNA detection can differentiate primary infection from long-term postinfectious shedding.[68] Serologic methods have been developed to detect HBoV1-specific immunoglobulin (Ig)M and IgG antibodies using recombinant capsid antigens or virus-like particles.[48,51–53,144] It is known that antibodies to HBoV2-4 can cross-react with HBoV1 antigens, so reliable detection of an HBoV1-specific response may best be achieved by depletion of reactive antibodies to the other genotypes.[50] An IgG-avidity enzyme immunoassay has been used to distinguish between acute and past infections, or between primary and secondary infections,[53] although the same antigenic cross-reactions may occur. Detection of HBoV1 DNA in blood is more closely associated with symptoms than are positive respiratory samples alone,[62,106] and higher viral loads in respiratory specimens correlate with acute infections, fewer coinfections, and increased disease severity.[48,51,52,54,56,59,101,108] Therefore, acute HBoV1 infection can more accurately be made by detection of DNA in serum or high viral load ($>10^4$ HBoV1 copies/mL) in respiratory samples along with detection of IgM antibodies or an increase in IgG response in paired serum samples.[48] To overcome the ambiguity and limitations of qualitative HBoV1 DNA detection in respiratory specimens, it has also been shown that the presence of HBoV1 mRNA (ie, actively transcribing virus) is more likely to indicate a causative role of the virus in acute respiratory tract disease.[104,105]

TREATMENT AND PROGNOSIS

As for many respiratory viruses, there is no definitive treatment for CoV-associated illness, which is generally not needed owing to the mild nature of most CoV infections (eg, 229E, OC43, NL63, and HKU1). However, the severe disease and increased mortality caused by both SARS-CoV and MERS-CoV demonstrates the need for antiviral options. Interferon-α2b in combination with ribavirin has been shown to reduce viral replication, moderate host immunologic response, and improve outcomes in rhesus macaques infected with MERS-CoV.[145] In addition, in vitro data have demonstrated that ribavirin, interferon-α, interferon-β, and mycophenolic acid have activity against MERS-CoV.[22] However, many of these drugs are nephrotoxic and may not be an appropriate choice for patients infected with MERS-CoV, which can also cause acute renal failure.

Although no specific treatment exists for adenovirus, both cidofovir and ribavirin show in vitro activity as well as limited clinical efficacy.[122] Cidofovir is an acyclic nucleoside phosphate with activity against several DNA viruses. Ribavirin, a nucleoside analogue, has broad antiviral properties, but clinical data on its efficacy are conflicting. Owing to the paucity of clinical efficacy data and side effects of treatment, off-label use of either drug for adenovirus treatment is generally reserved for disseminated

infections in immunocompromised patients. Treatment of ARD in immunocompetent patients is generally only supportive in nature. A live, attenuated vaccine for Ad4 and Ad7 was approved in 2011 for use in military recruits aged 17 to 50 years entering basic training, which may provide some cross-protection against other adenovirus serotypes.

There are no specific antiviral treatments for RV-C or HBoV1 infections. Therapy is supportive, just as for most respiratory virus infections. Vaccines have not been successfully developed for these viruses. For RVs, this is primarily due to the numerous serotypes and limited cross-protections between serotypes. For RV-C and HBoV1, standard precautions should be taken to limit transmission by respiratory secretions.

SUMMARY

Novel viruses are continuously discovered with surveillance programs and the application of new molecular techniques. Moreover, new introductions of viruses in areas where they had never been previously detected represent a challenge for diagnostic virology laboratories. To date the MERS epidemic is localized to the Middle East. However, MERS-CoV is the second CoV to emerge as a major human respiratory pathogen in the last 10 years. Further studies are needed to ascertain the full scope of disease and epidemiologic risk factors. Research aimed at refining the pathogenesis of MERS-CoV will be important in informing potential chemotherapy and interventional strategies. Although much was learned through the SARS epidemic, MERS-CoV belongs to a different lineage and appears to be more pathogenic. Surveillance efforts will need to continue to identify novel coronaviruses in bats and other potential hosts. Early detection of novel viruses, including coronaviruses, is an important strategy in limiting the spread of newly emerging viruses.

The combination of an immunologically naïve population and the emergence of an adenovirus with greater virulence and transmissibility has led to both military and civilian outbreaks involving Ad14. It is clear that Ad14 can cause outbreaks of respiratory disease that are largely recognizable by the severity of disease and increase in pneumonia and mortality. Mild respiratory infections attributable to Ad14 may go undiagnosed, because of either lack of testing altogether or lack of type-specific testing for Ad14. Therefore, it will be important to continue surveillance efforts, including adenovirus serotyping, to assess the full spectrum of Ad14-associated disease.

Until recently most VRTIs in infants and young children were attributed to established pathogens such as RSV, parainfluenza virus, and adenovirus. There is now evidence that newly identified species such as RV-C and viruses such as HBoV1 can be significant respiratory pathogens in young children. RV-C has been detected sometimes as a passenger and sometimes as a pathogen in acute respiratory tract disease; with monoinfection associated with high rates of morbidity. The pathogenic role of HBoV1 has been challenged, but diagnosis of acute infection using a combination of methods, including PCR of blood, quantitative PCR or detection of mRNA in respiratory secretions, and serology, has demonstrated that HBoV1 is responsible for a significant amount of respiratory tract disease in young children. The ability of newer diagnostic assays to simultaneously detect multiple respiratory viruses, including the newly identified ones, will help to clarify virus-host interactions that are still partially unknown, elucidate appropriate infection control measures, and monitor for respiratory outbreaks.

Accurate and timely diagnosis of viral infections is key to optimizing patient management, appropriate use of antivirals, reducing unnecessary tests and superfluous antibiotics, and implementing infection control precautions. Identification and

genotyping of novel viral pathogens also affects public health initiatives aimed at curtailing widespread outbreaks.

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