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Respiratory Viruses

Stuart Weston and Matthew B Frieman, University of Maryland School of Medicine, Baltimore, MD, United States

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Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| ARDS         | Acute respiratory distress syndrome |
| HAdV         | Human adenovirus |
| HBoV         | Human bocavirus |
| HCoV         | Human coronavirus |
| HMPV         | Human metapneumovirus |
| HRSV         | Human respiratory syncytial virus |
| HRV          | Human rhinovirus |
| ICU          | Intensive care unit |
| LRTI         | Lower respiratory tract infection |
| RT-PCR       | Reverse transcription polymerase chain reaction |
| SARS         | Severe acute respiratory syndrome |
| URTI         | Upper respiratory tract infection |
| WHO          | World Health Organization |

Introduction

Respiratory viruses are the leading cause of disease in humans, worldwide. While severe morbidity from respiratory virus infection occurs mostly in children, there is risk to healthy adults, the aged and immunocompromised as well. A wide range of virus families are able to infect and cause disease through the respiratory tract, and generally there is little treatment available other than supportive care. There is a critical need for understanding pathogenesis of infection and the development of therapeutics for this wide-ranging category of viruses.

Most common respiratory viruses cause mild cold symptoms in healthy individuals, resulting in significant loss of productivity. The recently emerged severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) demonstrate the ability for zoonotic emergence. Owing to the ability to spread via the respiratory route, newly emerging pathogens such as these have the potential for pandemic spread. By increasing our knowledge of these pathogens, we will be able to develop approaches to protect against the common cold and more severe, potentially pandemic, respiratory diseases.

Respiratory Syncytial Virus

The Virus

Human respiratory syncytial virus (HRSV) was first isolated from chimpanzees in 1956, and subsequently from infants suffering severe lower respiratory tract disease the following year. The virus is now recognized as a leading cause of childhood respiratory tract infection across the globe. By age 3, nearly all children will have been infected by the virus. HRSV is a member of the Orthopneumovirus genus within the family Pneumoviridae of the Mononegavirales order. Viruses within the Mononegavirales order have a nonsegmented, negative sense, single stranded RNA genome (Table 1). The HRSV genome is approximately 15.2 kilobases in size, comprising 10 genes, which encode 11 proteins (for a schematic view of the genome organization, see Fig. 1). HRSV virions are pleomorphic particles, with either spherical or filamentous forms, around 120–200 nm in size. Virions are encased in a host-derived lipid bilayer, from which three viral proteins protrude; F, G and SH. Within the virion, the genome is bound to the viral nucleoprotein (N) in a ribonuclear protein (RNP) complex. Similar to other negative sense RNA viruses, HRSV particles carry an RNA-dependent RNA-polymerase (L). The virion also contains the phosphoprotein (P) and the matrix protein (M2-1).

HRSV has two glycoproteins involved in cellular entry, F and G. G is responsible for receptor binding, but infection is possible in the absence of this protein, albeit at lower efficiency. F is a type I fusion protein and is essential for infection. The precise cellular receptor for HRSV has been debated, with heparan sulfate been suggested for many years. More recent work has suggested that this receptor was an artifact of working with immortalized cell lines, and that the receptor on more physiologically relevant human airway epithelial cells is the chemokine receptor CX3CR1, which is found exclusively on the apical surface of
these cells. The precise trigger for F-mediated membrane fusion is unknown, but is pH-independent because when F is expressed at the plasma membrane of cells it can cause syncytia formation at neutral pH. This syncytia formation is characteristic of HRSV infection and is from where the virus' name is derived. HRSV is generally thought to fuse with the plasma membrane, although there have also been suggestions that the virus is internalized through macropinocytosis prior to fusion with an endosomal membrane. Once membrane fusion is completed the viral genome is deposited to the cytosol. The negative sense RNA genome is transcribed by the actions of the L, P, N and M2-1, giving a set of mRNAs to direct the synthesis of viral proteins. The viral mRNAs are produced in a gradient with decreasing amounts of transcript to the 3' end of the genome. The genes transcribed from the 5' end at the highest abundance are the nonstructural proteins NS1 and NS2 that function to suppress the innate immune response of the infected cell. Over time, the polymerase complex switches from producing mRNA transcripts to producing full-length copies of the viral genome, the antigenome. This switch is in part regulated by the M2-2 protein. The antigenome is used as a template to direct replication of the negative sense genome that associates with N to form the RNP complex. Progeny virions

### Table 1

A comparative table of the major features of the viruses discussed in this article

| Virus | Family | Genome type | Genome size | Virion structure | Major clinical disease |
|-------|--------|-------------|-------------|------------------|------------------------|
| RSV   | Pneumoviridae | –ssRNA | 15.2 kb | 120−200 nm, enveloped | Bronchiolitis, wheezing |
| HMPV  | Paramyxoviridae | –ssRNA | 13 kb | 150−200 nm, enveloped | URT symptoms |
| HPN   | Paramyxoviridae | –ssRNA | 15 kb | 150−200 nm, enveloped | Croup, bronchiolitis |
| HRV   | Picornaviridae | +ssRNA | 6−7 kb | 30 nm, nonenveloped | URT symptoms |
| LP-HCoV | Coronavirusid | +ssRNA | 27−32 kb | 120 nm, enveloped | URT symptoms |
| SARS-CoV & MERS-CoV | Coronavirusid | +ssRNA | 27−32 kb | 120 nm, enveloped | LRT, ARDS |
| Adv   | Adenoviridae | dsDNA | 35 kb | 90 nm, nonenveloped | URT, conjunctivitis, gastroenteritis, myocarditis |

RSV, respiratory syncytial virus; HMPV, human metapneumovirus; HPN, human parainfluenza virus; LP-HCoV, low pathogenicity coronaviruses (HCoV-299E, -OC43, -NL63, -HKU1); SARS-CoV, severe acute respiratory syndrome coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; Adv, adenovirus; URT, upper respiratory tract; LRT, lower respiratory tract; ARDS, acute respiratory distress syndrome.

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**Fig. 1** Schematic representation of genomes of respiratory viruses. The genomes of the discussed respiratory viruses are displayed with approximate size of the viral gene features. The genome features of each virus are color coded with red for viral polymerase, blue for structural/envelope associated proteins, and yellow for replication factors and viral genome associated proteins. For coronavirus genomes, the 5' two-thirds are similar however the 3' one-third is unique to each family, as noted. The green gene products denote virus specific genes unique to each coronavirus species with HCoV-229E as an example of the seasonal coronavirus family. **I**TR, inverted terminal repeats; **IRES**, internal ribosome entry site; **RDRP**, RNA-dependent RNA polymerase.
assemble at the plasma membrane through an interaction of M with the RNP complex and the viral glycoproteins, prior to budding and release. In polarized epithelial cells virion release is directed toward the apical plasma membrane (for a schematic view of the life cycle, see Fig. 2).

Along with directing viral entry into cells, F and G are the main proteins presented to the immune system. HRSV is divided into two serotypes, group A and B, largely based on differences in the G protein, and the differing antibody responses this produces. Within these serotypes there are various genotypes. Group A has 11 genotypes, GA1–7, NA1, NA2, SAA1 and ON1, while group B has 23 genotypes, GB1–4, SAB1–4, URU1, URU2, BA1–12 and THB. Both serotypes and multiple genotypes have been found to co-circulate, although often one serotype will be dominant in a given season. It is thought this genetic and serological diversity is a major reason for the abundance of re-infection throughout life and lack of prolonged immunity.

Fig. 2  A schematic view of the life cycle of the four major viral families that cause respiratory diseases. (A) Paramyxoviridae and Pneumoviridae have very similar replicative life cycles and illustrated is a view of the HRSV replication life cycle. (1) HRSV attaches to cell surface receptors and has pH-independent fusion. It is generally thought that HRSV fusion occurs at the cell surface, but may also be within endosomes. Some members of the Paramyxoviridae have receptor-mediated endocytic uptake and fuse at internal membrane sites (as is depicted in the Coronaviridae life cycle). (2) Following release of the negative sense RNA genome to the cytosol, positive sense RNA is produced for protein production and genome replication. (3) Viral proteins are produced, with soluble proteins in the cytosol and the membrane interacting glycoproteins being synthesized at the endoplasmic reticulum (ER). (4) Glycoproteins are trafficked from the ER to the plasma membrane. (5) Viral structural proteins and genomic material associate with glycoproteins at the plasma membrane. (6) Nascent virions bud from the plasma membrane. (B) Picornaviridae, such as HRV, bind to cell surface receptors and trigger endocytic uptake. (2) Virions are delivered to endosomes and release their genomes into the cytosol. (3) The positive sense RNA is used to produce viral proteins. Not depicted, the positive sense RNA is used as a template to produce an antigenome which is used for replication of genomic RNA. (3) New viral particles are formed on intracellular double membrane compartments. (4) Viral particles are released from the cell through lysis. (C) Coronaviridae are internalized to cells through receptor-mediated endocytosis or have direct fusion at the plasma membrane following proteolytic cleavage of the spike glycoprotein. (2) Cathepsins in acidified endosomal compartments cleave spike to promote membrane fusion to release genomic material to the cytosol. (3) New viral proteins are produced, and the viral genome is replicated similarly to other positive sense RNA viruses. (4) Soluble viral structural proteins and the glycoproteins assemble new virions on membranes in the ER-Golgi intermediate compartment (ERGIC). The new virions that are produced bud into these vesicles. (5) Virus containing vesicles leave the cell through the exocytic pathway to release virus to the extracellular space (6). (D) Adenoviridae are taken into cells through endocytic mechanisms. (2) Virus particles are released from the endosome and a partially uncoated virus associates with the microtubule network through dynein motors to traffic to the nucleus. (3) Final viral uncoating occurs at nuclear pore complexes. (4) Double stranded DNA is released into the nucleus and treated similarly to cellular DNA to produce new viral proteins. (5) Viral particles assemble in the nucleus and are released by cell lysis (6).
Epidemiology

HRSV is the leading cause of lower respiratory tract infection (LRTI) in children across the globe. Moreover, pneumonia is the leading cause of mortality in children with HRSV being the most common single cause. Estimates suggest that each year there are over 30 million HRSV infections and over 60,000 childhood deaths worldwide. Approximately 99% of HRSV-associated deaths are in developing countries. HRSV has annual seasonal outbreaks in winter/early spring in temperate regions and during the rainy seasons of tropical regions. The peak of spread in temperate climates is during mid-December until early February. Along with changing climactic conditions and the influence this has on behavior (e.g., increased indoor crowding), protective antibodies may influence the seasonality of HRSV infection. It has been suggested there is a 6 month waning period of protective antibodies, leading to susceptibility come the following season.

It is estimated that around 30% of infants infected with HRSV require medical attention, with 2%–3% of these cases needing hospitalization. Bronchiolitis is the most common cause of hospitalization. Bronchiolitis occurs in roughly 10% of newborns within the first year of life, with between 60% and 90% of cases being due to HRSV infection.

HRSV is predominantly transmitted through close contact, either in the form of large aerosol particles, or through contaminated surfaces and fomites. The virus is stable on hands for half an hour or more, and up to 5 h on surfaces. Following initial contamination, there is usually a 4 to 6 day incubation period, although this can be between 2 and 8 days.

Pathogenesis and Clinical Features

The clinical manifestation of HRSV infection ranges from mild upper respiratory tract infection (URTI), often with otitis media, to more severe LRTI. Approximately a third of patients infected with HRSV will progress to LRTI. The most common presentation of LRTI in infants is bronchiolitis, but pneumonia and croup are also seen. The virus replicates in epithelial cells throughout the respiratory tract causing necrosis of ciliated cells, syncytia formation, peribronchiolar inflammation and impairment of secretion, all of which play a role in airway obstruction and lung hyperinflation, typical of bronchiolitis. HRSV infection is particularly severe in infants because their under-developed lungs are unable to compensate for the damage induced by viral replication and the inflammatory response. Re-infection usually displays reduced severity of disease. In healthy adults, infection usually causes mild, nonspecific features of viral URTI, such as coryza, sore throat, fever and malaise. In the elderly, immunocompromised or those with underlying health conditions, the symptoms are often more severe.

Many factors associated with risk of infant hospitalization have been found such as preterm birth, chronic lung disease, congenital heart disease, low birth weight, acquired or congenital immunodeficiency along with others. However, around half of infants hospitalized by HRSV infection are otherwise healthy. To date, very few protective factors have been found, although breast feeding, and the level of maternally derived antibodies correlate with a decreased likelihood of hospitalization. There is debate over whether there are any viral determinants of disease severity. Various studies suggest that serotype A viruses are more virulent, while others suggest serotype B causes more severe disease. Other studies show no particular impact of viral serotype on disease severity. However, it is established that a higher viral load is associated with increased risk of severe disease; a higher viral load on day 3 of hospitalization was found to be associated with an increased risk of admittance to the intensive care unit (ICU) and respiratory failure.

The immune response has been found to play a role in disease severity. A skew toward a Th2 cytokine response leads to more severe disease. This appears to have been responsible for the failure of a HRSV vaccine trialed in the 1960s. The formalin-inactivated vaccine designed to provide protection actually exacerbated the symptoms of circulating HRSV infection with 80% of recipients to be hospitalized, resulting in two deaths. One suggestion for the enhanced severity was that the vaccine promoted a Th2 response upon subsequent natural infection, whereas a primary infection by circulating HRSV and subsequent re-infection would be skewed toward producing a Th1 response.

Finally, there have been suggestions that HRSV hospitalization is associated with long-term sequelae. Infants hospitalized by HRSV infection have been suggested to have an increased likelihood of developing persistent wheezing, exacerbations of asthma and in some cases develop inflammatory airway disease.

Diagnosis

Nasopharyngeal aspirates, nasal washings, swabs and lower respiratory tract samples can all be used for viral detection. From these samples, virus can be recovered from various immortalized cell lines. Most commonly used are HeLa, although other epithelial derived cells such as A549 and A549 can also recover virus. All culture methods have a 3 to 7 day turnaround time so are not suitable for diagnostics.

Rapid antigen detection tests (RADT) have been developed such as enzyme-linked immunosorbent assays (ELISA) and chromatographic immunosayssays (CIA). These are both easy to use and have a rapid (under 10 min) turnaround time making them applicable at the point of care. Immunofluorescence assays can also be used from sloughed respiratory cells collected from a patient. This approach is slower than the RADTs but has a higher degree of sensitivity.

Overall, the most common, and sensitive, diagnostic tool is reverse transcription polymerase chain reaction (RT-PCR). Using real time methods, RT-PCR can be used to generate quantitative results to assess viral load as well as detect the virus. As discussed
previously, high viral load is associated with increased disease severity, so assessing viral load may be beneficial to judge likelihood of severe disease developing.

**Management and Control**

Currently, the majority of HRSV infection treatment is supportive. Most national recommendations emphasize the need for appropriate delivery of supplemental oxygen and hydration, in cases requiring treatment. The treatment of bronchiolitis is more extensive, but again supportive. Bronchodilators can be used such as alpha- and beta-adrenergics, anticholinergics and nebulized epinephrine, however, there is no conclusive evidence that these have a positive impact on disease outcome. Aspiration of respiratory secretions, or mechanical ventilation can be used to prevent hypoxemia, and in the most severe cases, extracorporeal membrane oxygenation can be used.

Ribavirin is currently approved for treatment of HRSV infection, but there is limited evidence for efficacy with meta-analyses suggesting that the investigations performed have lacked appropriate sample sizes to draw firm conclusions. The lack of conclusive evidence regarding efficacy, coupled with the side effects associated with ribavirin treatment means the drug is only administered to infants at the highest risk of severe disease. Ribavirin use in adults is off-license and limited to severely immunocompromised patients.

Passive immunization with humanized monoclonal antibodies is currently the most effective means of control for HRSV infection but its use is limited due to cost. Palivizumab is a monoclonal antibody (mAb) that targets the F protein of HRSV to block infection. The mAb is administered prophylactically by monthly intramuscular injection through the HRSV season in infants at highest risk of severe disease (5 monthly injections). A second generation mAb, Motavizumab, was developed but found to be no more efficacious than Palivizumab. Other companies have worked on the development of mAb therapy; Suptavumab went to a phase III clinical trial but was discontinued when primary end point measures were not met, and MEDI8897 is currently undergoing clinical development. Additionally, the approach of using nanobodies to target the F protein is under clinical development.

Vaccination would be the gold-standard for prophylactic protection, and has been long sought after, although hampered by the initial attempts. As discussed previously, in the 1960s a formalin-inactivated vaccine against HRSV was trialed in infants, but discontinued as it was found to enhance disease severity. This outcome has limited the development of an appropriate HRSV vaccine ever since. Other factors compound the issues of vaccine development; the vaccine would need to be administered at a very young age when the immune system is poorly developed, maternal antibodies may interfere, natural infection does not provide durable immunity, and it is unethical to test the vaccine efficacy in the appropriate target population of newborns/infants. However, much work is going into the development of an HRSV vaccine with four categories of vaccine being investigated; live-attenuated vaccines, subunit vaccines, vector-based vaccines and particulate vaccines.

In addition to work toward developing a vaccine to target HRSV, much effort is being placed in the development of antiviral compounds. Many different small molecules targeting various aspects of the viral life cycle are under development. Many fusion inhibitors are being trialed, along with compounds that target the G attachment protein, the L polymerase function (nucleoside and nonnucleoside inhibitors), the viral mRNA guanylylation activity of the L protein and inhibitors of the N protein.

In summary, currently, the majority of treatment for severe HRSV infection is supportive toward the respiratory symptoms caused by viral infection. Monoclonal antibody therapy appears to be effective, but its use is limited by cost and the need for monthly injections, making it unviable in the developing countries where 99% of HRSV-associated deaths occur. Various antiviral compounds and vaccine strategies are being trialed, but at the time of this writing, none have been approved for use in humans.

**Human Metapneumovirus**

**The Virus**

Human metapneumovirus (HMPV) is a negative sense, single stranded RNA virus of the Pneumoviridae subfamily within the Paramyxoviridae. It was first isolated in 2001 in the Netherlands through analysis of samples from children with respiratory tract infection of unknown etiology. HMPV is now recognized as the second most common cause of LRTIs in children after HRSV, which is in the same viral order (Mononegavirales). Retrospective studies have found HMPV to be present in the human population for at least 50 years. The virus can be divided into two serotypes, A and B, based on alleles of the surface proteins.

The approximately 200 nm diameter viral particles of HMPV are enveloped and infect epithelial cells in the nose and lungs (Table 1). Overall, replication of HMPV is similar to that previously described for HRSV. Infection proceeds through the attachment of glycoprotein (G) to heparan sulfate and other glycosylated proteins. The fusion (F) protein then interacts either with integrins or a currently unknown receptor to induce endocytosis. The F protein is activated by low pH in endosomal compartments to promote fusion between viral and cellular membranes for release of genetic material to the cytosol. The negative sense RNA genome is then used to direct production of new viral particles, which bud from the plasma membrane, as described previously for HRSV.
Epidemiology

HMPV is associated with approximately 20% of respiratory tract infections in children worldwide. Serologic surveys show that by 5–10 years of age, between 90% and 100% of children have been infected by HMPV. The median age of initial HMPV infection is close to 12 months of age, older than HRSV.

HMPV infections mostly occur in late winter and early spring, generally following the influenza and HRSV season. Infection causes mild URTI in healthy children and adults, however, in the immunocompromised and elderly, severe symptoms are commonly observed. Despite the seroprevalence of HMPV in young children, re-infection is common due to either insufficient antibody generation or infection with a different serotype.

Pathogenesis and Clinical Features

The clinical features of HMPV infection are similar to those presented during an HRSV infection, causing mild upper, or severe lower respiratory tract infections. Studies in humans have found diffuse alveolar damage, soughing of epithelial cells, eosinophilic cytoplasmic inclusions, syncytia and hyaline membrane formation. Common symptoms are fever, cough, tachypnea, dyspnea, hypoxia, rhinitis and sore throat. It has been reported that approximately 50% of infants present with otitis media. Hospitalization from HMPV infection is most common in infants aged 6–12 months, with bronchiolitis and pneumonia being the most frequent causes. Higher viral load is significantly associated with disease severity. Infection inhibits the immune response which can delay clearance of the virus from the respiratory tract and is probably the reason for low level neutralizing antibody development.

Diagnosis

Detection of HMPV is by RT-PCR from nasal or throat swabs. Serum from patients can also be tested. Virus from nasal secretions can be grown in cell culture and immunofluorescence used for detection.

Management and Control

Virus transmission is through respiratory secretions and contaminated surfaces. As such, spread can be limited by general hygiene countermeasures. No treatments are licensed although ribavirin has been used in severe cases. No clinical trials have been performed to examine the efficacy of ribavirin in humans, but it has been shown to be effective in vitro and in mouse models of infection. As previously discussed, monoclonal antibody therapy is used for HRSV infection and therefore may have potential for protection against HMPV infection, although this is at an early stage of development. Monoclonal antibodies have been shown to be protective in mouse, hamster and cotton rat models of infection, but have yet to be thoroughly examined in humans. Fusion inhibitors and RNAi approaches are also being explored. There is no HMPV vaccine, although similarly to HRSV, multiple approaches are being explored. Currently, palliative care is recommended to allow for clearance of the virus and recovery from the respiratory inflammation resulting from infection. Oxygen therapy, corticosteroids and mechanical ventilation can all be used for symptomatic treatment.

Human Parainfluenza Virus

The Virus

Human parainfluenza viruses (HPIV) are nonsegmented, negative sense, single stranded RNA viruses of the Paramyxovirus genus (like HMPV). HPIV was discovered in the 1950s as the cause of LRTI in children that were not the result of the already known influenza virus. Based on genetic and antigenic diversity, HPIV is divided into four types, HPIV-1, 2, 3 and 4, with HPIV-4 having subtypes a and b. These types are further subdivided into the respiroviruses, HPIV-1 and HPIV-3, and the rubulaviruses, HPIV-2 and HPIV-4.

The genome of HPIV is a single, negative sense strand of RNA of approximately 15 kilobases in a pleomorphic enveloped virion that ranges from 150 to 200 nm in diameter (Table 1). The virion envelope contains a glycoprotein that has both hemagglutinin and neuraminidase enzymatic activities (HN protein). HN is involved in entry of the virion via attachment of the hemagglutinin to sialic acids on the surface of permissive cells. Entry for HPIV occurs at the plasma membrane when the fusion protein (F) is cleaved by surface proteases causing a conformational change that promotes fusion of the viral envelope and plasma membrane. The viral genome, wrapped in a nucleocapsid, is released into the cytoplasm following the membrane fusion reaction and replication proceeds as described for HRSV.

Epidemiology

HPIV infections occur worldwide in young children with risk factors being malnutrition, vitamin deficiency, and respiratory irritants. Of the 5 million respiratory tract infections in the United States each year, HPIV is responsible for over one-third of
cases. Serologic surveys have found that by the age of 6–10 years, most children are seropositive for HPIV, demonstrating the widespread pathogenicity of this virus family. HPIV-1 to 3 are more prevalent than HPIV-4. Little is known about HPIV-4 since it is not often isolated from patients. HPIV-1 is strongly linked to croup with approximately 50% of cases being caused by the virus. Other severe symptoms include bronchiolitis, pneumonia and wheezing. HPIV-3 is second only to HRSV as a cause of pneumonia and bronchiolitis in young children.

HPIV-1 and HPIV-2 outbreaks occur in opposite years with each peaking in a year that the other wanes. The reason for this is unknown. Biennial fall outbreaks are common for both viruses with infections in children under age 5 years. Limited symptomatic infection is found in adults. HPIV-3 infections generally occur during the spring and summer months but can be found at low levels through the year. Of the HPIV viruses, HPIV-3 is most often associated with morbidity and mortality. HPIV-4 is detected at low levels throughout the year. The viruses are spread by fomites and large droplets with minimal persistence on solid surfaces.

Pathogenesis and Clinical Features

HPIV infection occurs primarily in children and is associated with upper and lower respiratory tract infections. Symptoms range from mild common cold with fever, to more severe disease such as croup, bronchiolitis and pneumonia. Classic “flu-like” symptoms are rarely seen in young children but are more common in adult infection. Croup presents with a hoarse barking cough and inspiratory stridor. This is the key clinical diagnostic phenotype for HPIV infection. Croup is the most common cause of infant hospitalization, with HPIV-1 and HPIV-2 most frequently the causative agents, although HPIV-3 has also been found. Bronchiolitis is also common in HPIV infection, especially during the first year of life. Immunocompromised children are at the greatest risk of severe HPIV infection, and in this population, it can be fatal. Minor risks of neurologic disease, apnea and bradycardia have been reported for HPIV infection. HPIV re-infection can occur throughout life with the elderly and immunocompromised at the greatest risk.

Diagnosis

Virus can be found in respiratory secretions, but the major diagnosis is from the bark-like cough and the stridor observed by chest x-ray where a “steeple sign” can be seen due to narrowing of the subglottic airway. RT-PCR is a common molecular diagnostic after infection of cell monolayers to amplify virus.

Management and Control

No treatments are licensed although acetaminophen or ibuprofen are often used for treating fever. For croup, supportive care is available including taking the child outside into cool air, having them breathe steamy air from a shower and calming them down to slow their breathing. Additionally, corticosteroids and nebulizers can be used to treat respiratory symptoms.

Rhinovirus

The Virus

Human rhinoviruses (HRV) are in the Enterovirus genus of the Picornaviridae family and cause common cold symptoms. They are the most commonly isolated respiratory virus from people with mild URTI, responsible for between a half and two-thirds of all colds. HRV is considered one of the most common human infectious agents worldwide.

Rhinoviruses have very broad genetic diversity, with over 150 different strains identified by either serology or sequencing. The phylogeny can be divided into HRV-A and HRV-B, which are the most common strains found in humans, and HRV-C, which is less common but is linked to more severe disease. HRV causes a lytic infection of the epithelial cells lining the airways with increased infection in the upper versus lower airways. This segregation is thought to be due the virus having better replication kinetics at 34°C compared to 37°C, with the nasal epithelium being at a lower temperature compared to deeper parts of the airways.

HRV's are single stranded positive sense RNA viruses with a genome size between 7 and 8 kilobases (for a schematic view of the genome organization, see Fig. 1). HRV virions are small, approximately 30 nm diameter, nonenveloped viruses (Table 1). Viral particles have an icosahedral capsid structure consisting of 60 copies each of VP-1, 2, 3 and 4. The viral particles are stable for days on surfaces and resistant to many common disinfectants.

Receptor usage by HRV depends on the subtype; minor group HRV-A bind to low-density lipoprotein receptor (LDLR) family members, the major group HRV-A and HRV-B bind to intracellular adhesion molecule 1 (ICAM-1), and HRV-C binds cadherin-related family member 3 (CDHR3). HRV is taken up from the cell surface by receptor-mediated endocytosis and a conformational change occurs in the capsid upon exposure to low pH to promote pore formation and release of genomic material into the cytosol. The RNA genome has a 5' internal ribosomal entry site (IRES) that initiates translation of the viral RNA. The genome is translated as a single polypeptide that is subsequently cleaved by host and virally encoded proteases to yield 11 viral proteins required for
replication. Newly transcribed viral RNAs can either be used for protein production or packaged into new virions for release (for a schematic view of the life cycle, see Fig. 2).

**Epidemiology**

HRV causes common colds around the world and humans are the only host of the virus. Adults average between 2 and 3 colds per year, with more than half of these due to HRV infection. Children typically suffer a higher frequency of colds, with estimates being up to 12 infections per year. HRV transmission is seen year-round, but cases of HRV-A and -B are seen to have two peak periods in April/May and September/October. There is an association of incidence with children returning to school. HRV-C cases appear to peak in winter months.

Virus is transmitted by droplets or fomites with close contact generally needed for cross infection between individuals. Viral particles can survive for several hours on skin which can facilitate human-to-human transmission. The virus is highly infectious, with only a small infectious dose needed to initiate disease in a human. The incubation period for infection is less than 2 days and usually requires 7–10 days for clearance.

**Pathogenesis and Clinical Features**

HRV infection is via the aerosol route with infection primarily occurring in the nasal or oral mucosa and eye conjunctiva. The primary site of viral replication is in airway epithelial cells. HRV infection can disrupt epithelial barrier function which can, in turn, allow translocation of pathogens across this barrier to cause disease complications. The host response to HRV is swift with an increase in systemic chemokines and cytokines that limit virus spread but causes clinical symptoms. Infection is usually mild and self-limiting, although more severe disease such as pneumonia has been seen in the elderly and immunocompromised.

HRV symptoms start with sore throat and runny nose and can develop to cough, sneezing and congestion. Other symptoms can include fatigue, body aches and loss of appetite. Patients can have several colds a year due to the wide spectrum of serotypes in the environment and the limited immune response to each strain that is produced during infection. HRV is also associated with asthma exacerbations in addition to causing common colds.

**Diagnosis**

HRV diagnosis is by clinical symptoms and by RT-PCR of nasal secretions. Some clinical microbiology laboratories will grow samples from nasal swabs in cell culture for analysis. The vast genetic differences between the large number of isolates makes ELISA analysis difficult due to the variability in surface antigen alleles present in the environment. Usually RT-PCR is targeted to conserved regions of the genome for diagnosis in hospitals.

**Management and Control**

There are no licensed therapeutics and only supportive care is given for HRV infection. Many antiviral drugs have been proposed, however, all lack potency in humans. For instance, zinc has been tried as an over the counter therapeutic but with limited success. Vaccine development is problematic due to the high variability of surface proteins, although current efforts are directed to regions of conservation between many of the serotypes. Future work on prophylaxis is ongoing.

**Coronaviruses**

**The Viruses**

Coronaviruses are part of the *Coronaviridae* family in the *Nidovirales* order that contain the largest viral RNA genomes currently known. The first two human coronaviruses (HCoV) were identified in the 1960s and are known as HCoV-229E and -OC43. These two viruses, and coronaviruses in general, were given relatively little attention until the severe acute respiratory syndrome (SARS)-CoV outbreak in 2003. Subsequently, three further HCoVs were identified. HCoV-NL63 was found in a child suffering bronchiolitis in the Netherlands, while HCoV-HKU1 was discovered in adult cases of pneumonia in Hong Kong in 2005. Finally, an outbreak of Middle East respiratory syndrome (MERS)-CoV was first identified in 2012. These six HCoVs can be broadly divided into two groups; HCoV-229E, -OC43, -NL63 and -HKU1 can be described as low pathogenicity (LP) HCoVs that typically cause mild respiratory infection. While SARS- and MERS-CoV can be considered highly pathogenic HCoVs. The unifying virology of these pathogens will be considered first, then they will be divided when discussing their epidemiology, pathogenesis, clinical features, diagnosis, management and control.

The *Coronaviridae* family can be broken into two subfamilies, the Coronavirinae and Torovirinae. The Coronavirinae has four groups, alpha, beta, gamma, and delta. HCoV-229E and -NL63 are alpha-coronaviruses, while the other four HCoVs are beta-coronaviruses (Table 1). The family of viruses have positive sense RNA genomes ranging from 27 to 32 kilobases (kb) (for a
These polyproteins contain nonstructural proteins (nsp), with pp1a comprising nsp1 direct translation to produce viral proteins. ORF1a and ORF1b are translated to express the polyproteins pp1a and pp1ab.

occurs at the plasma membrane or within endosomal compartments is unclear. Debated. HCoVs have been found to fuse both at the plasma membrane following cleavage by TMPRSS2, or within acidified vesicles. The precise and most physiologically relevant site of membrane fusion is debated. HCoVs have been found to fuse both at the plasma membrane following cleavage by TMPRSS2, or within acidified endosomes following cleavage by cathepsins. Data appear to suggest that both routes of entry can be used, but that at least for clinical isolates of the LP-HCoVs there may be more efficient infection through cleavage by TMPRSS2, although whether fusion occurs at the plasma membrane or within endosomal compartments is unclear.

Once viral and cellular membranes fuse, the viral genome can enter the cytosol. Since the genome is positive sense, there is direct translation to produce viral proteins. ORF1a and ORF1b are translated to express the polyproteins pp1a and pp1ab. These polyproteins contain nonstructural proteins (nsp), with pp1a comprising nsp1-11 and pp1ab additionally having nsp12-16. Within these polyproteins there are two proteases, papain-like protease (PLpro) and main protease (Mpro) that release the nsps by proteolytic cleavage. Liberated nsps then regulate production of subgenomic RNAs that encode the structural and accessory genes, and controls replication of the viral genome, both of which occur through negative sense RNA intermediates.

After translation from subgenomic RNA, the structural proteins E, M and S insert to the endoplasmic reticulum membrane. Subsequently, these proteins are trafficked to the endosomal reticulum-Golgi intermediate compartment (ERGIC) where virion assembly occurs. M is the main driver of virion assembly as it is responsible for interaction with the E protein to form VLPs and interacts with N for encapsulation of the viral genome. Following the interaction of the four structural proteins and the viral genome, there is inward budding of cellular membrane to the ERGIC to produce a virion. These virions then exit the cell through the Golgi exocytic pathway and fusion of a cellular vesicle at the plasma membrane. In polarized cells, such as those in the airways, there is preferential but not exclusive, release of progeny viral particles at the apical plasma membrane (for a schematic view of the life cycle, see Fig. 2).

Low Pathogenicity Coronaviruses

Epidemiology

The four LP-HCoVs are considered to be the second most frequent causes of the common cold behind rhinoviruses. These viruses display seasonality with spread occurring in winter and spring months in temperate regions, although summer activity has also been observed. During the season, LP-HCoVs can be responsible for anywhere between 1% and 35% of acute respiratory infections. Seroprevalence levels are seen to raise through age 5 years. By adulthood, there is about 40% seroprevalence for LP-HCoVs, in the United States. Epidemiological studies of these four viruses have often found cases of co-infection with other respiratory viruses, yet the relevance of this to clinical outcome has yet to be thoroughly examined.

Pathogenesis and Clinical Features

Studying the pathogenesis of LP-HCoVs has been hindered by a lack of small animal models that support replication and/or reproduce disease. The pathogenesis of HCoV-229E has been examined in volunteers that were experimentally infected and subject to ultrastructural studies. This work demonstrated that viral infection causes epithelial cell damage, ciliary loss and cytolysis beginning at 3 days postinfection. Virus is seen to be shed for approximately 5 days, starting 48 h after initial infection, coincidently with symptom onset. Symptoms associated with LP-HCoVs are similar to those of other respiratory pathogens; cough, sputum, rhinorrhea, tachypnea, sore throat and occasionally fever. Disease severity can be worse in the young, elderly and those with underlying health conditions, but usually infection is self-limiting and clears within a week. HCoV-NL63 has been associated with cases of croup; a study in Germany determined that up to 45% of children under 3 years of age infected with HCoV-NL63 developed croup.

Diagnosis

The most often used technique for diagnosis of LP-HCoVs is RT-PCR. It is also possible to isolate virus in cell culture, and serological assays can be used.
Management and Control
LP-HCoV infections are usually self-limiting and often not associated with severe disease. As a result, treatment of infection is usually supportive care and symptomatic relief. There are currently no direct acting antivirals against LP-HCoVs, nor any vaccines.

Highly Pathogenic Coronaviruses—SARS-CoV
Epidemiology
The SARS-CoV outbreak began in the Guangdong province of China in November 2002. However, the outbreak was not fully appreciated until early 2003 when a single infected patient traveled from China to stay at Hotel Metropole in Hong Kong. This patient, staying on the 9th floor, managed to infect over 10 people, who subsequently spread the virus throughout Asia and Canada. One of these patients traveled to Hanoi, Vietnam and was responsible for a hospital-based outbreak of 38 cases. It was from here that the highly contagious nature of this novel pathogen was reported to the WHO by Carlo Urbani, who himself became infected and died from the virus in March 2003. Within weeks of the transmission out of Hong Kong, SARS-CoV had spread to 27 countries and infected thousands of people, with about a third being healthcare workers. The virus responsible for SARS was identified in April 2003. By July 2003, the outbreak was halted through public health measures, although a handful of additional cases were detected in December 2003 to January 2004. In a little over a year, SARS-CoV infected 8098 people and was responsible for 774 deaths, with a predicted economic impact of over 30 billion USD in the Far East alone. No cases of SARS-CoV have been reported since.

SARS-CoV emerged through zoonotic transmission from the natural reservoir host, horseshoe bats. The outbreak was however facilitated by an intermediate, amplification host, of palm civets which were kept in live animal markets in China. These animals appear to be incidental hosts since serological analyses failed to detect antibodies in animals in the wild or in breeding facilities. The virus initially transmitted from animals into the human population and was found to then adapt to facilitate efficient human–human spread. Many surveillance studies have since been performed, and multiple SARS-like viruses have been found in bats, suggesting the potential for re-emergence of a similar pathogen.

Human–human transmission occurs through aerosol droplets or contaminated surfaces and fomites. It has been shown that the virus can persist for up to 2 days on environmental surfaces. Many cases were nosocomial as the majority of virus shedding only occurs after the onset of illness (around 5 days postinfection). Approximately 33%–42% of SARS cases were in healthcare works, while 22%–39% of cases were through transmission between family members. The R0 for the virus is between 2 and 4, but measures to limit spread were highly effective and brought this down to around 0.4 to quell the outbreak.

Pathogenesis and Clinical Features
The incubation period for SARS-CoV infection is typically between 4 and 6 days. Initial presentation is general flu-like symptoms such as fever, myalgia, and lethargy, with respiratory symptoms such as cough and sore throat developing between 2 and 7 days later. The infection then progresses with more severe respiratory symptoms such as shortness of breath, pneumonia and acute respiratory distress syndrome (ARDS). Infection causes diffuse alveolar damage, lung edema, hyaline membrane and syncytium formation. Approximately half of SARS cases resulted in hypoxemia around 9 days after onset of symptoms. Many of these patients required admittance to the ICU and mechanical ventilation. A higher viral load was found to be associated with poorer clinical outcome.

Patients display widespread immune cell infiltration to the lungs. Immunopathology plays a major role in the more severe forms of SARS-CoV infection; indeed, ARDS is heavily associated with upregulation of proinflammatory cytokines and chemokines. The immunopathology of the disease is best evidenced by the fact that the most severe symptoms occur as virus titer begins to decline.

In addition to respiratory disease, SARS-CoV infection was associated with gastrointestinal symptoms such as diarrhea and vomiting. The precise cause for this is unclear and there is no evidence of fecal–oral transmission of the virus. Virus was also detected in the urine of up to 30% of patients.

The median age of SARS patients was under 45, but symptoms were typically more severe in the elderly. The case fatality for SARS-CoV infection was much higher in older patients with 43% of patients over 60 dying from infection, compared to 13% of patients under 60. About 10%–30% of patients had comorbidities associated with infection and the mortality rate in this population was 46%.

Diagnosis
Samples for diagnosis can be recovered from respiratory secretions, feces and urine. There is a higher viral load in the lower respiratory tract, so samples from that area are more reliable, but more invasive and difficult to obtain. RT-PCR, along with serological assays were used to confirm SARS-CoV infection. Virus can be isolated with Vero E6 cells, and indeed this was the approached used to determine that SARS was caused by a novel coronavirus.
Management and Control

The SARS-CoV outbreak was halted by public health intervention, not through any direct acting antiviral or vaccine. Measures such as quarantine in negative pressure rooms, handwashing, disinfection of surfaces, appropriate personal protective equipment and avoidance of contact with bodily fluid all helped to block spread of infection.

Much of the care given to SARS patients was supportive to mitigate issues of hypoxemia and ARDS. In numerous cases ribavirin along with pegylated interferon was used, although this intervention was not thoroughly tested, and retrospective studies have shown that the efficacy is debatable. Corticosteroids and thymosins were also used, but again, no thorough clinical trials were performed, and the role any of these interventions played in disease outcome remains unclear. However, convalescent patient plasma use did seem to be associated with a reduced frequency of poor outcomes when given before day 14 of infection.

To date, there are no approved direct acting antivirals against SARS-CoV. However, many compounds are currently under investigation for treating MERS-CoV infection with many of these being shown to also be effective against SARS-CoV (discussed further in the following section). Protease inhibitors did appear to have some degree of efficacy during the SARS-CoV outbreak. A number of patients received lopinavir, ritonavir or a combination of both. These drugs are licensed for use as HIV-1 protease inhibitors and are part of highly active antiretroviral therapy. Again, the use of these compounds did not undergo rigorous clinical investigation, but when used in combination with ribavirin, with or without corticosteroids, a reduction in viral load was detected and symptoms were found to subside, with disease progression being milder.

Similar to antiviral drug therapy, there is no approved vaccine against SARS-CoV infection. Multiple approaches were undertaken, however, none were successfully trialed in humans. With the emergence of MERS-CoV, there has been a renewed push for countermeasures against HCoV infection with some approaches looking to produce broadly acting vaccines. However, to date these studies are at early stages.

Highly Pathogenic Coronaviruses—MERS-CoV

Epidemiology

In June 2012, around 10 years after the emergence of SARS-CoV, a novel coronavirus was identified in the Middle East when a 60-year-old man died of acute pneumonia and renal failure in Saudi Arabia. MERS-CoV was isolated from the sputum of this patient by scientists at Erasmus Medical Center in the Netherlands and was initially called HCoV-EMC, before being re-named MERS-CoV. Retrospective studies determined that the virus had been responsible for the hospitalization of 11 patients in Jordan in April 2012. In contrast to SARS-CoV, there has been no explosive spread of MERS-CoV. The largest outbreak occurred in spring 2014 which saw around 500 hospital acquired cases in Saudi Arabia. May 2015 also saw a large outbreak in South Korea initiated by a patient that had traveled to the Middle East. This outbreak saw 186 infections across 16 hospitals with 36 deaths. The epidemic was halted largely through quarantine measures, with around 17,000 people being isolated.

While MERS-CoV has reached 27 different countries, the vast majority of cases are confined to the Middle East. Again, in contrast to SARS-CoV, MERS-CoV continues to sporadically cause disease and in early 2018 there have been over 2100 cases, responsible for around 750 deaths. MERS-CoV therefore has a significantly higher case fatality ratio than SARS-CoV and has caused fewer cases over a longer period of time.

Similar to SARS-CoV, MERS-CoV has close ancestors in bats. MERS-CoV is also found extensively in dromedary camels in the Middle East, Northern and Eastern Africa. It is estimated that in certain areas, the seroprevalence of MERS-CoV antibodies in dromedary camels may be as high as 90%. The ubiquity of MERS-CoV in camels is most likely responsible for the continued, sporadic emergence of cases due to close contact between humans and camels, particularly in the Middle East. It is worth noting that MERS-CoV is found extensively in camels in regions of Africa, yet there have been very few reported cases of human infection on this continent. There may be various reasons for this such as reduced surveillance, differences in the interaction between humans and camels, or the spread of a virus with less virulence. In addition to camels, MERS-CoV has been found in sheep, goats, cows, water buffalo and alpaca, suggesting the virus could become more widespread.

MERS-CoV does not seem to have adapted to spread from human-to-human effectively, unlike SARS-CoV. The majority of MERS-CoV cases are through zoonotic events. Most human-to-human transmission of MERS-CoV has been nosocomial, with estimates suggesting between 62% and 79% of cases in a hospital setting, compared to 12%–21% between family members. This nosocomial transmission likely relates to the fact that there is only significant virus shedding following the onset of symptoms. Poor human-to-human transmission of the virus is reflected in the fact the R0 value is currently estimated to be around 0.7.

Of all MERS cases to date, around 66% have been in males. However, there does not seem to be any sex-based differences in the case fatality ratio. A likely explanation may be cultural and as a result of the relative levels of interaction with the animal reservoirs of the virus. The median age of MERS patients is about 50 years, older than seen for SARS cases. Age appears to be associated with an increased risk of infection. The clearest risk factors are underlying comorbidities. Diabetes is the leading comorbidity, seen in 68% of cases, but chronic renal disease (49%), obesity (34%), choriionic cardiac disease (28%) and other lung diseases are also highly associated. Approximately 75% of MERS patients have underlying conditions, and the case fatality ratio in this population is close to 60%.
Pathogenesis and Clinical Features

MERS-CoV infection causes a spectrum of disease, from mild respiratory symptoms, usually confined to the upper respiratory tract, to more severe disease impacting the lower airways. The virus infects cells throughout the lungs via the receptor DPP4, which is found on type I and II pneumocytes, nonciliated bronchial epithelial cells, endothelial cells and some hematopoietic cells. The receptor is found at lower abundance in the upper respiratory tract, leading to higher viral load in the lower airways. DPP4 is also found in the kidneys, intestine, liver, thymus and bone marrow.

The incubation period for the virus is 2–14 days with the median being 5–7 days. For patients that suffer severe disease, there is usually a 4-day period of symptoms before hospitalization. MERS initially starts with flu-like symptoms, but then rapidly progresses to pneumonia and ARDS in severe cases (a more rapid progression than seen in SARS patients). Similar to SARS-CoV infection, there can be gastrointestinal involvement with diarrhea, abdominal pain and vomiting often seen.

The full extent of the pathogenesis of MERS-CoV infection is still being established, and at the time of writing, only two postmortem examinations have been performed. The first of these was a full autopsy of a 45-year-old male who died in Abu Dhabi in April 2014. Prior to hospitalization, this patient had a 4-day history of fever, rhinorrhea and productive cough and was diagnosed with bronchitis. He developed a persistent cough and shortness of breath and was diagnosed with pneumonia before being admitted to the ICU. His condition worsened, he developed kidney injury and renal failure and died 3 days later. An autopsy was performed 10 days postmortem. The patient had clear signs of pleural effusion, pericardial effusion, abdominal effusion, edematous and consolidated lungs and generalized congestion. Additionally, there was diffuse alveolar damage, denuding of bronchiolar epithelium, hyaline membrane formation, alveolar fibrin deposits, type II pneumocyte hyperplasia, syncytia formation and immune cell infiltration to the lungs. Extensive damage to the kidney was also seen, but there was no evidence of virus dissemination to extra-pulmonary sites suggesting other mechanisms for this damage. The lung pathology seen in this patient was similar to that seen in SARS patients.

The second postmortem examination was of a 33-year-old male who died in Riyadh in September 2015. This patient was additionally suffering from T-cell lymphoma, which had previously been treated with chemotherapy and radiotherapy. He had been admitted to hospital in Riyadh in May 2015 for further chemotherapy and possible stem cell transplantation. The patient initially presented with cellulitis and sepsis in July. His condition worsened as he developed thrombocytopenia and neutropenia along with a fever and productive cough with chest infiltrates revealed by X-ray. At this stage the patient’s sputum suggested bacterial pneumonia and was negative for MERS-CoV. But 20 days later, the patient tested positive for MERS-CoV and with increasing hypoxemia was admitted to the ICU and given mechanical ventilation. The patient died 4 days later from refractory shock and hypoxemia. Tissue samples were collected within 45 min of death, but no full autopsy was performed. Samples demonstrated severe acute hemorrhagic pneumonia, diffuse alveolar damage and immune infiltrates, and many other similarities to the previously described autopsy. Electron microscopy examination revealed the presence of virus particles in pneumocytes, alveolar macrophages, renal tubular epithelial cells and macrophages infiltrating skeletal muscles. In contrast to the previous case, there was evidence of systemic spread of MERS-CoV. However, this patient may be an atypical example of infection owing to potential exacerbation of symptoms as a result of the immunosuppression from lymphoma and chemotherapy.

Currently, a full understanding of the pathogenesis of MERS is limited by the lack of human postmortem examination and the ongoing development of appropriate animal models.

Diagnosis

The WHO have developed guidelines for the diagnosis of MERS-CoV infection. These state that sampling should be from the lower respiratory tract during the acute phase of illness and use RT-PCR to detect the presence of two parts of the MERS-CoV genome; a region upstream of the E gene (UpE) and in the ORF1ab gene. Serological tests can also be used to test for MERS-CoV infection with ELISA, immunofluorescence, and microneutralization assays all employed.

Management and Control

Currently, the best forms of control for MERS-CoV infection are public health interventions. Quarantine methods, appropriate personal protective equipment, avoiding contact with bodily fluids, and general hygiene measures can all reduce spread of infection. Moreover, limiting exposure to the animal reservoir may aid in reducing infection.

There is currently no approved vaccine against MERS-CoV, although much research is ongoing. Inactivated, live-attenuated, vector, subunit, DNA and recombinant vaccine strategies are all being explored. Along with vaccine approaches in humans, camel vaccinations are being developed to look to reduce zoonotic transmission of the virus.

Along with vaccination approaches, immunotherapy measures are being explored. Passive immunotherapy with convalescent patient plasma has been tested, although there is currently a lack of surviving patients with high antibody titers to test this approach effectively. Neutralizing antibodies targeting the spike protein of MERS-CoV are also being examined but have yet to be fully trialed in humans. Along with antibodies targeting the spike protein of the virus, monoclonal antibodies targeting the DPP4 receptor are also being examined.

There are also no approved direct acting antivirals. However, many avenues are being explored to inhibit various aspects of MERS-CoV infection. Lopinavir and ritonavir have been used in MERS patients alongside interferon and ribavirin, however, as in...
SARS patients, the efficacy remains unclear. Other protease inhibitors that were initially examined as anti-SARS-CoV compounds are being explored but have yet to be fully evaluated in vivo. Repurposing of currently approved drugs is a common theme among the currently examined antiviral compounds. Antimalarials such as chloroquine, amodiaquine, or mefloquine have all been found to have antiviral activity against SARS- and MERS-CoV. A widely used antiarrhythmic agent, loperamide, was found to be inhibitory to coronavirus replication through in vitro screening. Cyclophilin inhibitors currently used as immunosuppressive agents can inhibit replication as can various kinase inhibitors in use for cancer treatment. However, most of these compounds have only been tested in vitro or have limited in vivo examination. Nucleoside analogs are being explored to inhibit the RNA-dependent RNA-polymerase such as GS-57344 and BCX4430.

Overall, the best approach for control of MERS-CoV is public health measures to limit infection. There are no approved therapies or vaccines, but extensive research is taking place to move toward such approaches. Much of this work is also looking for broad-activity of any compound to not only protect against MERS-CoV infection, but also any novel coronavirus that may emerge in the future.

### Adenoviruses

#### The Viruses

Adenoviruses are a large family of double strand DNA viruses. There are seven different species, A–G, with over 50 distinct serotypes. The viruses are in the Mastadenovirus genus, family Adenoviridae. The first identification of an adenovirus came in 1953 when a transmissible agent was found in adenoid tissue, giving rise to the name.

Adenovirus virions are nonenveloped, have icosahedral symmetry and are around 90 nm in size. The viral capsid is made up for 252 capsomere proteins that are divided into three types, hexons (240 copies), penton bases and penton fibers (12 base/fiber copies) (Table 1). Each vertex of the icosahedral virus has a penton base/fiber protruding from it. These three proteins are all exposed to the immune system and are the targets for neutralizing antibodies. The genome inside the virion is linear, nonsegmented double stranded DNA of between 26 and 46 kilobase pairs (for a schematic view of the genome organization, see Fig. 1).

Virion entry to cells and uncoating to release the genome is a complex, multistep process. Initially, the virus binds to a cellular receptor through the penton fiber. Two receptors are used depending on the viral species, with most human adenoviruses (HAdV) using the coxsackie B and adenovirus receptor (CAR). HAdV species B utilizes CD46 instead. Certain strains of HAdV have also been suggested to use desmoglein-2, sialic acids or GD1a glycan as receptor molecules.

Following initial interaction with surface receptors, the virus binds to a second receptor in the integrin family of proteins, in particular αvβ integrin, through the penton base. Binding to the two receptors begins the uncoating process. Interaction between penton fibers and CAR triggers drift of the virus along the plasma membrane in an actomyosin-2 dependent fashion. This drift exerts a pulling force on the virus which, if also attached to relatively immobile integrins will start to exert pressure on the virion. As such, penton fibers begin to shed from the virus at the cell surface.

Along with promoting the initial stages of virus disassembly, interaction with integrins triggers an endocytic response of the cell, causing uptake of virions either in a clathrin-dependent or macropinocytic manner. In the endosomal system, further rearrangement of the capsid occurs to allow release of protein VI. HAdV protein VI is responsible for rupturing endosomes to allow for release of the partially disassembled virion into the cytosol.

Once in the cytosol, HAdV associates with the microtubule network through the dynein motor protein and is trafficked to the nucleus. The remaining capsid proteins then interact with the nuclear pore complex and the joint interaction between microtubule motors, nuclear porins and viral capsid proteins leads to the final stages of uncoating to deposit the double stranded DNA genome into the nucleus.

The HAdV genome is treated similarly to cellular DNA and transcribed to give mRNAs that are translated to produce the viral proteins. Transcription/translation occurs in early and late phases. Early gene expression produces proteins that are nonstructural and promote viral replication. For instance, E1A and E1B which bind to Rb and p53, respectively, promote progression to the cell cycle to allow co-option of cellular DNA replication machinery for replication of the viral DNA. These early genes also play roles in expression of the late genes such as the virally encoded DNA polymerase. In the late phase, structural proteins are produced to assemble new virions. Assembly occurs in the nucleus where the viral DNA is encased in new capsid. Progeny virions are released through cell lysis (for a schematic view of the life cycle, see Fig. 2). However, it is also possible for HAdV to enter into a latent life cycle; this life cycle was how adenovirus was first discovered in adenoid tissue.

#### Epidemiology

HAdVs cause a spectrum of disease depending on the species but respiratory infections are among the most common, especially in children under 5 years. It is estimated that globally, about 5%–7% of pediatric respiratory tract infection are from HAdV. Most of these infections are mild and self-limiting causing general common cold symptoms. However, there can be more severe disease such as pneumonia, bronchitis and croup. Along with respiratory infection, HAdV infection can cause conjunctivitis, gastroenteritis, hepatitis and myocarditis.

Of the various species of adenovirus, B, C and E have been found to infect the respiratory tract. Infections by respiratory HAdVs occurs globally and do not appear to show seasonality. When outbreaks do occur, they are often in small populations such as in...
hospitals and particularly in military installations. Most infections occur in young children or immunocompromised adults, but there have been severe outbreaks in healthy adults. The reasons for the spectrum of disease severity are not fully clear, but it is hypothesized that it may be down to emergence of novel strains of HAdV. For instance, 2007 saw the emergence of a novel HAdV-14 that initially spread in US military personnel and then in civilian populations that caused pneumonia and ARDS. Antibody responses to HAdV infections have been shown to be effective and long lived, suggesting that the emergence of strains that can avoid preexisting immunity may indeed play a role in more severe infections.

Respiratory HAdVs are transmitted in aerosol form through coughs and sneezes and through contaminated surfaces. Adenoviruses have a high degree of environmental stability, they are unperturbed across a pH range of 5–9 and are resistant to isopropyl alcohol, ether, and chloroform. HAdV can survive on surfaces for weeks at room temperature and even longer at colder temperature.

Pathogenesis and Clinical Features

Respiratory tract infection by HAdV typically causes mild, self-limiting, common cold symptoms. The most common manifestations of HAdV infection are cough, chest pain, headache, malaise, pharyngeal symptoms and fever. The virus has a lytic life cycle, so causes damage to the respiratory tract through cell death and can cause infiltration of immune cells. More severe forms of disease can occur, particularly in the young or immunocompromised that are associated with pneumonia or bronchitis. Children under the age of 5 years can often develop fever, pharyngitis and otitis media, which can occur in up to 30% of cases.

Patients are seen to develop a neutralizing antibody and T-cell immune response to HAdV infection that protects in a type-specific manner. However, because of the various species of HAdV, re-infection is common. Owing to the immune response many re-infections can be asymptomatic, and indeed, up to 50% of HAdV infections are thought to be asymptomatic due to the combination of latency and the immune response.

Diagnosis

Virus can be found in respiratory, ocular and ear secretions, but there is a need for clinical correlation when virus is detected because asymptomatic shedding is common. Virus can be grown out of samples using various cell lines such as HEp-2, HeLa and A549 and can be easily detected through the cytopathic effect infection causes. PCR is the most sensitive method for detection, but again requires clinical correlation due to asymptomatic HAdV infection. Point of care immunochromatography tests are also available.

Management and Control

Owing to the usually mild and self-limiting nature of HAdV infection, there is little impetus for intervention. No treatments are currently licensed, although cidofovir has been suggested to have therapeutic potential in pediatric patients. A live-attenuated oral vaccine is licensed for use in US military recruits attending basic training, but this is not available to the public. General countermeasures of hygiene and surface disinfection with virucidal agents are routinely used to limit spread.

Human Bocavirus

The Virus

Human bocavirus (HBoV) was first identified in 2005 by screening of nasopharyngeal aspirates for novel viruses causing respiratory disease. A random PCR amplification strategy followed by sequencing and bioinformatics identified a previously unknown member of the Parvoviridae that became known as human bocavirus. The name derives from there being genetic similarities to two already defined paroviruses, bovine parvovirus 1 and canine minute virus. Since the initial identification, three further subtypes of HBoV have been found in stool samples (HBoV2, 3 and 4).

HBoV is a member of the Parvoviridae which are small single stranded DNA viruses. Paroviruses are among the smallest known viruses both in terms of genome, around 5–5.5 kilobases, and particle size, ranging from 18 to 26 nm (Table 1). Virions are nonenveloped and made up of 60 capsid proteins. The HBoV genome contains three ORFs (ORF1–3). There are four nonstructural proteins encoded from ORF1, NS1–4, and an additional nonstructural protein, NP1 encoded from ORF2. The final ORF encodes the three capsid proteins, VP1–3.

The precise infectious life cycle of HBoV has yet to be fully characterized. It has been suggested that sialic acids act as the receptor at the cell surface, however, the virus has proven hard to grow in many cell lines which could suggest other receptor requirements. Uptake from the cell surface is thought to be through a clathrin-mediated endocytosis. HBoV escape from the endosomal system is dependent on a phospholipase A2 domain in the VP1 protein, although the precise mechanism of escape is unclear. Replication occurs in the nucleus, and similar to other paroviruses, HBoV requires many cellular DNA replication components. The virus can be released from cells through a lytic process, but can also be persistent in an episomal form, the determinants of these life cycles is unclear.
Epidemiology

HBoV has been found to have a worldwide distribution, with infection occurring year-round, although there does appear to be more cases in winter and spring months. A large meta-analysis of epidemiological studies conducted between 2005 and 2016 suggested that HBoV is found in about 6% of respiratory infection cases. This study also showed that HBoV is often detected in conjunction with other respiratory viruses, with over 50% of cases being co-infection. This high degree of co-infection has caused some suggestion that HBoV may not cause disease itself and just be present in a certain percentage of the human population. However, HBoV has been detected in cases of acute respiratory infection where no other causative agents could be found.

The greatest disease burden for HBoV appears to be in children between 6 and 24 months of age. Studies suggest that HBoV can be found in up to 20% of children suffering acute respiratory infection. The virus has also been associated with community acquired pneumonia in young children.

There are some limitations to the current knowledge of HBoV epidemiology. Virus has been found to shed for up to 12 months after acute infection. Thus, detection may not necessarily indicate a causative link to disease. Moreover, it has been suggested that the routine use of DNA and PCR to detect HBoV may not be appropriate due to prolonged shedding and persistence. An alternative approach of using RT-PCR to look for a spliced form of the capsid mRNA that is only present during active replication. Using this approach, HBoV was still associated with community acquired pneumonia in young children, but at much lower levels than when using DNA; approximately 10% of cases were suggested to be HBoV positive by DNA, compared to 2% by mRNA.

Pathogenesis and Clinical Features

The pathogenesis of HBoV infection is not well understood owing to the lack of an animal model and difficulties of in vitro study, although new culture systems are being developed. The understanding of HBoV pathogenesis is also clouded by the fact that the virus has a high prevalence of detection in patients infected with other viruses known to cause respiratory infection.

Studies in human airway epithelial cell culture models have suggested that HBoV infection is highly disruptive. HBoV was found to perturb tight-junction barriers, cause a thinning of epithelium, a loss of cilia and epithelial cell hypertrophy. HBoV has also been found to cause cell death, with both apoptotic and pyroptotic mechanisms suggested. It could therefore be speculated that HBoV infection in humans may disrupt the respiratory tract epithelia and cause an inflammatory response which could contribute to pathogenesis.

Symptoms associated with HBoV range from mild common cold to more severe disease. The most commonly reported symptoms in clinical studies are cough, fever, rhinorrhea, asthma exacerbation, bronchiolitis, acute wheezing and pneumonia. Viremia has also been observed in patients and viral genetic material has been detected in urine and stool. HBoV has been suggested to be associated with gastrointestinal symptoms such as nausea, vomiting and diarrhea.

Diagnosis

HBoV is most commonly detected by PCR. Virus can be detected either from respiratory secretions or stool samples. Patients have also tested positive for HBoV specific antibodies and ELISA tests can be performed.

Management and Control

The study of HBoV is largely in its infancy and there is still some debate about whether the virus is an etiologic agent of acute respiratory infection. As such, there are no antivirals or vaccine candidates against the virus.

Additional Viruses With Respiratory Transmission

Measles Virus

Measles virus (MV) is in the Morbillivirus genus, family Paramyxoviridae. Similar to the other Paramyxoviruses discussed in this article, measles has a nonsegmented, negative sense, single stranded RNA genome, which is roughly 16 kilobases in length. This genome encodes six structural proteins: nucleoprotein (N), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin (H) and large (L). In addition, there are two nonstructural proteins, V and C, which are encoded within the P gene. MV particles are spherical and roughly 150–300 nm in diameter. Virions are enveloped and have the F and H proteins protruding on the surface that regulate cell entry. Inside the virion, the RNA genome is bound by the N protein which associates with the RNA-dependent RNA-polymerase L, and the cofactor P, as a ribonuclear protein complex. This complex is surrounded by the M protein.

Cell entry of MV is regulated by the H and F proteins. H is responsible for receptor binding. Wild type MV bind to CD150/SLAM that is found on the surface of B and T lymphocytes, immature thymocytes, monocytes and dendritic cells. In contrast, the attenuated form of MV used for vaccination uses CD46 that is found ubiquitously on nucleated cells. MV can also utilize nectin-4. Following receptor engagement, fusion of the viral envelope and cellular membranes is mediated by the F proteins and occurs at neutral pH. The genome is then replicated in a similar fashion to that described previously for other paramyxoviruses.
MV is a highly contagious pathogen, with an R0 value of between 12 and 18. Indeed, it is estimated that in an immunologically naïve population, 90% of people in close contact with an infected individual will contract the virus. MV has been shown to spread by respiratory droplets and aerosolized particles, but infects immune cells resident in the lungs, rather than respiratory epithelial cells, as a result of the tropism imposed by CD150/SLAM usage. Symptoms typically develop 9–12 days following exposure. Initial symptoms present with a fever, cough, conjunctivitis and coryza. Over time, further, highly characteristic, systemic symptoms appear such as Koplik’s spots in the mouth and an erythematous, maculopapular rash. Further complications can occur such as diarrhea, blindness, encephalitis and pneumonia. After spreading through the blood stream in lymphocytic cells, MV returns to, and infects, airway epithelial cells from the basolateral side through nectin-4, allowing for respiratory shedding and onward transmission.

A highly effective, live-attenuated vaccine is available. The vaccine was originally developed by John Enders in 1963 and is now routinely given to children before the age of 18 months, along with vaccines against mumps and rubella (MMR). Immunity to measles virus is lifelong and the virus is on a target list for eradication by the WHO.

**Enterovirus-D68**

Enterovirus-D68 (EV-D68) was first identified in California in 1962 from children suffering severe respiratory tract infections and pneumonia. The virus is in the *Picornaviridae* family. Like other picornaviruses, EV-68 virions are small at around 18–30 nm, made up of four structural proteins and are nonenveloped. The genome is approximately 7.5 kilobases and produces 11 proteins that are released from a single polyprotein.

EV-D68 appears to require sialic acid for infection, and in vitro studies have suggested that sialic acid binding causes virus uncoating. ICAM-5/leucencephalin has also been suggested as a receptor. Once inside cells, EV-D68 replicates similarly to other positive sense RNA viruses, and particularly the already discussed rhinovirus, another member of the *Picornaviridae*.

From initial identification in 1962, until the early 2000s, EV-D68 infection was considered rare. However, in recent years there have been large scale outbreaks of the virus, especially in the United States which has seen epidemics in 2014 and 2017. Infection is typically associated with respiratory symptoms, either mild common cold, or more severe pneumonia. For a period, EV-D68 was referred to as rhinovirus 87 by some. In contrast to many other enteroviruses, EV-D68 is sensitive to low pH and therefore does not effectively infect through the gastrointestinal tract, leaving the respiratory tract as the primary site of infection. Similar to rhinovirus, EV-D68 has been found to replicate better at lower temperatures. In addition to respiratory symptoms, EV-D68 has been associated with cases of acute flaccid myelitis, similarly to poliovirus, suggesting the virus may be capable of infection at sites other than the lungs.

To date, there is no effective antiviral therapy or vaccine against EV-D68 and care is supportive and symptomatic.

**Enterovirus 71**

Enterovirus 71 (EV71) is another *Picornavirus*. EV71 was also identified in California, a number of years after EV-D68 in 1969. EV71 utilizes P-selectin glycoprotein ligand-1 and scavenger receptor class B member 2 as receptors for infection. EV71 is best known for causing hand-foot-mouth disease, largely in the Asia-Pacific region, and in some cases severe neurological symptoms. However, the virus is also associated with respiratory disease such as pharyngitis, bronchiolitis, croup and pneumonia, mostly in young children. As with most other viruses discussed in this article, there is currently no treatment or antiviral therapy for EV71, but research is ongoing in the area.

**Conclusion**

Respiratory viruses are the most frequent causes of human disease worldwide. Each year, this broad group of pathogens is responsible for a huge number of deaths and economic loss through days of sickness. Moreover, owing to the nature of respiratory transmission, this category of viruses has the potential for wide-ranging spread. SARS-CoV, a virus that reached 27 countries within a matter of weeks, highlights the explosive outbreak potential of respiratory pathogens. Emergence of novel respiratory viruses carries with it the fear of pandemic disease spread. To date there is generally a lack of antiviral therapy or vaccination against any of these viruses, further fueling fears for far reaching spread. By developing a deeper understanding of how these viruses replicate, cause disease and transmit, we will be able to be prepared for, and counter, the next great pandemic respiratory virus.

**Further Reading**

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