155 Coronaviruses, Including Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS)

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SHORT VIEW SUMMARY

Definition
- The coronaviruses (CoVs) commonly cause mild but occasionally more severe community-acquired acute respiratory infections in humans. CoVs also infect a wide variety of animals, and several CoVs (e.g., severe acute respiratory syndrome [SARS], Middle East respiratory syndrome [MERS]) have crossed the species barrier, producing outbreaks of severe human respiratory disease. While SARS-CoV was eradicated, MERS-CoV continues to circulate in human and camel populations. As of March 10, 2019, 2374 cases of laboratory-confirmed MERS were reported to the World Health Organization, with 823 deaths.

Epidemiology
- Community-acquired CoV infections cause about 15% of common colds. They are typically epidemic in the winter months. MERS has occurred in patients in the Arabian Peninsula and those who recently traveled from this locale.

Microbiology
- CoVs are members of the Nidovirales order, single-stranded, positive-sense RNA viruses with a large genome. They mutate and also recombine frequently.

Diagnosis
- Laboratory diagnosis is best accomplished by finding viral RNA through polymerase chain reaction.

Therapy
- There are no accepted effective antiviral drugs for CoVs.

Prevention
- Prevention is through epidemiologic methods and the use of appropriate respiratory precautions in hospital settings. The SARS epidemic and MERS outbreaks were controlled through careful case identification, quarantine, and use of barrier precautions.

The family Coronaviridae, within the order Nidovirales, presently contains two subfamilies, the Coronavirinae and the Torovirinae. However, increased recognition of the genomic diversity of viruses within the Nidovirales order makes it likely that nidovirus, coronavirus, and torovirus taxonomy will require modification. Coronaviruses (CoVs) are a large group of viruses infecting mammals and birds and producing a wide variety of diseases. They have been divided into four genera, two of which contain viruses infecting humans (see later). All human coronaviruses (HCoVs) are primarily respiratory pathogens. During the winter of 2002–2003, an alarming new disease appeared: severe acute respiratory syndrome (SARS), which was quickly attributed to a new CoV, the SARS-CoV. The outbreak originated in southern People's Republic of China, with evidence that the virus was first derived from bats and was transmitted to humans through intermediate hosts, probably the palm civet *(Paguma larvata)* and raccoon dog *(Nyctereutes procyonoides)*.[1–3] The SARS epidemic was controlled through a massive effort at case identification and containment, and the last known case occurred in mid-2004. In retrospect, the emergence of SARS is consistent with what is known about CoVs as a group: They are important pathogens in animals causing a wide variety of diseases through a wide variety of pathogenic mechanisms, and they have been noted to mutate frequently and infect new species.[4,5]

More recently, a related but different CoV producing severe respiratory disease has emerged, the Middle East respiratory syndrome coronavirus (MERS-CoV). MERS-CoV was grown in June 2012 from a sputum sample obtained from a man in Saudi Arabia who died of overwhelming pneumonia. The virus was quickly identified as a new CoV most closely related to several bat CoVs.[6] This report was followed by a number of other reports identifying a total of 2374 infected individuals, most of whom had acute respiratory symptoms, severe in most and fatal in 823 (as of March 10, 2019).[7,8]

Human-to-human transmission has been documented but appears to be inefficient except in hospital settings.[9–11] The animal reservoir of MERS-CoV is believed to be camels, although evidence suggests that bats may be infected with related viruses.[12–14] Infection of camels on the Arabian peninsula and throughout Africa is widespread, and several cases of camel-to-human transmission have been reported, generally from juvenile camels.[12,15]

HISTORY

Community-Acquired Respiratory Coronaviruses, Severe Acute Respiratory Syndrome, and Middle East Respiratory Syndrome

In 1965, Tyrrell and Bynoe[16] cultured a virus obtained from the respiratory tract of a boy with a common cold by passage in human embryonic tracheal organ cultures. The media from these cultures consistently produced colds in volunteers. The agent was ether sensitive but not related to any known human virus. Subsequently, electron microscopy of fluids from infected organ cultures revealed particles that resembled infectious bronchitis virus of chickens.[17] At about the same time, Hamre and Procknow recovered a cytopathic agent in tissue culture from medical students with colds.[18] The prototype virus was named 229E and was found on electron microscopy to have a similar or identical morphology (Fig. 155.1).

Using techniques similar to those used by Tyrrell and Bynoe, McIntosh and colleagues[19] reported the recovery of several infectious bronchitis–like agents from the human respiratory tract, the prototype of

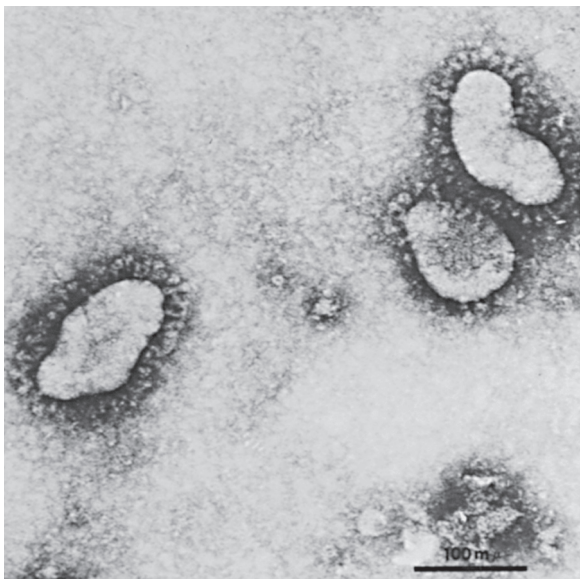

FIG. 155.1 Coronavirus strain HCoV-229E, harvested from infected WI-38 cells (phosphotungstic acid stain). *(From McIntosh K, Dees JH, Becker WB, et al. Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease.* Proc Natl Acad Sci U S A. *1967;57:933–940.)*

which was named OC43 (OC for organ culture). At much the same time, mouse hepatitis virus and transmissible gastroenteritis virus of swine were shown to have the same morphology on electron microscopy.[20] Shortly thereafter, the name *coronavirus* (the prefix *corona* denoting the crownlike appearance of the surface projections) was chosen to signify this new genus.

The number of animal CoVs quickly grew, including viruses causing diseases in rats, mice, chickens, turkeys, various other bird species, cattle, several wild ruminants, beluga whales, dogs, cats, rabbits, and pigs, with manifestations in the respiratory and gastrointestinal tracts, central nervous system, liver, reproductive tract, and other locations. Through sequencing and antigenicity studies, the animal CoVs and HCoVs initially were divided into three groups: group 1, which contained HCoV-229E, as well as numerous animal viruses; group 2, which contained HCoV-OC43 plus the closely related animal viruses, bovine CoV and mouse hepatitis virus; and group 3, which included only avian viruses related to infectious bronchitis virus (Fig. 155.2). Current taxonomy divides the subfamily Coronavirinae into four genera: *Alphacoronavirus* (which includes viruses previously in group 1); *Betacoronavirus* (which includes viruses previously in group 2, most notably SARS-CoV and MERS-CoV); *Gammacoronavirus* (which includes viruses previously in group 3); and *Deltacoronavirus* (which includes several newly described avian and swine viruses).[21]

SARS was first identified in Guangdong Province of the People's Republic of China in November 2002 and spread from there to Hong Kong and then throughout the world.[22] A CoV was independently and

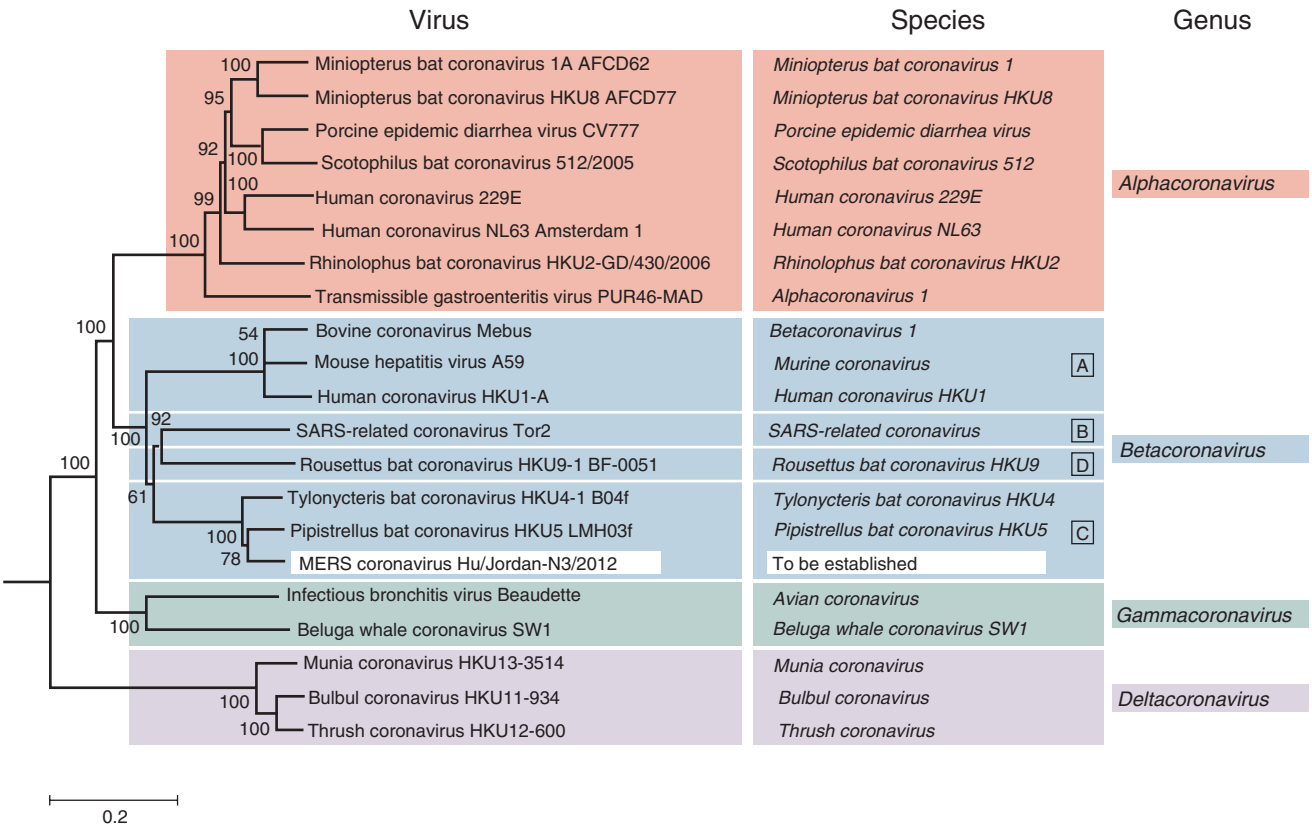

FIG. 155.2 Phylogenetic relationships among members of the subfamily Coronavirinae. A rooted neighbor-joining tree was generated from amino-acid sequence alignments of Coronaviridae-wide conserved domains in replicase polyprotein 1 (ADRP, nonstructural protein [nsp]3; Mpro, nsp5; RdRP, nsp12; Hel, nsp13; ExoN, nsp14; NendoU, nsp15; O-MT, nsp16) for 21 coronaviruses, each a representative of a currently recognized coronavirus species. Five of the six known human coronaviruses (HCoV-229E, HCoV-NL63, HCoV-HKU1, SARS-CoV, and MERS-CoV) are indicated. HCoV-OC43 is closely related to bovine coronavirus, which is shown in the figure. Equine torovirus Berne served as the outgroup. Virus names are given with strain specifications; species and genus names are in italics as per convention. The tree shows the four main monophyletic clusters, corresponding to genera *Alpha-, Beta-, Gamma-,* and *Deltacoronavirus* (color coded). Also indicated are betacoronavirus lineages A through D (corresponding to former CoV subgroups 2A through D). Bootstrap values (1000 replicates) are indicated at branch points. The tree is drawn to scale (scale bar, 0.2 amino-acid substitutions per site). *(From de Groot RJ, Baker SC, Baric RS, et al. Middle East respiratory syndrome coronavirus [MERS-CoV]: announcement of the Coronavirus Study Group.* J Virol. *2013;14:7790–7792.)*

almost simultaneously isolated from SARS patients by several laboratories and found by sequencing to be only distantly related to previously characterized CoVs.[23–26] The SARS outbreak stimulated a rapid and intense public health response coordinated by the World Health Organization (WHO), and by July 2003, transmission had ceased throughout the world. Despite this effort, however, 8096 probable cases had occurred in 29 countries, with 774 deaths.[22]

With the identification of the SARS-CoV, the HCoV field became much more active. Sensitive molecular methods were developed to detect RNA from viruses identical or closely related to HCoV-229E and HCoV-OC43 in the respiratory tract, and two new species were discovered: NL63, an alphacoronavirus, and HKU1, a betacoronavirus.[27–29] HCoV-NL63 was found independently by three groups, two in the Netherlands and, somewhat later, the third in New Haven, Connecticut.[30] In all three cases, positive samples were from infants and children with respiratory disease. Notably, HCoV-NL63 and HCoV-229E were estimated to have originated from a common bat precursor and diverged approximately 1000 years ago.[31,32] CoVs related to HCoV-229E have been isolated from camels.[33] All CoVs are susceptible to recombination, but HCoV-OC43 may be especially susceptible, with multiple recombinant strains identified in a confined geographical setting.[34] HCoV-HKU1 was found in Hong Kong in an adult with respiratory disease. These two new HCoV strains subsequently have been found worldwide and appear to have pathogenicity similar to that of HCoV-229E and HCoV-OC43, with the possible exception that NL63 is more frequently found in children with croup.[35,36]

The MERS-CoV was found when a man was admitted in June 2012 to a hospital in Jeddah, Saudi Arabia, with overwhelming acute pneumonia and renal failure. A sample of sputum grew a cytopathic virus that, on sequencing, proved to be a CoV, classified as a *Betacoronavirus* and most closely related to two bat CoVs, HKU4 and HKU5.[6] MERS-CoV continues to cause new infections, with all but a few of them sporadic or hospital-based and in individuals living or traveling in the Middle East.[37,38]

In the remainder of this chapter, the group of respiratory HCoVs first discovered in the 1960s and containing HCoVs 229E, OC43, NL63, and HKU1 are referred to as community-acquired respiratory (CAR) HCoVs to distinguish them from the SARS-CoV and the MERS-CoV.

Gastrointestinal Coronaviruses and Toroviruses

In view of the prominence of CoVs in animal enteric diseases, there have been extensive efforts to identify enteric HCoVs. There are numerous reports of CoV-like particles (CoVLPs) found by electron microscopy in human fecal matter, but these particles have been difficult to characterize further. Efforts to detect CoV RNA in feces using polymerase chain reaction (PCR) and primers for respiratory HCoVs have had limited success and have failed to associate CoVs with gastrointestinal disease.[39,40] In one instance, a human enteric CoV with high identity to bovine CoV was isolated from a child with diarrhea and shown to cause diarrhea when reintroduced into calves, demonstrating its pathogenic potential.[41]

Toroviruses were, like CoVs, first described in animals. They were first detected in the feces of cattle (Breda virus) and horses (Berne virus).[42,43] While previous publications suggested that particles resembling toroviruses could be detected in human fecal material using electron microscopy,[44] there are presently no reports definitively showing the existence of human toroviruses.

DESCRIPTION OF THE PATHOGENS

The CoV nucleic acid is RNA, approximately 30 kb in length, of positive sense, single stranded, polyadenylated, and infectious. The RNA, the largest known viral RNA (Fig. 155.3), codes for (in order from the 5′ end) a large polyprotein that is cleaved by virus-encoded proteases to form several nonstructural proteins, including an RNA-dependent RNA polymerase, methyltransferases, and a helicase, followed by either four or five structural proteins intermingled with a variable number of nonstructural and minor structural proteins.[4] The first of the major structural proteins is a surface hemagglutinin-esterase (HE) protein, present on HCoVs OC43 and HKU1 and some animal betacoronaviruses, that may play some role in the attachment or release of the particle, or both, at the cell surface. The gene for the HE protein contains sequences similar to the hemagglutinin of influenza C virus, likely evidence of an interfamily recombinational event that occurred many years ago. Notably, the HE receptor binding activity of HCoV-OC43 and probably also of HKU1 was progressively lost along with a decrease in HE-associated esterase activity during evolution in humans. These changes most likely reflected adaptation to the human sialic acid receptor after introduction into humans from zoonotic sources.[45] The next gene encodes the surface glycoprotein that forms the petal-shaped surface projections and is responsible for attachment and the stimulation of neutralizing antibody. This is followed by a small envelope (E) protein, a membrane glycoprotein, and a nucleocapsid protein that is complexed with the RNA. There are several other open reading frames, which are unique to each strain of CoV. While their coding functions are not clear, many of them are probably involved in immune evasion.[46,47] The strategy of replication of CoVs is similar to that of other nidoviruses, in that all messenger RNAs form a nested set with common polyadenylated 3′ ends, with only the unique portion of the 5′ end being translated.[4] As in other RNA viruses, mutations are common in nature, although the mutation rate is much lower, approximately 2×10^{-6} per site per replication cycle.[48] Unlike other RNA viruses, CoVs encode a 3′→5′ exonuclease that has proofreading activities, playing a critical role in maintaining replication fidelity in cell cultures and in animals.[49] CoVs are also capable of genetic recombination if two viruses infect the same cell at the same time.

All CoVs develop exclusively in the cytoplasm of infected cells (Fig. 155.4). They bud into cytoplasmic vesicles from membranes of the pre-Golgi endoplasmic reticulum. These virus-filled vesicles are then extruded by the exocytic secretory pathway with the small E protein critical for this process.[50] The resultant virus particles have a diameter of 70 to 80 nm on thin-section electron microscopy and 60 to 220 nm on negative staining. They are pleomorphic, with widely spaced, petal-shaped projections 20 nm long (see Fig. 155.1).

The cellular receptor for 229E and most other alphacoronaviruses is aminopeptidase N (APN).[51] Interestingly, NL63, the other known human alphacoronavirus, uses as its cellular receptor angiotensin-converting enzyme 2 (ACE2),[52] the same receptor as is used by the SARS-CoV.[53] Mouse hepatitis virus, a betacoronavirus related to strain OC43, uses as its receptor a member of the carcinoembryonic antigen family.[54] HCoV-OC43 and bovine CoV, which is closely related to HCoV-OC43, bind to 9-*O*-acetylated neuraminic acid as part of the entry process.[55] The host cell receptor for MERS-CoV is dipeptidyl peptidase 4, which, like ACE2 and APN, is an ectopeptidase that is abundantly expressed in the respiratory and enteric tracts.[56,57] This preferential usage of large host ectopeptidases for CoV entry is notable but not understood.

All the CAR HCoVs grow only with difficulty in tissue culture. Despite this, both 229E and NL63 were discovered because they produced a detectable cytopathic effect, the first in human embryonic kidney[18] and the second in LLC-MK2 cells.[27] Both the SARS-CoV and the MERS-CoV were initially isolated and grew readily in Vero cells.[6,25] HCoVs OC43 and HKU1 have been grown in tissue culture after laboratory adaptation or in primary ciliated human airway epithelial cells.[58,59] Detection of all these viruses in clinical specimens is most conveniently and sensitively achieved using PCR.

The enteric CoVs have been difficult to cultivate in vitro. All but a few strains have been detected only by electron microscopy of human fecal material. Two strains obtained from an outbreak of necrotizing enterocolitis in Texas were reported to contain four or five proteins with apparent molecular weights similar to those of other CoVs but not related antigenically to known human or animal strains.[60] The evidence favors the view that these isolates, as well as particles antigenically related to HCoV-OC43, are members of the family Coronaviridae, although their association with human disease is not proven.

EPIDEMIOLOGY

CAR Coronaviruses

Evidence of CAR CoV infections has been found wherever in the world it has been sought (e.g., Japan,[61] Ghana[62]). In temperate climates, CAR CoV infections occur more often in the winter and spring than in the summer and fall. The contribution of CAR CoV infections to the total

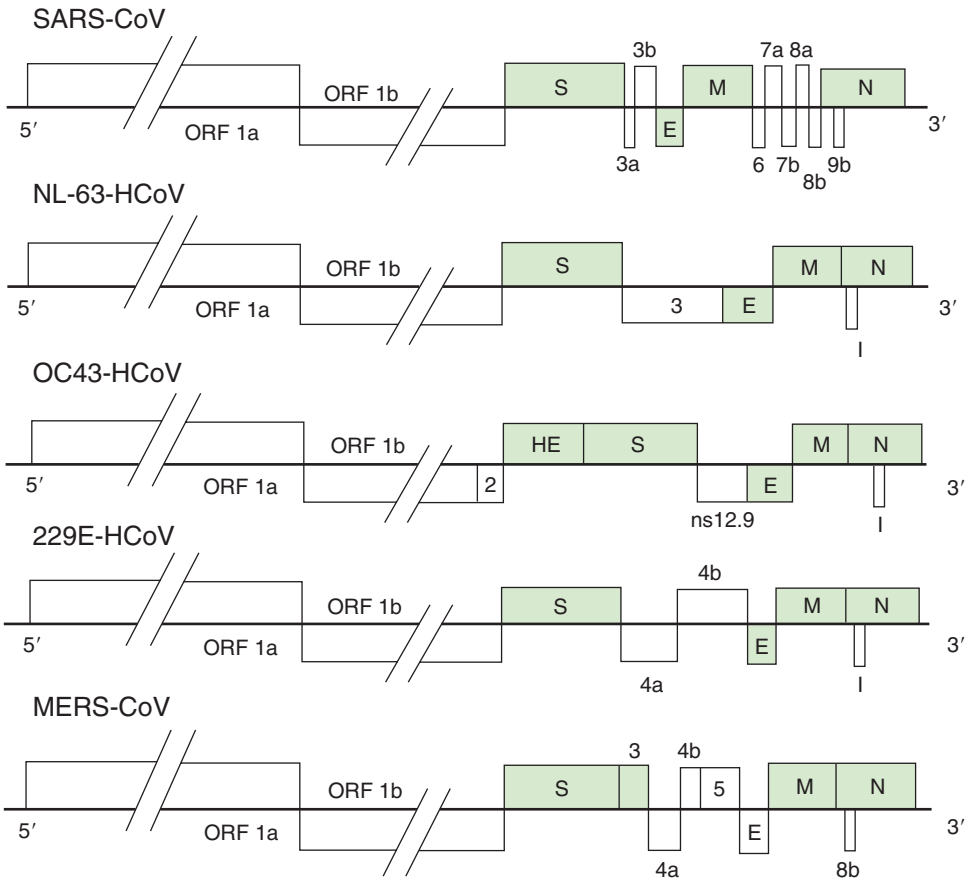

FIG. 155.3 Genome organization of representative human coronaviruses. All coronavirus genomes have the same basic structure and mechanism of replication.[4] The 5′ end of each genome encodes a leader sequence, which is attached to each virus-specific messenger RNA transcript by a novel mechanism of discontinuous replication. The first two-thirds of each genome encode replicase-associated genes. Gene 1 is translated as two large polyproteins, with the first expressed from ORF1a and the second from ORF1a/b following a −1 frameshift event. These polyproteins are then cleaved into individual proteins by two virus-encoded proteases. The major structural genes, the hemagglutinin-esterase *(HE),* surface *(S),* envelope *(E),* transmembrane *(M),* and nucleocapsid *(N)* proteins, are indicated in green. The nonreplicase, accessory genes located at the 3′ end of the genome are indicated with open boxes. The functions of these proteins are largely not known, and there is no sequence homology between accessory proteins of different coronaviruses. Some of these proteins are virion associated, but none is required for virus replication. The open reading frames (*ORFs*) encoding these proteins are numbered in order of appearance from the 5′ end of the genome, with the exception of ns12.9 of human coronavirus *(HCoV)*-OC43. I is an internal protein expressed from an alternative reading frame located within the N gene. It is equivalent to severe acute respiratory syndrome coronavirus *(SARS-CoV)*–specific protein 9b and the Middle East respiratory syndrome coronavirus *(MERS-CoV)*–specific protein 8b. *(Figure prepared by Rahul Vijay.)*

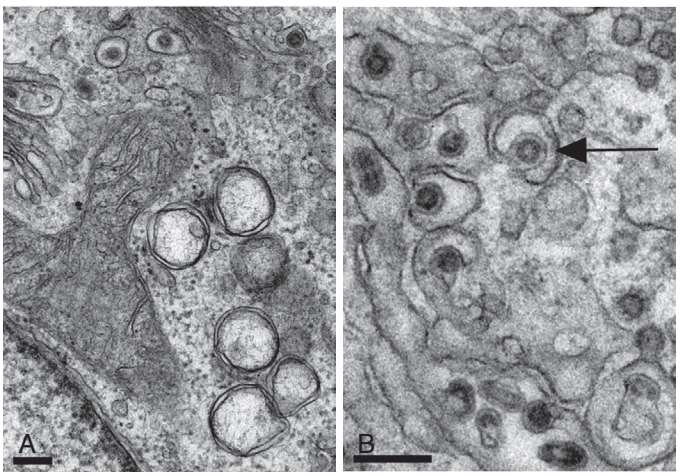

FIG. 155.4 Coronavirus strain 229E in Huh-7 cells. Huh-7 cells infected with HCoV-229E and fixed at 18 hours postinfection. (A) Double membrane vesicles, which are sites of virus replication, are shown. (B) Sites of virus assembly and budding *(arrow)* are shown. *(Images courtesy Drs. Eric Snijder and Montserrat Bárcena, Leiden University Medical Center, The Netherlands).*

number of upper respiratory illnesses may be as high as 35% during times of peak viral activity. Overall, the proportion of adult colds produced by CAR CoVs may be reasonably estimated at 15%,[63] with HCoV-NL63 and HCoV-OC43 being more common than HCoV-229E or HCoV-HKU1 in infants.[63,64]

Early studies of HCoV-OC43 and 229E in the United States demonstrated periodicity, with large epidemics occurring at 2- to 3-year intervals.[65] Similar studies of NL63 and HKU1 have not been done, but it seems from the available data that they also vary widely in incidence from year to year and place to place. Reinfection is common and may be due to the rapid diminution of antibody levels after infection.[66–68] Infection occurs at all ages but is most common in children. The ratio of symptomatic to total infections varies between 50% and 90%, depending on the age of the population studied, the method of virus detection, and the definition of "infection."[67,68] Among adult volunteers, 72% of those infected with HCoV-229E developed colds.[69]

MERS Coronavirus

Middle East respiratory syndrome (MERS) was first identified in 2012 in a man from Jeddah, Saudi Arabia, who developed pneumonia in June and died of respiratory and renal failure.[6] A virus was grown from a sputum sample that was subsequently sequenced and found to be a betacoronavirus most closely related to bat CoVs HKU4 and HKU5.

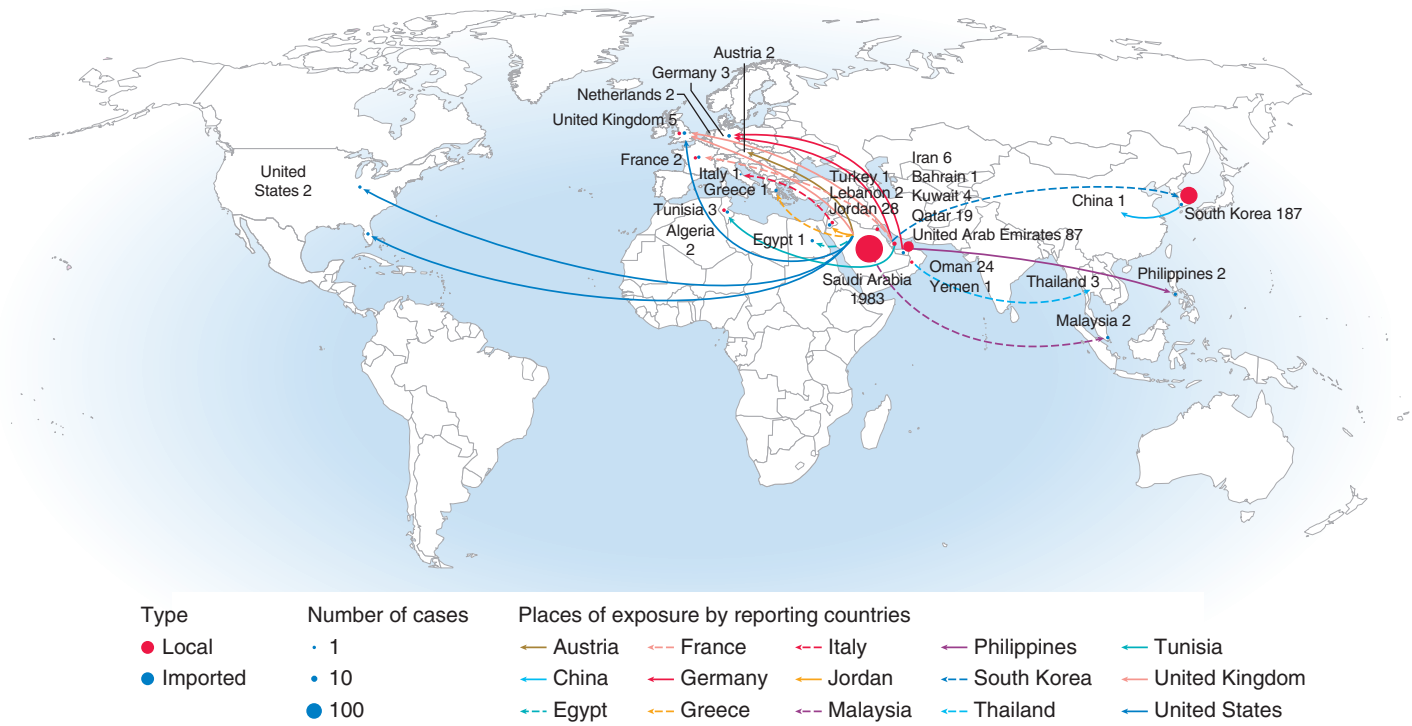

FIG. 155.5 Distribution of confirmed cases of Middle East respiratory syndrome coronavirus by reporting country, March 2012 to March 2019. This map is based on one published by the World Health Organization and shows the predominance of cases reported from the Kingdom of Saudi Arabia, with other cases arising in the Middle East, as well as the routes of travel during importation to other countries *(colored arrows). (Modified from World Health Organization. Middle East respiratory syndrome coronavirus [MERS-CoV].* http://www.who.int/emergencies/mers-cov/en/*. Accessed December 18, 2017.)*

Between then and March 10, 2019, a total of 2374 cases occurred, all infected by this virus, now termed the *Middle East respiratory syndrome coronavirus*.[70] The vast majority of these have been acquired and diagnosed in the Kingdom of Saudi Arabia, with most of the remainder in the United Arab Emirates, Qatar, Jordan, Oman, Lebanon, Iran, and Kuwait (Fig. 155.5). Cases originating in the Arabian peninsula have also occurred in travelers to Egypt, Tunisia, Germany, Italy, Great Britain, Greece, Malaysia, the Philippines, the United States, and the Republic of Korea, with secondary cases sometimes occurring in those locations through close family or hospital spread. In the United States, these include two unrelated MERS cases.[37,38,71]

WHO, the CDC, the Saudi Arabian Ministry of Health, and the Korean Centers for Disease Control and Prevention have published case definitions as well as surveillance instructions to aid in epidemiologic control of the MERS-CoV.[7,8,72,73] The majority of MERS-CoV transmission in the early years after virus identification reflected nosocomial spread.[74] Spread commonly occurred in emergency rooms and during aerosol-generating procedures. Environmental MERS-CoV contamination can be commonly detected in the vicinity of MERS patients, indicating that fomite and contact spread could occur and emphasizing the need for careful surface hygiene management.[75] However, as better infection control measures have been followed in hospitals, approximately 50% of cases are now believed to be primary cases, often acquired from camels.[76] (Of note, only a minority of presumptive primary cases describe camel contact.[11]) Virus may spread from camels through exposure to nasal or other body secretions, via the consumption of raw camel milk, or via environmental contamination[12] since virus can survive on hard surfaces for at least 48 hours.[77] Spread within family settings outside of the hospital is uncommon.[78]

In May and June 2015, a large outbreak of MERS occurred in South Korea; the index case was in a traveler returning from the Arabian Peninsula.[79] By the end of the outbreak, 186 cases, including 38 deaths, had been reported in Korea among household and hospital contacts. The large number and severity of cases resulted from several factors, including lack of timely diagnosis, "doctor-shopping," "super-spreading events," patterns of familial caregiving, and inadequate hospital infection control measures.[80,81]

Studies of MERS prevalence rely on virus detection at the time of acute infection or on measurements of anti–MERS-CoV antibodies. Prevalence studies may underestimate the numbers of exposed patients because antibody titers either do not develop (subclinical infection) or are transient (mild pneumonia).[78,82] Measurements of human MERS-CoV T-cell responses, although technically challenging, may provide better estimates of prevalence since they tend to decline less rapidly in both SARS and MERS survivors.[83,84]

Camels are almost certainly the major if not sole source for human infections. High percentages of dromedary camels that are currently or were previously infected have been detected throughout the Arabian peninsula, Africa, central Asia, and Pakistan.[12] Serologic studies demonstrated the presence of MERS-CoV antibodies in camels in Africa since at least 1983,[85] even though the first human infection was not diagnosed until 2012. MERS-CoVs have only been rarely detected in other animal species, with no evidence that they are involved in transmission to humans. Circulating MERS-CoVs with 98% to 99% identity were detected in camels and patients in the Kingdom of Saudi Arabia,[13] and in some cases virtually identical viruses were detected in patients and their contact camels.[15,86] While camels are the probable source for human MERS, bats may be the original source for the virus. Viruses related to MERS-CoV have been isolated from bats in Africa.[14,87]

SARS Coronavirus

The SARS epidemic began in Guangdong Province in the People's Republic of China in mid-November 2002.[22] It came to worldwide attention in March 2003 when cases of severe, acute pneumonia were reported to WHO from Hong Kong, Hanoi, and Singapore. Disease spread in hospitals to health care workers, visitors, and patients; among family members; and, on occasion, in hotels, apartment complexes, markets, and airplanes. Worldwide spread was rapid but focal. The

largest numbers of cases were reported from the People's Republic of China, Hong Kong, Taiwan, Singapore, and Toronto, Canada. The overall case-fatality rates in these locations ranged from 7% to 17%, but persons with underlying medical conditions and those older than 65 years of age had mortality rates as high as 50%. There was no mortality in children or in adults younger than the age of 24 years.[88]

In response to the global spread and associated severe disease, WHO coordinated a rapid and effective control program that included isolation of cases, careful attention to contact, droplet and airborne infection control procedures, quarantine of exposed persons in some settings, and efforts to control spread between countries through travel advisories and travel alerts. Presumably as a result of these efforts, global transmission ceased by July 2003. A few subsequent cases of SARS were detected, but all were either a result of laboratory spread or individual cases related to presumed contact with civet cats or other intermediate hosts. The last known case occurred in mid-2004.[89]

Spread of SARS to humans is thought to have occurred primarily through droplet or contact transmission, with a possible role for fomites. In most instances, an individual case transmitted to very few others, although super-spreading events were well documented,[90] likely involving small-particle airborne transmission.[91] Spread in hospital settings appeared to be surprisingly efficient, but it could be effectively suppressed with the enforcement of droplet and contact precautions and airborne precautions during aerosol-generating procedures.[92] Containment measures were efficacious, in part, because patients were most contagious only after lower respiratory disease developed.[93] The chain of spread was finally broken in the People's Republic of China, the last country to experience epidemic spread, in July 2003.

It now seems almost certain that the human epidemic began with the spread of a closely related bat virus first to palm civets or other animals sold in live wild game markets and then to humans in Guangdong Province in the People's Republic of China, and that the virus adapted itself through mutation and possibly recombination, until it transmitted readily among humans.[3,94–96] The virus that spread worldwide came largely from a single infected individual who traveled from Guangdong Province to Hong Kong and infected a large number of individuals before himself succumbing to the disease. In contrast, the virus that was epidemic in the People's Republic of China was more variable.

Gastrointestinal Coronaviruses

Although an etiologic role is not proven, enteric CoVLPs have been most frequently associated with gastrointestinal disease in neonates and infants younger than 12 months. Particles have been found in the stools of adults with the acquired immunodeficiency syndrome.[97,98] Asymptomatic shedding is common, particularly in tropical climates[99] and in populations living in poor hygienic conditions.[100] The particles can be detected for prolonged periods and without any apparent seasonal pattern.[101–103]

PATHOGENESIS

CAR Coronaviruses

CAR CoVs (HCoV-229E, OC43, NL63, HKU1) generally replicate in ciliated (HCoV-OC43, NL63, HKU1) and nonciliated (HCoV-229E) epithelial cells of the nasopharynx,[104] probably producing both direct cell degeneration[105] and an outpouring of chemokines and interleukins, with a resultant common-cold symptom complex similar to that produced by rhinovirus infection.[106] The incubation period is, on average, 2 days, and the peak of respiratory symptoms, as well as viral shedding, is reached at approximately 3 or 4 days after inoculation.[69]

The pattern of virus replication of CoVs is at least in part determined by virus-receptor interactions. The two best-defined receptors for the CAR CoVs are APN for strain HCoV-229E and ACE2 for NL63.[51,52]

MERS Coronavirus

Understanding of the pathogenesis and pathology of MERS has been hampered by a lack of surgical and autopsy specimens, largely for cultural and religious reasons. Autopsies from an immunocompetent patient in the United Arab Emirates and an immunocompromised patient in the Kingdom of Saudi Arabia revealed severe changes in the lungs, including hyaline membrane formation, alveolar fibrin deposition, and alveolar septal edema.[107,108] Hemorrhagic changes were observed in the lungs of the immunocompromised patient. Viral protein was detected by immunohistologic staining in the lungs of the patient from the United Arab Emirates. Particles resembling HCoVs were seen by electron microscopy in both lungs and kidney of the immunocompromised patient, but immunohistology was not recorded.

SARS Coronavirus

The pathogenicity of SARS includes systemic spread. Although the lung is the primary focus of the disease process, there are often signs of involvement in other organ systems, including diarrhea, leukopenia, thrombocytopenia, and, most notably, pan-lymphopenia.[109] Virus has been detected in respiratory secretions, blood, stool, and urine specimens and in lung, spleen and lymph nodes, brain, kidney, and intestine tissues when examined at autopsy.[110,111] On the basis of PCR testing, virus titer is highest during the second week of illness[112] and can often be detected in the stool into the third week of illness or longer.[26] Pulmonary symptoms may worsen late in the course of the illness, with the development of adult respiratory distress syndrome.[112] There may also be late evidence of liver and kidney involvement.

The pulmonary pathology of infection by the SARS-CoV has been described extensively,[25,111,113,114] but less has been published about the pathology in other organ systems.[110,111,115] The extrapulmonary pathologic changes found most consistently at autopsy are extensive necrosis of the white pulp of the spleen and a generalized small vessel arteritis. In the lung, there is hyaline membrane formation, interstitial infiltration with lymphocytes and mononuclear cells, and desquamation of pneumocytes in the alveolar spaces. Giant cells are a constant finding and usually have macrophage markers. In bronchoalveolar lavage, biopsy, and autopsy specimens, viral particles and viral RNA and protein have been noted in type I and II pneumocytes.[110,116]

CLINICAL MANIFESTATIONS

CAR Coronaviruses

Administration of antigenically distinct CAR CoV to volunteers produced illness with similar characteristics.[16,69,117] A summary of these characteristics is given in Table 155.1, in which a comparison is made with colds produced by rhinoviruses in similarly inoculated volunteers. The incubation period of CoV colds was longer and their duration somewhat shorter, but the symptoms were similar. Asymptomatic infection was sometimes seen and, indeed, has been a feature of both serologic surveys and PCR-based studies of natural infection of infants, children, and adults.[118,119]

More serious respiratory tract illness is probably also caused by all four strains of CAR HCoV. The evidence for this is not conclusive, but it seems likely that all strains can produce pneumonia and bronchiolitis in infants,[35,39,120,121] otitis and exacerbations of asthma in children and young adults,[122–124] pneumonia in healthy adults,[125] exacerbations of asthma and chronic bronchitis in adults,[126–128] influenza-like illness, serious bronchitis and pneumonia in the elderly,[63,67,129,130] and pneumonia in the immunocompromised host.[131,132] HCoVs are found in asymptomatic individuals of all ages, and, when accompanied by illness, are also sometimes accompanied by infections with other potential respiratory pathogens. Infection without disease and coinfection during disease are features of many respiratory pathogens, including rhinoviruses, adenoviruses, human metapneumovirus, human bocavirus, and parainfluenza viruses, but also (although less frequently) respiratory syncytial virus and influenza virus, making pathogenicity difficult to prove. Because infections with CAR HCoVs are so common, however, it is possible that they are responsible for a significant portion of these serious lower respiratory tract diseases, even though the basic pathogenicity of HCoVs (judging from volunteer studies) is similar to that of rhinoviruses, and clearly less than that of respiratory syncytial virus, influenza viruses, and certain adenovirus types. There is some evidence that HCoV-OC43 is more pathogenic in the elderly than HCoV-229E[133] and that NL63 differs from the other CAR HCoVs in preferentially causing childhood croup.[36]

MERS Coronavirus

MERS-CoV predominantly, if not solely, initiates infection via the respiratory tract. The median incubation period is 7 days, with a

TABLE 155.1 Clinical Features of Colds Produced by Experimental Infection With Four Viruses

	CORONAVIRUSES		RHINOVIRUSES	
FEATURE	**229E**	**B814**	**Type 2 (HGP or PK)**	**DC**
No. of volunteers inoculated	26	75	213	251
No. (%) getting colds	13 (50)	34 (45)	78 (37)	77 (31)
Incubation period (days)				
Mean	3.3	3.2	2.1	2.1
Range	2–4	2–5	1–5	1–4
Duration (days)				
Mean	7	6	9	10
Range	3–18	2–17	3–19	2–26
Maximum no. of handkerchiefs used daily				
Mean	23	21	14	18
Range	8–105	8–120	3–38	33–60
Malaise (%)	46	47	28	25
Headache (%)	85	53	56	56
Chill (%)	31	18	28	15
Pyrexia (%)	23	21	14	18
Mucopurulent nasal discharge (%)	0	62	83	80
Sore throat (%)	54	79	87	73
Cough (%)	31	44	68	56
No. (%) of volunteers with colds of indicated severity				
Mild	10 (77)	24 (71)	63 (80)	36 (47)
Moderate	2 (15)	7 (20)	12 (15)	28 (36)
Severe	1 (8)	3 (9)	4 (5)	13 (17)

From Bradburne AF, Bynoe ML, Tyrrell DAJ. Effects of a "new" human respiratory virus in volunteers. Br Med J. *1967;3:767–769.*

range of 2 to 17 days.[9,79] MERS-CoV causes a spectrum of illness ranging from subclinical disease to lethal pneumonia.[134–136] Although infections with severe respiratory involvement have occurred at all ages, the elderly and those with underlying conditions (diabetes, renal disease, immunosuppression) are the most often severely or fatally affected.[134,135,137] MERS requiring admission to the intensive care unit cannot be distinguished by clinical, radiologic, or standard laboratory criteria from other causes of severe pneumonia, so laboratory-based diagnosis is critical.[138,139] Outcomes are worse in critically ill MERS compared to non-MERS patients, with mortality rates of approximately 58% to 78% in patients admitted to the intensive care unit.[134,135,138,139] Health care workers typically develop mild disease, although MERS-CoV has also caused lethal disease in this group.[140] Patients usually present with nonspecific symptoms, including fever, shortness of breath, cough, fever, and diarrhea. Radiographic examination of MERS patients revealed a ground-glass appearance, initially located in the lung periphery. The presence of a pleural effusion was associated with a worse outcome.[141–143]

Acute renal injury occurs commonly in patients in the Arabian peninsula with severe disease, but less so in Korean patients, possibly reflecting differences in the extent of comorbidities such as diabetes and hypertension.[37,38,135,136,138] The MERS-CoV host cell receptor, dipeptidyl peptidase 4, is expressed at high levels in the kidney,[144] raising the possibility that direct infection of this organ contributes to renal disease. Particles resembling coronaviruses were seen by electron microscopy in the renal proximal tubular cells of a fatal case of MERS in a man who had recently received chemotherapy for T-cell lymphoma.[108] MERS patients often presented with hematologic abnormalities, including leukopenia, lymphopenia, and thrombocytopenia and elevated lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase, although this was less common than observed in patients with SARS.[37,138,145] As in patients with SARS, expression of proinflammatory mediators such as interferon-α, interleukin-6, and chemokine (C-X-C motif) ligand 1 is prolonged in severe compared to mild cases. Virus-specific antibody responses developed more rapidly and robustly in MERS patients who survived the infection than in those who died.[146]

Pediatric patients with MERS generally develop mild or subclinical disease, although fatal disease in patients with underlying disease has been reported. MERS in pregnancy has been associated with fetal and/or maternal demise.[147–150]

SARS Coronavirus

SARS is generally initiated through the respiratory tract. After an incubation period that is usually 4 to 7 days, but could be as long as 10 to 14 days, the disease begins, usually with fever and other systemic (influenza-like) symptoms, with cough and dyspnea developing a few days to a week later.[22,151] Approximately 25% of patients have diarrhea. Interestingly, upper respiratory symptoms such as rhinorrhea and sore throat usually do not occur.[112,151–154] The chest radiograph is frequently abnormal, showing scattered airspace opacification, usually in the periphery and lower zones of the lung.[155] Spiral computed tomography demonstrates both ground-glass opacification and consolidation, often in a subpleural distribution.[155–158]

Lymphopenia is common,[112,113,159] with normal or somewhat depressed neutrophils. Paradoxically, neutrophilia was associated with poor outcomes.[109] Creatine kinase is often abnormal, as are lactate dehydrogenase and aspartate aminotransferase. Levels of proinflammatory cytokines were elevated at early times during infection in patients with severe clinical disease[160] and decreased in those patients who resolved the infection.[161]

Approximately 25% of patients develop severe pulmonary disease that progresses to adult respiratory distress syndrome, most commonly in patients older than 50 years or with underlying disease such as diabetes, cardiac disease, and chronic hepatitis.[112,154,159,162] The overall mortality rate is between 9% and 12%, with the highest rates in the elderly and adults with underlying liver disease. In some patients, clinical deterioration occurred during the second week of illness, as virus levels decreased, suggesting that disease was partly immune mediated.[112,154,159,162] Clinical improvement was associated with the onset of a virus-specific antibody response.[161]

Pediatric disease is significantly less severe than adult disease, although the features are similar.[163] Disease during pregnancy is severe, with high mortality in both the mother and fetus.[164] Congenital transmission has not been described.

Gastrointestinal Coronaviruses

The nature of the illness associated with enteric CoV infection is much less clear. One study found a significant association of gastroenteritis in infants 2 to 12 months of age with the presence of CoVLPs in the stool.[165] Another study, confined to infants in a neonatal intensive care unit, found highly significant associations between the presence of CoVLPs in the stool and the presence of water-loss stools, bloody stools, abdominal distention, and bilious gastric aspirates.[103] Finally, CoVLPs have been associated with at least three outbreaks of necrotizing enterocolitis in newborns.[60,103,166] Efforts to detect HCoV RNA by PCR using primers that would detect the known CAR HCoVs in stool have been disappointing, with most HCoV-positive samples also containing rotavirus or norovirus.[39,40,167,168] Of note, all of the studies that associate CoVLPs with gastrointestinal disease were done before the development of diagnosis by PCR.

Neurologic Syndromes

Like many other viruses, CoVs have been sought as possible etiologic agents in multiple sclerosis. The search has been stimulated by the capacity of JHM, a well-studied strain of mouse hepatitis virus, to produce in mice and rats an immune-mediated chronic demyelinating encephalitis histologically similar to multiple sclerosis.[169] HCoV-OC43[170,171] and HCoV-229E[172] have been detected in brain tissue from multiple sclerosis patients using virus isolation,[170] in situ hybridization, immunohistology,[171] and PCR.[172] The strongest support for CoV-mediated infection of the brain comes from a study in which HCoV-OC43 was identified in neurons in an 11-month-old patient with severe combined immunodeficiency and lethal encephalitis.[173] Except for this one report, evidence is lacking to establish an etiologic or pathogenic association of CoVs with human central nervous system disease.

LABORATORY DIAGNOSIS

CAR Coronaviruses

Although some human CAR CoVs grow in tissue culture directly from clinical samples and although antigen detection systems have been developed for both HCoV-OC43 and HCoV-229E,[174,175] laboratory diagnosis of CoV respiratory infections is best accomplished by molecular methods. Reverse-transcriptase PCR (RT-PCR) systems have been developed using many different primers and detectors.[176–182] From a clinical point of view, a single generic test for respiratory CoVs would be desirable, and such tests have been developed. However, when tested side by side with specific systems, the generic systems have somewhat lower sensitivity.[120]

MERS Coronavirus

MERS-CoV was originally isolated in Vero and LLC-MK2 cells, and standardized methods using RT-PCR–based examination of respiratory and other clinical samples have been published.[183,184] These methods can detect as little as 10 to 15 copies of viral RNA. Peak virus titers were detected at approximately 14 days after infection, and viral RNA could be detected for greater than 21 days in patients with severe disease.[185] MERS-CoV infection can be diagnosed beginning 2 to 3 weeks after the onset of illness using serologic methods, enzyme-linked immunosorbent assay (ELISA) initially, with confirmation by indirect immunofluorescence assays (IFA) and neutralization assays.[186] The transient nature of the antibody response in MERS patients with subclinical or mild disease has diminished the diagnostic utility of this approach.[78,82]

SARS Coronavirus

Although SARS-CoV was grown from respiratory tract specimens in Vero E6 and fetal rhesus monkey kidney cells, the more sensitive and rapid RT-PCR assays were most widely used to detect infection. Virus was detected by RT-PCR in upper and lower respiratory tract, blood, stool, and urine specimens. Early in the illness, specimens were found positive only in approximately one-third of patients.[112] Use of samples from multiple sources increased the yield. Virus was detected most frequently during the second week of illness.[112,187]

Antibody tests have been developed using tissue culture–grown virus and ELISA and IFA. Immunoglobulin M antibody can be detected in most patients for a limited period of time, and immunoglobulin G antibody appears first approximately 10 days after onset of fever in patients with good outcomes and becomes essentially universal after 4 weeks.[112,161]

THERAPY

Given the severity of SARS, clinicians throughout the world empirically treated most patients with corticosteroids and intravenous or oral ribavirin.[188] It is now known that ribavirin has little activity against SARS-CoV in vitro, and corticosteroid usage may have resulted in worse outcomes.[189,190] Lopinavir-ritonavir and intravenous immune globulin were also used in some patients, without conclusive evidence that they were helpful or harmful. There is anecdotal evidence of the benefit of either interferon-α or interferon-β treatment. However, no therapy has proven efficacy, and therapy continues to be largely supportive.[190]

Similarly, treatment of MERS-CoV infection at present depends entirely on supportive measures. No antiviral drugs are recommended,[191,192] although several studies have indicated that MERS-CoV is more sensitive to interferon-α or interferon-β than is SARS-CoV.[193,194] Combinations of interferon alfa-2b and ribavirin inhibit MERS-CoV in vitro, and administration of the combination improved outcomes in MERS patients at 14 but not 28 days.[195] A new broad-spectrum drug, GS-5734, has demonstrated efficacy against SARS-CoV and MERS-CoV in cells and experiment animals and may be a useful therapeutic option.[196] Standard droplet precautions should be used, with aerosol precautions during certain high-risk procedures.[197]

PREVENTION

Rigorous application of hospital infection control procedures, particularly those directed at contact and droplet spread, was shown to have a major beneficial effect on the spread of the SARS-CoV.[92] The containment of the global SARS outbreak is a testament to the power of the cooperation and collaboration engendered by WHO to address a major public health threat. Similarly, standard droplet precautions are recommended for patients with suspected or confirmed MERS-CoV infections, with aerosol precautions during certain high-risk procedures.[196,198,199] Suitable barrier precautions are especially critical in the case of MERS patients since a large fraction of infections result from nosocomial transmission.[11,74]

Vaccines for animal CoVs have been developed and widely used with variable efficacy. Vaccines are likely to be key in disrupting MERS-CoV camel-to-human and interhuman transmission. A variety of vaccination strategies, including inactivated, subunit, live-attenuated, DNA, and nanoparticle vaccines, are being pursued.[200,201] Vaccines will need to be carefully evaluated because an inactivated SARS-CoV vaccine and a feline infectious peritonitis surface glycoprotein vaccine caused immunopathologic disease after challenge.[202–204] Monoclonal antibodies that neutralize MERS-CoV have been identified and proposed for use therapeutically.[205,206] Given the small number of human cases and the high prevalence of virus in camels, vaccination of juvenile camels may be the preferred approach to protecting human populations.[207] Such vaccines are under development.

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The complete reference list is available online at Expert Consult.

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