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Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV)

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Abstract

First reported in September 2012, the Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV) has resulted in 206 laboratory-confirmed deaths, with a 42% mortality rate as of 27 March 2014. Closely related coronaviruses have been isolated in bats, but most evidence suggests that humans have become infected directly from camels in a number of separate transmission events, with limited human-to-human transmission reported thus far. The majority of cases originated in the Middle East (predominantly Saudi Arabia), including all the index cases. Clinical manifestations primarily involve fever, chills, and rapidly progressive respiratory failure, often resulting in an acute respiratory distress syndrome, with a minority of patients reporting gastrointestinal symptoms, as well. The majority of critically ill patients are older males with medical co-morbidities, and a large number of minimally symptomatic cases likely go undetected. Unfortunately, attempted therapies have all been unsuccessful thus far, and treatment remains supportive care.

Introduction

The first introduction to the medical community of what would later be called the Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV) was made by an Egyptian microbiologist, Ali Mohamed Zaki, on ProMED-mail on 20 September 2012. In his posting, he described the previous isolation (June 2012) of a coronavirus from sputum, which was identified by a pancoronavirus reverse transcriptase (RT) PCR assay from a 60-year-old male patient with acute respiratory and renal failure of unknown etiology in the city of Jeddah, Saudi Arabia. Three days later, another posting on ProMED-mail appeared, describing a 49-year-old male Qatari national with a history of travel to Saudi Arabia who developed similar fulminant symptoms of respiratory failure in a London hospital. These cases then generated interest regarding an earlier, unsolved outbreak that occurred in March and April 2012 in Zarqa, Jordan, involving 11 patients, including 8 health care workers (HCWs), and resulting in 2 deaths. PCR testing of banked samples later confirmed these cases as MERS-CoV infection (1,2).

Zaki sent his sample to Ron Fouchier's virology laboratory at Erasmus University Medical Center in Rotterdam, The Netherlands, for further study. The virus was named HCoV-EMC/2012 in November 2012; HCoV for human coronavirus and EMC for Erasmus Medical Center. The name MERS-CoV came about in May 2013. Although the custom of naming a disease after a geographic location is not uncommon, this name was not without controversy, as many complained that it stigmatized the Middle East (3).

Where Are We Now, and Who is Affected? The Epidemiology of MERS-CoV

As of 27 March 2014, there have been 206 laboratory-confirmed cases of MERS-CoV infection with 86 deaths, for a mortality rate of 42%, as detailed by the World Health Organization (WHO) MERS-CoV summary and literature update (4). The median age of infected patients was 52 years, with a significant predominance of men affected, at a 2:1 male/female ratio. A large majority of patients had underlying co-morbidities: 76% in an earlier update by the WHO MERS-CoV research group. The most common

Erratum

In the June 15th, 2014 issue of CMN (Vol. 36, No. 12), the address of the author, Marie Pezzlo, was incorrect. It should be 393 Lookout Dr., Laguna Beach, CA and not 93 Lookout Dr.

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co-morbidity is chronic kidney disease (13.3%), followed by diabetes mellitus (10%), heart disease (7.5%), and immunocompromised status (3%). Almost 2/3 of patients (63.4%) experienced severe respiratory disease. Although only 11% of reported cases were asymptomatic, the true number of minimally symptomatic or asymptomatic cases in the community is likely much greater. Interestingly, some stark differences have been found between primary/index cases and secondary cases. Primary cases have been in older individuals (average age, 58 years) and have occurred predominantly (reported to be as high as 80%) in men; secondary cases have been in individuals with an average age of 45 years, with 58% men affected. In addition, the difference in severity has been astounding: 90.2% of primary cases have been classified as severe or fatal disease as opposed to 49.5% of secondary cases (4,5).

Affected countries in the Middle East include the Kingdom of Saudi Arabia (the majority of cases to date), Jordan, Kuwait, Oman, Qatar, and the United Arab Emirates. Additional cases have occurred in the European countries France, Germany, Italy, and the United Kingdom, as well as the African country Tunisia and, recently, the United States (4). All European, U.S., and African cases have either been imported from the Middle East or are secondary cases. Since the first detected cases in March and April 2012 in Zarqa, Jordan, the number of detected cases did not increase significantly until around April 2013. This is in part due to the expansion of aggressive screening recommended in the fall of 2012 by the Saudi Arabian Ministry of Health (MoH), not only of hospitalized patients with fever and respiratory symptoms, but also of their close contacts (5,6).

Transmission

Thus far, no evidence has been found for sustained community transmission of MERS-CoV, secondary attack rates among family members have been low, and nosocomial spread to HCWs and other patients has been reasonably limited. Clusters among family members, as well as hospital clusters among patients and HCWs, have been reported in a number of countries. Studies of these clusters have allowed the calculation of an estimated incubation time of 5 to 14 days (6). The true prevalence of the disease is difficult to estimate, as there are likely a number of cases that have been asymptomatic or minimally symptomatic in the community that went unrecognized, especially in younger, healthier patients. Attempts to identify some of these cases have been made, including in a large prospective study by Memish et al. (7) aimed at the widespread screening of MERS-CoV contacts in Saudi Arabia using PCR of respiratory tract samples. Over a 12-month period starting in September 2012, 5,065 individuals were screened, with detection of 108 cases (99 adults and 9 children), for a case detection rate of approximately 2%. Of the total population screened, the majority of positive cases were hospitalized patients with suspected infection, and the rest were almost evenly split between HCWs with exposure to confirmed MERS-CoV cases and family contacts. The authors note that cases were likely missed, given that serology was not used in the screening process to screen for resolved infections. Although the case detection rate for HCWs was only around 1.1%, it should be noted that the MoH in Saudi Arabia had been aggressive in their infection control practices, including the recommendation for contact and airborne precautions early in the epidemic.

Hajj pilgrims in 2012 were screened in an attempt to identify minimally symptomatic or asymptomatic cases in the community. Gautret et al. (8) systematically surveyed 154 French pilgrims returning from the Hajj in Saudi Arabia by PCR testing of nasal swabs, completed prior to their departure from France and then 3 days prior to their return. Although greater than 80% suffered from respiratory symptoms during their Hajj pilgrimage, none tested positive for MERS-CoV, suggesting low transmissibility of the virus. Notably, the average age of the cohort was 59 years, and over 50% had chronic disease comorbidities, including greater than 25% with diabetes mellitus.

Nosocomial transmission has occurred in a number of hospitals, including a German hospital, as presented by Buchholz et al. (9). The authors reported a contact investigation involving a large number of HCWs exposed to a patient with MERS-CoV infection that further supports the notion that nosocomial transmissibility of MERS-CoV is limited, even without appropriate isolation precautions or personal protective equipment. The index patient was transferred to the German hospital approximately 19 days after his symptoms began and was not diagnosed with MERS-CoV infection until 1 month after his initial admission in Qatar. The authors identified 123 contacts of the patient, 85 of whom underwent serological testing by a 2-stage approach. Notably, no specific respiratory precautions had been taken; protective measures were generally limited to gloves, occasionally gowns, and surgical face masks during suctioning. Nine HCWs had performed aerosol-generating procedures within the 3rd or 4th week of the patient's illness. Notably, none of the 85 contacts, most of whom were HCWs, tested positive for MERS-CoV.

Transmission among family members is nicely exemplified by Memish et al. (10), who present a family cluster involving 4 patients in Riyadh, Saudi Arabia, in November 2012. The index case was a 70- year-old male with diabetes mellitus, coronary artery disease, and hypertension who developed MERS-CoV infection manifested as acute respiratory and renal failure and who died on hospital day 11. Two of his sons, both smokers, contracted the disease as well; one died within a few days of admission, while the other recovered after a pneumonia-like presentation and did not require intubation. The fourth patient was the healthy 16-year-old grandson of the index patient and son of the above-mentioned patient who died. He recovered and was classified only as a probable case, given that he did not test positive by PCR. His illness was characterized by flu-like symptoms, along with diarrhea and abdominal pain, with imaging that revealed bilateral hilar infiltrates; he made a full recovery.

These 4 patients lived among a total of 28 family members within a large house, mostly in separate apartments; the family included 9 children. The authors nicely detailed the amount of contact among family members in general and during the family members' illnesses. They noted that the men and adolescent males shared

meals, socialized, and worshipped together, notably separate from their wives and children. When the index patient became ill, he was tended to at home by women in the household and the 2 sons who became ill; however, once he was hospitalized, the wives rarely visited, while the sons continued to tend to their father before they themselves fell ill. Similarly to the wives, the children had very limited contact once the male patients were hospitalized. The fact that the women and children did not become ill suggests that infectivity may be limited early in the course of illness. Notably, none of the other 24 family members developed any respiratory symptoms during this period (9).

Clinical Presentation

Assiri et al. (11) reviewed 47 laboratory-confirmed cases of MERS-CoV disease reported from the Kingdom of Saudi Arabia between 1 September 2012 and 15 June 2013. The cases included 2 clusters, one involving 3 people residing in the same villa and the other comprising 23 cases from a hospital outbreak in the Al-Hasa region of Saudi Arabia. None of the patients had concomitant blood or respiratory viral, fungal, or bacterial infection, and the majority received oseltamivir and broad-spectrum antibiotics, including coverage for atypical bacteria. The case fatality rate among this cohort was 60%, split evenly between males and females. Comorbid medical disorders were found in the vast majority of patients (96%). The most common comorbid disorders included diabetes mellitus in more than one-third of the patients (68%), chronic kidney disease in almost half (49%), hypertension (34%), and chronic heart disease (28%); only one patient was on long-term immunosuppression with steroids. The authors noted a few important factors in regard to these statistics. First, diabetes is common in Saudi Arabian citizens over the age of 50 years, and second, the Al-Hasa outbreak involved a hemodialysis unit, thereby inflating the number of patients with chronic kidney disease.

The predominant presenting symptoms in this cohort of patients were fever (98%), chills or rigors (87%), cough (83%, with slightly more than half dry and the rest productive), and dyspnea (72%). Less common presenting symptoms included myalgias (32%), diarrhea (26%), nausea (21%), and vomiting (21%). Most of these patients required intensive care (89%), and more than 2/3 required mechanical ventilation (72%); the median time to ventilation was 7 days and to death was 14 days. Common laboratory abnormalities included elevated lactate dehydrogenase in roughly half (49%), 36% with thrombocytopenia, and 34% with lymphopenia. Hepatic abnormalities were uncommon, with only 15% with elevated aspartate transaminase and 11% with elevated alanine aminotransferase. All patients had abnormalities on chest X ray, with many different patterns observed, including patchy infiltrates, interstitial changes, consolidation, nodular or reticular opacities, and pleural effusions. Limited experience with survivors in the rehabilitation phase revealed prolonged weakness, dyspnea, occasional anxiety, and depression. Pulmonary lung function testing completed 2 months after hospital discharge revealed a mild or moderate restrictive pattern (11).

Of note, this cohort of patients is likely not representative of the MERS-CoV-infected population as a whole. The recommendation by the Saudi Arabian MoH to aggressively screen inpatients with fever and respiratory illness led to the diagnosis of many cases among older patients with many comorbidities. Second, this cohort includes patients involved in a large hospital outbreak that were in the intensive-care unit and/or on hemodialysis prior to becoming infected with MERS-CoV (11).

Additional laboratory abnormalities noted in other series included anemia; hyponatremia; hypoalbuminemia; elevated C-reactive protein and procalcitonin levels; and organ dysfunction, including pericarditis and disseminated intravascular coagulation. Markers of renal function, such as blood urea nitrogen and creatinine levels, are generally not available in some of the large published cohorts, but a recent WHO summary statement (12) noted that some severe cases developed renal failure during their clinical course. A number of patients with MERS-CoV infection developed bacterial superinfection with both gram-positive and gram-negative organisms, as well as a few patients with viral pulmonary coinfection, including influenza virus H1N1 infection and type 2 parainfluenza.

Payne et al. (13) published a case of a second-trimester stillbirth in a pregnant subject with MERS-CoV infection confirmed by 3 serologic methods. The patient was a 39-year-old Jordanian female with close contact with 2 other cases (one of whom died from MERS-CoV infection) who developed acute respiratory symptoms concurrent with vaginal bleeding and abdominal pain, followed by delivery of a stillborn fetus. Although the authors were able to demonstrate only an association between MERS-CoV and the stillbirth, causality is suggested by biological plausibility, as well as the adverse birth outcomes noted during the severe acute respiratory syndrome (SARS) and influenza virus H1N1 epidemics.

Coronaviruses

MERS-CoV is an enveloped, positive-sense, single-stranded RNA virus of the order *Nidovirales*, family *Coronaviridae*, genus *Betacoronavirus*, lineage C, clade 2c. There are 4 coronavirus genera (*Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*); the genus *Alphacoronavirus* contains HCoV-229E and NL63, both causes of the "common cold," while the genus *Betacoronavirus* contains the other 2 coronaviruses that cause the common cold (OC43 and HKU1), SARS-CoV, MERS-CoV, and 2 common bat coronaviruses, HKU4 and HKU5 (12). Notably, the 4 coronaviruses HCoV-OC43, 229E, NL63, and HKU1 account for 5 to 30% of all respiratory infections, and up to roughly 21% of the general population have antibodies (14). Coronaviruses can infect a large number of animals, including livestock such as poultry, bats, camels, pets, and humans.

Possible Animal Reservoirs

The horseshoe bat was found to be the natural reservoir of the SARS coronavirus, making bats a reasonable place to start the search for a likely animal host for MERS-CoV. In 2006, Woo et al. (15) performed a coronavirus surveillance study of bats in the Hong Kong Special Administrative Region and found 6 different novel coronaviruses among 13 species of bats in that region alone. The finding that SARS-CoV had originated and that MERS-CoV may have originated in bats is not surprising given a number of

factors. The investigators noted that bat behavior includes the ability to fly as high as 5,000 meters and roosting with large numbers of other bats. Annan et al. (16) found that juvenile and lactating female bats were much more likely to harbor coronaviruses than adult or non-lactating females, arguing that bat maternity colonies likely play a role in the amplification of coronaviruses. In addition, a given bat species can become infected with multiple species of coronaviruses. These factors can facilitate viral genetic exchange among coronaviruses, not only in other bats, but in other species of animals. Given that coronaviruses have a high frequency of recombination and high mutation rates, infection of bats creates a "perfect storm" for the creation of novel coronaviruses that can adapt to infect humans.

The search is proceeding in earnest for bat coronaviruses similar in genetic identity to MERS-CoV. In Asia, the search targeted the common bat coronaviruses HKU4 in the lesser bamboo bat (*Tylonycteris pachypus*) and HKU5 in the Japanese house bat (*Pipistrellus abramus*), as they represent 2 of only 3 known coronaviruses in *Betacoronavirus* clade 2c, along with MERS-CoV (17). Lau et al. (18) found that these 2 species had approximately 90 to 92% sequence overlap in a portion of their RNA polymerase gene, *RdRp*, and even less in a sequence of the S and N genes, concluding that neither of these coronaviruses is likely to be a direct ancestor of MERS-CoV and likely diverged a number of centuries ago from a common ancestor. Annan et al. (16) targeted bat species in Ghana, as well as 4 countries in Europe (Germany, The Netherlands, Romania, and Ukraine), also using the *RdRp* gene target. They found novel coronaviruses from *Betacoronavirus* clade 2c in both regions of the world, with one novel coronavirus isolated from a common pipistrelle bat (*Pipistrellus pipistrellus*) differing from MERS-CoV by only 1.8% in amino acid identity.

In South Africa, Ithete et al. (19) discovered a novel betacoronavirus from an adult female *Neoromicia zuluensis* bat that differed from MERS-CoV by only 1 amino acid exchange (0.3%) in the translated 816-nucleotide *RdRp* gene fragment. The authors noted that this coronavirus is more closely related to MERS-CoV than any other known coronaviruses, including those found in Ghana and the above-mentioned HKU4 and HKU5 coronaviruses from Asia, suggesting an African origin of MERS-CoV or its closest coronavirus ancestor.

Finally, in Bisha, Saudi Arabia, Memish et al. (20) tested samples from 96 bats of 7 species near the home of an index case patient. A coronavirus from a *Taphozous perforatus* bat showed 100% nucleotide identity in a small translated region of the *RdRp* gene with the index case patient's isolate.

Camels have been hypothesized to be a primary reservoir for MERS-CoV, and a number of intriguing studies clearly demonstrated that camels can be infected by the virus and suggested, but do not clearly prove, direct camel-to-human transmission. Reusken et al. (21) provided evidence of infection with MERS-CoV by means of positive serology in 2 cohorts of dromedary camels, one from Oman and the other from the Canary Islands of Spain. All 50 of the Omani camels tested positive by saturating reactivity by microarray, and all 50 had high MERS-CoV-neutralizing capacity. In contrast, only 8.5% of the 105 Spanish camels displayed neutralizing capacity. The authors noted that these 2 cohorts are not related, as the Spanish camels were imported from Africa during or before the 15th century (after which time importation from Africa to the region was banned), whereas the Omani camels were likely imported from Africa much later; how MERS-CoV was introduced into these cohorts and whether there is a distant connection between them remain unclear. Similarly, Perera et al. (22) found a seroprevalence rate of greater than 90% in dromedary camels in Egypt using a novel spike pseudoparticle neutralization assay.

In Saudi Arabia, Alagaili et al. (23) performed a nationwide survey of 203 dromedary camels using both serologic and molecular diagnostic methods in 2013. They found that 73% of all camels surveyed had antibodies to MERS-CoV by ELISA with Western blot confirmation. In addition, they found that 35% of juvenile camels and 15% of adult camels were PCR positive, primarily by the nasal swab method. Only 3 rectal swabs and none of the serum or whole blood samples were positive. The authors argued that isolating the virus primarily in nasal secretions suggested an airborne route of transmission. In addition, the authors tested a number of samples from camels archived since 1992, the majority of which were positive. Samples available from most years were very limited, aside from 1994, of which 93% of 123 camels tested were positive, suggesting that MERS-CoV has been circulating in camels since at least 1992.

Finally, a number of camels residing on a small farm in Qatar have been linked to 2 cases of human MERS-CoV infection as part of an outbreak investigation in October 2013 (24). Both patients had regular contact with animals on the farm, including camels, and neither had recently traveled outside of Qatar prior to becoming ill. Six of the 14 camels tested positive for MERS-CoV by 2 nasal swab PCR assays (meeting the international criterion that 2 of 3 PCR tests must be positive), and all 14 camels were antibody positive by immunofluorescence and neutralizing capacity. Sequencing was performed, which revealed only a 1-nucleotide difference in the open reading frame 1a (ORF1a) region between the camel and human cases and an 8-nucleotide difference between the camel and an early human isolate from the Erasmus Medical Center laboratory in Rotterdam, The Netherlands. As noted by the authors, this provided evidence of MERS-CoV infection of camels but did not delineate camel-to-human (versus human-to-camel) transmission or if both the camels and humans were independently infected by a third source (such as bats) (24).

Host Factors

A number of recent studies have identified the human targets of MERS-CoV, from the organs affected to the specific cellular receptors to which the spike proteins of MERS-CoV bind. Chan et al. (25) studied the ability of MERS-CoV to infect different human and animal cell lines in vitro by infecting 28 cell lines, including 15 human cell lines. Their criteria for infection by MERS-CoV included the measurement of mean viral loads higher baseline levels by RT-PCR, expression of nucleoprotein by immunofluorescence, and demonstration of cytopathic effect. The results showed that 7 of the 15 human cell lines met these criteria, including cells

from the lower airway, kidney, intestinal tract, and liver, as well as histiocytes. These findings correlate with the clinical findings of severe pulmonary disease in most patients and with acute renal injury, gastrointestinal symptoms, and mild hepatitis noted in a minority of patients. Notably, MERS-CoV did not infect any upper airway cell lines. Interestingly, MERS-CoV was able to infect primate, pig, and civet cat cell lines, but the authors' infection criteria were not met by canine, feline, rodent, chicken, and insect cell lines. Similarly, Muller et al. (26) infected a number of animal cell lines with MERS-CoV. They noted that all of the bat cell lines tested were readily infected.

Chan et al. (27) utilized fresh biopsy specimens of human bronchi and lung parenchyma to demonstrate and quantify infection with MERS-CoV. They also used SARS-CoV and a common human coronavirus, HCoV-229E, as control groups. In human bronchial specimens, they utilized viral antigen staining and RT-PCR targeting a region upstream of the E gene and detected a 2-log-unit increase in MERS-CoV within 72 hours of infection; notably, SARS-CoV infected the tissue, but without a significant increase in viral titer from baseline. In lung parenchymal tissue, they detected a significant amount of MERS-CoV antigen throughout the lungs, with a 2-log-unit increase within 48 hours of infection, results similar to those after SARS-CoV infection. In contrast, coronavirus 229E did not infect either the bronchial or lung parenchymal tissue.

Next, the authors used immunohistochemical methods (cellular staining with specific cell markers) and electron microscopy to identify MERS-CoV antigen in specific cells in the bronchi, lung parenchyma, and vessels of the lung. MERS-CoV notably localized to nonciliated bronchiolar-type epithelial cells in the bronchi, type I and type II pneumocytes and epithelial cells within the lung parenchyma, and finally endothelial cells within the medium-size interstitial vessels of the lung. Notable cells that did not stain included macrophages, ciliated cells of the lung, and goblet cells. The authors postulated that infection of the endothelial cells of the medium-size interstitial vessels may provide the means of extrapulmonary dissemination (27).

Finally, Chan et al. (27) focused on identifying which cells underwent apoptosis as induced by MERS-CoV and by what mechanism (direct or indirect). They again used transmission electron microscopy, along with staining of extensive expression cleaved caspase 3 (a protease heavily involved in apoptosis), as well as MERS-CoV antigen, each in a different color. They demonstrated extensive apoptosis but noted that the apoptotic cells were different from the cells expressing viral proteins based on lack of co-staining, suggesting a paracrine mechanism of apoptosis rather than direct infection.

Raj et al. (28) were able to identify the cellular receptor to which MERS-CoV binds, known as dipeptidyl peptidase 4 (DPP4). In general, coronaviruses bind to host cell surface receptors using a specific domain of their spike (S) entry protein; SARS-CoV uses the metallopeptidase angiotensin-converting enzymes 2 (ACE2). DPP4 is a type II transmembrane glycoprotein present on the cell surface that has many functions, including roles in glucose metabolism, T cell activation, chemotaxis modulation, cell adhesion, and apoptosis. Primary sites of expression in humans include the kidney, small intestine, and liver and prostate epithelial cells, as well as activated leukocytes. These investigators proved their hypothesis by a number of methods, using cell lines including Vero and Huh-7 (human liver) cells. These methods included inhibition of Vero cell infection with soluble DPP4, blockage of infection of Huh-7 cells after pre-incubation with polyclonal DPP4 antiserum before virus inoculation, and finally transformation of previously non-susceptible Cos-7 cells into cells capable of MERS-CoV infection through transfection with a human DPP4 expression plasmid. In addition, they demonstrated that a number of common coronaviruses, including CoV-229E and OC43, were unable to infect human cells expressing DPP4.

Diagnostic Laboratory Testing

The primary means of testing for MERS-CoV infection is by RT-PCR, which can be positive from bronchoalveolar lavage, nasopharyngeal aspirate, sputum, tracheal aspirate, and urine specimens (11). A laboratory-confirmed case is defined as either two positive PCR assays with different targets or one positive PCR assay along with one positive PCR sequencing product (usually from the *RdRp* gene target) (6). Corman et al. (29) developed two early RT-PCR tests using the Rotterdam virus isolate from Ron Fouchier's laboratory as a template. One targeted an upstream region of the E protein gene (UpE), while the other targeted a section of ORF termed ORF1b. There was no cross-reactivity in either of these assays with any of the four common human respiratory coronaviruses (OC43, NL63, 229E, and HKU1), with SARS-CoV, or with a large number of common respiratory viruses (including parainfluenza serotypes 1 to 4, respiratory syncytial virus (RSV), human metapneumovirus, and influenza virus H1N1 or H3N2).

Additional RT-PCR assays have been developed including ORF1a, nucleocapsid (N), and spike (S) gene targets (6,29). The UpE and ORF1a assays are considered highly sensitive, while the ORF1b assay is less so; the UpE assay is recommended for screening, followed by use of the ORF1a or ORF1b assay for confirmation.

Serum neutralizing antibody titers can also be helpful and can identify past infection, and they are expected to rise fourfold when taken between 14 and 21 days apart (11). Buchholz et al. (9) utilized a two-staged serologic approach for screening in their contact investigation. They first used an indirect immunofluorescence assay (IFA) for broad screening, followed by confirmation with both recombinant IFA and serum neutralization testing.

Growth on viral culture from various specimens, including respiratory, fecal, urine, and tissue, has been demonstrated but is not practical, as participating laboratories would require biosafety level 3 precautions. Infection of various cell lines, including Vero and LLC-MK2 cells, produces cytopathic effect, including syncytium formation (11).

Animal Models

Two groups of investigators independently infected rhesus macaques with MERS-CoV (the first intratracheally and the second through a variety of routes) (30,31). Yao et al. (30) reported

that juvenile rhesus macaques (aged 2 to 3 years) demonstrated a transient increase in body temperature and decrease in water intake, as well as significant changes in lung imaging. X rays revealed localized infiltration and interstitial markings, and autopsy findings showed congestion and scattered nodules with microscopic multifocal interstitial pneumonia and exudative changes. Interestingly, no extrapulmonary lesions were observed, including within the kidneys, despite the observed moderate incidence of renal failure in human infection. PCR was notably positive from lung homogenate but negative from oropharyngeal, nasal, and cloacal swabs and from additional organs. Neutralizing antibodies appeared on day 7 after infection and peaked at day 14. None of the infected monkeys died. Munster et al. (32) performed a similar experiment but used older monkeys (aged 6 to 12 years), and they reported findings of reduced appetite, cough, tachypnea, piloerection, and hunched posture, as well as serum laboratory results that included leukocytosis. In addition, their pathologic results included "multifocal to coalescent bright red lesions throughout the lower respiratory tract" with progression to "dark reddish purple areas… with fibrous adhesions, consolidation, and edematous and atelectatic areas in the lungs."

Attempts to develop a small-animal model, which would provide an easier and less expensive means of studying the virus than using primates, have been unsuccessful, primarily due to lack of the DPP4 receptor in many small-animal species. Although many researchers have had considerable difficulty infecting mice, hamsters, guinea pigs, or ferrets with MERS-CoV (33), Zhao et al. (34) created a mouse model by means of transduction with a recombinant, non-replicating adenovirus expressing the DPP4 receptor and were able to successfully use their model to study not only clinical disease, but also possible therapies and a potential vaccine.

Infection Control Efforts

The MOH in Saudi Arabia has been aggressive in its infection control efforts and proactive surveillance of possible contacts of confirmed MERS-CoV cases. Soon after the first case report of MERS-CoV infection in September 2012 in Saudi Arabia, the MoH recommended aggressive screening of all inpatients with unexplained respiratory symptoms, as well as active screening of their contacts, leading to an increased case detection rate including asymptomatic or minimally symptomatic cases (7).

The MoH also targeted the Hajj of 2012 as an event that could lead to a potential epidemic, as it involved a large number of people gathered from around the world in close proximity. Control measures were implemented, including the recommendation to wear masks in over-crowded areas and that the elderly and those with chronic diseases (including diabetes and kidney disease), immunodeficiency, cancer, or terminal illness, as well as pregnant women and children, postpone their trip to the Hajj, or Umrah. The U.S. CDC agreed with this advice for U.S. travelers, while the WHO and European CDC did not agree with any travel restrictions. However, these public health agencies were unanimous in recommending protective measures aimed at limiting the spread of respiratory viruses among religious pilgrims, including cough etiquette and wearing face masks (35).

Treatment

Although no clearly effective treatments have yet been developed for MERS-CoV, there are a number of agents that have shown promise in vitro. Treatment options are currently limited to supportive care, including mechanical ventilation for respiratory failure and/or hemodialysis in the setting of renal failure. Extracorporeal membrane oxygenation has been attempted in at least 6 patients, but only 1 of the 6 survived. Corticosteroids have been used to prevent progression of adult respiratory distress syndrome to the fibrotic stage, but the benefit of this practice is unclear (6).

Interferon alpha 2b and ribavirin have been postulated to treat MERS-CoV infection, given their roles in the treatment of viruses such as hepatitis C virus, RSV, and Lassa fever virus. Ribavirin, a nucleoside analog, can inhibit RNA replication and has a wide spectrum of anti-viral activity. Falzarano et al. (36) designed an experimental model using infected Vero and LLC-MK2 cells to test the effects of ribavirin and interferon. They defined markers of treatment success, including reduction in both the number of viral genome copies and cytopathic effect. A dose-dependent reduction in genome copies was noted for interferon, reaching a 1.84-log-unit reduction at 5,000 U/ml, and for ribavirin, reaching a 2.04-log-unit reduction at 2,000 µg/ml. Similar success was noted in regard to reduction in cytopathic effect, with elimination at 1,000 U/ml for interferon and at 200 µg/ml for ribavirin. However, the authors noted that these concentrations are likely not safely achievable in humans. Once the two drugs were combined, though, they were able to achieve an 8- to 16-fold decrease in the doses required, making it theoretically possible to achieve therapeutic levels in humans.

Two small studies detail treatment of MERS-CoV infection with interferon and ribavirin in humans and rhesus macaques. Al-Tawfiq et al. (37) presented a retrospective observational study of 5 patients treated with ribavirin and interferon alpha 2b, none of whom responded to the treatment. Notably, all 5 patients were of advanced age (average age, 62 years) with significant comorbidities, and treatment was not started until 19 days after admission, due in part to difficulty with diagnosis. In contrast, Falzarano et al. (38) demonstrated promising results in infected rhesus macaques with this treatment combination. They demonstrated improvement in pulmonary edema, a decrease in serum and pulmonary pro-inflammatory markers, and a reduction in viral genome copies. These results may be quite difficult to replicate in infected humans, however, because treatment was started only 8 hours after inoculation with the virus.

Convalescent sera from patients recovered from SARS-CoV infection have also been postulated to have a beneficial role in the treatment of MERS-CoV infection. In Southern China, Chan et al. (14) measured and compared levels of MERS-CoV antibodies in 28 recovered SARS patients, 94 animal handlers (including animal market retailers and slaughterers of chickens, ducks, geese, pigeons, rabbits, cats, etc.), used as a high-risk surrogate for coronavirus infection, and 152 healthy blood donors. A significantly higher number of SARS patients had anti-MERS-CoV immunoglobulin G by indirect immunofluorescence testing (61%, with

titers ranging from 1:20 to 1:320, compared to 2.1% of the animal handlers, with titers of only 1:20 to 1:40, and none of the healthy blood donors). Using neutralization antibody testing, the authors found that 25% of SARS patients had low MERS-CoV neutralizing antibodies (titers of 1:20 or less) in comparison to none of the animal handlers or healthy blood donors. These results supported the hypothetical role of convalescent sera from recovered SARS patients in providing some degree of active antibody but suggest that intravenous immunoglobulin from healthy donors may not be helpful. Another option along the same line is to use convalescent serum from recovered MERS-CoV patients, as was tried with serum from recovered SARS patients with questionable success during the SARS-CoV epidemic (39).

DPP4 (also known as CD26), the functional receptor for MERS-CoV in bronchiolar tissue, is a putative therapeutic target through the inhibition of binding by MERS-CoV. The DPP4 inhibitors sitagliptin, vildagliptin, and saxagliptin, currently used for the treatment of diabetes mellitus, were unable to block the binding of MERS-CoV to the DPP4 receptor in vitro (40). On the positive side, adenosine deaminase, a natural DPP4 ligand, has been shown to inhibit MERS-CoV infection of ferrets transfected with human DPP4 (40), and both humanized monoclonal and polyclonal anti-CD26 antibodies have shown promise when used in in vitro studies (39).

Other ideas for treatment include the development of fusion inhibitors. These compounds would block the S2 subunit of the spike protein, which mediates membrane fusion with the host target cell. Promising therapeutic compounds have been developed by Lu et al., who have developed 2 peptides, HR1P and HR2P, that can inhibit both replication and fusion of MERS-CoV (41). Another promising development is the ability of cyclophilin inhibitors, such as mycophenolic acid and cyclosporine, to block the replication of MERS-CoV RNA in vitro (39). Vaccine design is in the early developmental stages and has been based on earlier vaccine candidates for SARS-CoV, designed to target the receptor-binding domain in the S1 component of the spike protein (42).

Conclusion

Since the first cases of MERS-CoV infection were identified retrospectively in Zarqa, Jordan, approximately 2 years ago, the virus has affected over 200 patients and killed over 80 of them. Progress has been made in diagnosing infection and limiting nosocomial spread, but no clearly effective treatment yet exists. It remains to be seen if the virus will disappear from humans, as SARS-CoV did, or gain increased transmissibility and lead to a worldwide pandemic.

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