Middle East Respiratory Syndrome

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SUMMARY

The Middle East Respiratory Syndrome (MERS) is a newly recognized highly lethal respiratory disease caused by a novel single stranded, positive sense RNA betacoronavirus (MERS-CoV). Dromedary camels, host species for MERS-CoV are implicated in the direct or indirect transmission to humans, although the exact mode of transmission remains unknown. First isolated from a patient who died from a severe respiratory illness in June 2012 in Jeddah, Saudi Arabia, as of 16 February 2015, 983 laboratory-confirmed cases of MERS-CoV (360 deaths; 36.6% mortality) were reported to the WHO. Cases have been acquired in both the community and hospitals with limited human-to-human transmission reported in the community. Whilst the majority of MERS cases have occurred in Saudi Arabia and the United Arab Emirates, cases have been reported from Europe, USA and Asia in people who traveled from the Middle East or their contacts. Clinical features of MERS range from asymptomatic or mild disease to acute respiratory distress syndrome and multi-organ failure resulting in death, especially in individuals with underlying co-morbidities. There is no specific drug treatment for MERS and infection prevention and control measures are crucial to prevent spread of MERS-CoV in health care facilities. MERS-CoV continues to be an endemic, low level public health threat. However, the concern remains that the virus could mutate to exhibit increased interhuman transmissibility, increasing pandemic potential. Our seminar presents an overview of current knowledge and perspectives on the epidemiology, virology, mode of transmission, pathogen-host responses, clinical features, diagnosis and development of new drugs and vaccines.

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Introduction

The first reported case of a new human disease, the Middle East Respiratory Syndrome (MERS) described a patient who died from a severe respiratory illness in a hospital in Jeddah, Saudi Arabia in June 2012. A previously unrecognized coronavirus (MERS-CoV) isolated from this patient\(^1\) had similarities to the coronavirus that caused the Severe Acute Respiratory Syndrome (SARS-CoV) epidemic in 2002-2003. The virus was initially designated as HCoV (human coronavirus)-EMC, but with global consensus was re-named MERS-CoV\(^2\). The genomic structure of MERS-CoV\(^3\) was delineated and dipeptidyl peptidase 4, DPP4 (also known as CD26) was identified as the host cell receptor for cell entry\(^4\). Reverse genetics systems facilitated studies of the MERS-CoV genome\(^5,6\), and molecular diagnostic tests were rapidly developed. The high mortality rates in family and hospital-based outbreaks, especially in patients with co-morbidities such as diabetes and renal failure\(^7-10\), and the respiratory droplet route of transmission of MERS-CoV evoked global concern and intensive discussion in the media. The numbers of reported MERS cases spiked during hospital based cluster outbreaks in the spring of 2013 and 2014; cases continue to be detected throughout the year at a low level. MERS-CoV was seen as a serious public health epidemic threat, since millions of pilgrims from 184 countries converge in Saudi Arabia each year for Hajj and Umrah pilgrimages. Fortunately, there have been no MERS cases associated with the 2013 and 2014 annual Hajj pilgrimages.

This Seminar reviews the current knowledge about MERS epidemiology, virology, clinical manifestations, pathogenesis, diagnosis, case management, therapeutic options and prophylactic interventions, including a discussion of the likelihood of widespread outbreak or epidemic spread.

MERS Case Definitions

Case definitions of ‘suspected’, ‘confirmed’ and ‘probable’ MERS were developed by WHO, the Centers for Disease Control and Prevention (USA)\(^11\), and the Ministry of Health of Saudi Arabia (MOH) (Supplementary Table 1). In addition to fever and pneumonia or acute respiratory distress syndrome (ARDS), the patient under investigation must have a history of travel to countries in or near the Arabian peninsula within 14 days prior to symptom onset or be in contact with a traveler from this region who developed a febrile respiratory illness. Confirmed cases have laboratory evidence of MERS-CoV infection, which generally is PCR-based. The case definition was recently updated (8 Dec 2014) by the MOH to include management of patients with healthcare-associated MERS-CoV pneumonia, those with acute febrile dengue-like illnesses and those with exposure to an infected patient and upper respiratory tract disease\(^12\).
Geographical distribution and surveillance

Although the first MERS case was reported from Jeddah in September 2012, retrospective analyses identified the first cases from an outbreak involving 13 individuals in March/April 2012 in Zarqa, Jordan. Since then MERS cases have been identified across the Arabian peninsula, in Asia, Europe, Africa and North America (USA). MERS cases reported from outside the Middle East invariably have a history of recent travel from the Arabian Peninsula or were a close contact of a primary case. Saudi Arabia has reported the majority of cases (as of 16 Feb 2015, 891 total cases and 372 deaths (41.8% mortality)). There are several proactive global surveillance and information systems related to MERS and guidelines for prevention, treatment and infection control measures are updated regularly by the WHO, CDC, MOH, ECDC and Public Health England (PHE). Surveillance has intensified over time, especially in the context of healthcare and community outbreaks, as it has become clear that infected patients may be asymptomatic or have acute febrile illnesses or upper respiratory tract disease.

Virology of MERS-CoV

Coronaviruses are large (28-32 kb) single stranded positive RNA viruses. Four coronavirus genera have been identified thus far, with human viruses detected in the alphacoronavirus (NL63 and HCoV-229E) and betacoronavirus (HCoV-OC43, HCoV-HKU1, MERS-CoV and SARS-CoV) genera. MERS-CoV and SARS-CoV belong to lineage c and lineage b betacoronaviruses, respectively. The genomic structures of the two viruses are very similar, with proteins involved in virus replication encoded at the 5' end and structural proteins expressed from the genes at the 3' end of the genome. Accessory proteins, which are not required for virus viability, are interspersed throughout the structural genes and may interfere with the host innate immune response in infected animals. MERS-CoV and SARS-CoV encode 5 and 8 different accessory proteins, respectively, that share no sequence similarity. These differences in accessory proteins, with presumed differential effects on induction and signaling of Type 1 interferon (IFN), may explain why MERS-CoV is more sensitive to IFN than is SARS-CoV. This difference in IFN sensitivity has therapeutic implications since Type 1 IFN has been used to treat patients infected with SARS-CoV or MERS-CoV.

Another feature of coronaviruses pertinent for understanding MERS-CoV (and SARS-CoV) is that these viruses exhibit high rates of mutation and recombination and a propensity to cross species. Although this was most famously illustrated by the spread of SARS-CoV from Chinese horseshoe bats to human populations during the 2002-2003 epidemic, other coronaviruses such as HCoV-OC43 and bovine coronavirus (BCoV), or feline coronavirus-II, canine coronavirus-II and transmissible gastroenteritis virus (swine virus) are very closely related, consistent with cross-species transmission. This ability to adapt to new environments raised concerns that MERS-CoV would gain virulence and enhanced ability to transmit from human-to-human as the MERS outbreak continued, but this has not occurred.

Both MERS-CoV and SARS-CoV bind to large ectopeptidases (DPP4, and angiotensin converting enzyme 2 (ACE2), respectively) to enter cells. Binding to the host cell...
receptor is a major determinant in pathogenesis, since in its absence no infection occurs. SARS-CoV most likely originated in bats and adapted to non-bat ACE2 variants as it crossed species to infect humans. Most notably, there were changes in the surface (S) glycoprotein that enhanced binding to the human ACE2 receptor. MERS-CoV has not mutated extensively during transmission in human populations; only a single mutation at position 1020 of the S protein has been consistently detected in human isolates. This amino acid is located in a region of the protein involved in fusion to host cell membranes but not in binding to DPP4. In contrast to SARS-CoV, MERS-CoV is able to bind DPP4 from a variety of species, facilitating spread to human and other populations and infect cells from a large number of species. Thus, in addition to camels and humans, non-human primates (NHP), rabbits, goats, sheep and horses, among other animals, are predicted to be susceptible to infection. This contrast in the role of S protein mutation in SARS-CoV and MERS-CoV adaptation to new populations probably reflects subtle differences in the mechanism of virus entry: coronavirus entry involves both binding to the receptor and subsequent release of a fusion peptide. The relative importance of each component is different in SARS-CoV vs MERS-CoV infections. Increased understanding of the relative roles of receptor binding and protease action will facilitate prediction of whether specific zoonotic coronaviruses will infect humans and the likelihood of adaptation.

Pathogenesis, Pathology and Immunity

In its most severe form, MERS-CoV causes an acute highly lethal pneumonia. Renal dysfunction/failure commonly occurs in infected individuals and could either be a consequence of hypoxic damage or direct infection of the kidney. The latter is a possibility because DPP4 is expressed at high levels in the kidney. Greater understanding of sites of infection requires examination and analysis of infected patient tissue samples. Unfortunately, no tissue samples are available largely for cultural/religious reasons, so that much of our understanding of pathogenesis has and will come from studies of experimentally infected animals. Several animals can be experimentally infected with MERS-CoV, including macaques, marmosets and camels. Macaques develop mild clinical disease with evidence of inflammatory cell infiltration on radiological examination, making these animals a useful model for nonlethal MERS. In contrast, MERS-CoV-infected marmosets develop severe interstitial pneumonia. Neutrophil and macrophage infiltration and alveolar edema were observed in infected lung tissue. Infected marmosets are useful models for severe MERS, but these animals are not readily available, limiting their utility. In another study, three camels that were infected experimentally with MERS-CoV developed mild rhinitis but not systemic disease. Virus was shed for several days, providing experimental evidence for the notion that camel-to-camel and camel-to-human spread occurred and contributed to the ongoing MERS outbreak.

A small rodent model would be most useful for MERS studies but mice are not susceptible to MERS-CoV. In one study, mice were sensitized to MERS-CoV infection using an adenovirus expressing human DPP4 (hDPP4). An advantage of this approach is that any strain of mouse can be made susceptible to MERS-CoV. Thus, whereas hDPP4-transduced immunocompetent mice develop only mild or no clinical disease, mice deficient in Type I interferon signaling develop more clinical disease and more extensive pathological changes.
in the lungs. Like infected macaques, these mice are most useful for vaccine and anti-virus studies. Mice transgenic for hDPP4 expression develop severe clinical disease after MERS-CoV challenge, but these mice also develop encephalitis, compromising their utility.

Little is known about what constitutes a protective immune response in MERS patients who recover, but based on studies of other coronaviruses, including SARS-CoV, coordinated innate and adaptive immune responses are required. MERS-CoV elicits attenuated innate immune responses with delayed pro-inflammatory cytokine induction in cell culture and in vivo, which may contribute to a dysregulated immune response. Parallel observations were made in SARS patients, in whom an ineffectual B and T cell response with prolonged cytokine expression was detected in those with severe disease, while a more rapid shutoff of the innate immune response and a potent anti-SARS-CoV antibody response was found in recovered patients.

The anti-SARS-CoV antibody response waned over time so that it was undetectable by 6 years after infection, while T cell responses could still be detected. Based on this information, it is likely that vaccines that result in only antibody responses will be useful in the short term, but may not provide long-lived protection against MERS-CoV.

**Epidemiology**

**Animal source**

The exact source and mode of transmission of MERS-CoV to humans remains undefined. Initial investigations revealed a possible bat origin for MERS-CoV, based on finding sequences related to MERS-CoV in several bat species. Other studies supporting a bat source showed that a bat coronavirus, *Tylonycteris* bat coronavirus HKU4 and MERS-CoV could both bind to human and bat DPP4 to enter cells. However, MERS-CoV has never been isolated from bats, so whether direct or indirect bat to human spread is actually important in transmission remains unknown.

Several other animal species in the Arabian peninsula have been assessed for serological evidence of previous MERS-CoV infection. One caveat with this type of serological studies is that it may detect other coronaviruses such as bovine coronaviruses, which are present in camel populations, or human coronaviruses that cause the common cold and are distantly related to MERS-CoV. Over time, serological assays have improved and sensitivity and specificity increased. In an early study, 100% of dromedary camels (*Camelus dromedarius*) in Oman and 14% in the Canary Islands (Spain) were positive for anti-MERS-CoV antibodies. Subsequent studies confirmed a high rate of seropositivity in camels in the Arabian peninsula, and also showed no evidence of infection of cows, goats or most likely, sheep.

One key question is whether MERS-CoV newly emerged into camel or human populations or whether the infection has been present for many years but was not detected. A retrospective analysis of stored human samples obtained in 2012 from blood donors and abattoir workers in Saudi Arabia showed no evidence of MERS-CoV seroreactivity. In marked contrast, anti-MERS-CoV antibodies were detected in archived sera obtained from dromedary camels in Saudi Arabia from 1993 and the United Arab Emirates in 2003.
Further, many camels in Saudi Arabia are imported from East Africa; additional studies showed that sera positive for MERS-CoV were detected in camels in East, West and North Africa from as early as 1992\textsuperscript{59, 60, 61}, indicating widespread circulation of MERS-CoV in camel populations for many years.

Transmission from camels to humans

Transmission from camels has been linked to human MERS-CoV infection (Figure 3), although only a minority of MERS patients have a history of camel exposure. However, interpretation of this lack of an obvious exposure may be confounded by less direct exposure; for example, patients may be exposed to infectious virus by consumption of unpasteurized camel milk, which is not uncommon in Saudi Arabia\textsuperscript{62}. Identical\textsuperscript{64, 65} or nearly identical\textsuperscript{64} viruses were isolated from geographically linked infected camels and patients. Interpretation of these types of studies is potentially complicated by the observation that RNA viruses in infected hosts, including MERS-CoV-infected dromedary camels, consist of swarms of closely related RNA molecules ('quasispecies')\textsuperscript{66}. Upon transmission to a new host, only 1 or a few members of the RNA virus swarm are transmitted, which make it difficult to isolate the exact same virus from both the donor and recipient.

The high anti-MERS-CoV seropositivity rate in adult camels and the high antibody titers in individual animals suggests that infection occurs at a young age with boosting occurring commonly\textsuperscript{53-55, 58, 62}. However, these conclusions have been disputed since the lack of camel contact in most human cases raises the possibility that transmission is primarily human-to-human or from another intermediate host and that some camel infections result from human-to-camel spread\textsuperscript{67}. Additional intermediate hosts may be involved; a CoV related to MERS-CoV was isolated from European hedgehogs (Erinaceus europaeus)\textsuperscript{68}. Together these results suggest that while many details of camel-to-human transmission are not clear, transmission from camels to human constitute the only confirmed zoonotic source for the human infection. Analyses of human populations in contact with camels in the horn of Africa, which is a source for many of the camels in Saudi Arabia, may identify individuals who are seropositive, as well as others who succumbed to the infection.

Whilst MERS cases continue to be reported intermittently at low levels throughout the year, some seasonal trends are observed. The first MERS case identified in April 2012, was followed by an increase in number of cases in April/May 2013, and a similar increase in April/May 2014. A small peak in case numbers also occurred in September-November, 2013 and 2014. The increase in the March to May period is consistent with the notion that transmission occurred primarily from newly infected young camels. Infection of this population may result from increased virus burdens in camel populations, occurring as a consequence of camel birthing and concomitant immunosuppression of pregnant dams. The increase in numbers of MERS cases in April/May 2014 in Saudi Arabia can also be attributed in part to breaches in infection control and in part to improved reporting and a high degree of awareness for widespread screening.
Human to human transmission

Human to human transmission of MERS-CoV was confirmed through epidemiological and genome sequence studies of cases associated with hospital and household MERS outbreaks\textsuperscript{10, 69}. In a hospital-based outbreak that occurred in April/May 2013 in Al-Hasa, an eastern province of Saudi Arabia, 23 patients in hemodialysis or intensive care units were infected with a single clade of virus, with a consequent mortality rate of 65\%\textsuperscript{10}. Spread was assumed to largely occur via large droplets and contact, although the possibility of airborne or fomite transmission was not eliminated. Most infections occurred as a result of person-to-person spread and emphasized the importance of using the appropriate contact and droplet precautions to prevent spread to other patients, healthcare workers and family members\textsuperscript{70}.

MERS-CoV isolated from single outbreaks are closely related\textsuperscript{26}. The genomic data to date are most consistent with human-to-human spread accompanied by periodic re-introduction of the virus into human populations. These analyses allow calculation of \(R_0\), the basic reproduction number, which for MERS-CoV is generally estimated to be no greater than 0.7\textsuperscript{71}. This number is substantially less than 1.0, the number associated with epidemic potential, making sustained transmission of the virus unlikely unless the virus mutates to transmit among humans more efficiently. By comparison, \(R_0\) for SARS-CoV was >1, consistent with sustained transmission during the SARS epidemic\textsuperscript{72, 73}.

Serious symptomatic disease occurs most commonly in patients with co-morbidities, such as diabetes, renal failure and underlying immunosuppression. However, it is also clear that patients without underlying diseases can become infected, although most develop asymptomatic or mild clinical disease\textsuperscript{52, 74, 75}. MERS-CoV transmission requires close contact between infected patients and susceptible individuals, but even under these conditions, only a limited amount of transmission has been documented\textsuperscript{52, 74-77}. In one study, Drosten et al showed that the rate of transmission from 26 MERS patients to 280 household contacts was approximately 4\%\textsuperscript{52}. All but one of these infected contacts developed subclinical disease, raising the possibility that the number of previously infected individuals in Saudi Arabia and other countries in the Arabian peninsula (and Africa) is much greater than now documented and lending support to efforts to increase surveillance in these countries.

MERS-CoV evolution

The phylogenetic relationship and evolution of MERS-CoV has been studied through whole genome sequencing of samples from MERS cases from several geographical backgrounds (Supplementary Figure 2). In one instance, 4 different MERS-CoV phylogenetic clades were identified in patients in Saudi Arabia in the period September to May 2013\textsuperscript{26}. However, by the end of the observation period, three of these clades were no longer circulating, consistent with \(R_0\)<1. Further, the extent of difference between these genetically distinct lineages of MERS-CoV makes it is unlikely that the infections were the result of a single continuous human-to-human transmission chain.
Clinical Features

The clinical manifestations of MERS-CoV infection range from asymptomatic infection to severe pneumonia with ARDS, septic shock, and multi-organ failure resulting in death. In contrast to SARS (Table 1), about 75% of patients with MERS had at least one co-morbid illness with fatal cases more likely to have an underlying condition (86% among fatal cases vs. 42% among recovered or asymptomatic cases, p<0.001). Index/sporadic cases in the first wave in 2013 were older (median age 59 years vs. 43 years, p<0.001), and more likely to suffer from severe disease requiring hospitalization (94% vs. 59%, p<0.001) in comparisons to the secondary cases. Cases specifically reported as “mild disease” or “asymptomatic” occurred only among secondary cases.

Based on data related to human-to-human transmission in several clusters, the incubation period has been estimated as >5 days, but could be as long as two weeks (median 5.2 days (95% CI: 1.9 to 14.7)). The median times from symptom onset of MERS to hospitalization, admission to an ICU and death were 4.0 (range 0-16, n=62), 5.0 (1-15, n=35) and 11.5 days (4-298, n=40), respectively.

Typically, MERS begins with fever, cough, chills, sore throat, myalgia and arthralgia, followed by dyspnoea and rapid progression to pneumonia within the first week, often requiring ventilatory and other organ support. While most patients with symptomatic MERS present with respiratory illness, immunocompromised patients may present with fever, chills and diarrhoea and later develop pneumonia due to MERS-CoV. Similar to SARS, at least one-third of patients with MERS had gastrointestinal symptoms, such as vomiting and diarrhoea. Risk factors for developing severe disease, in addition to an immunocompromised state, include comorbid illness such as obesity, diabetes, cardiac disease and lung disease. Concomitant infections and low albumin were found to be predictors of severe illness whereas age ≥65 years was the only predictor of mortality in a case series in Saudi Arabia.

The limited data available on viral dynamics and clinical course suggest that MERS-CoV infected patients have a shorter time from illness onset to clinical presentation and to a requirement for ventilatory support (median 7 days; range 3-11) than SARS patients, as well as higher respiratory tract viral loads during the first week of the illness.

Similar to SARS and other severe viral illnesses, common laboratory findings of MERS include leucopaenia, particularly lymphopenia. There were some cases with a consumptive coagulopathy and elevations in creatinine, lactate dehydrogenase and liver enzymes. Co-infection with other respiratory viruses (e.g. parainfluenza, rhinovirus, influenza A virus, herpes simplex virus, influenza B virus) has been reported while nosocomial bacterial infections (including Klebsiella pneumoniae, Staphylococcus aureus, Acinetobacter spp., candida sp.) occurred in patients receiving invasive mechanical ventilation.

Chest x-ray and tomographic findings of MERS are consistent with viral pneumonitis and ARDS, with bilateral hilar infiltration, uni- or bilateral patchy densities or infiltrates, segmented or lobar opacities, ground-glass opacities, and small pleural effusions in some
cases. Lower lobes are generally affected more than upper lobes early in the course of illness with more rapid radiographic progression than occurred in SARS\textsuperscript{1, 8, 9, 83}.

Reports from some MERS cases identified viral RNA in blood, urine and stool but at much lower viral loads than in the respiratory tract\textsuperscript{84}. MERS-CoV viral loads and genome fractions in upper respiratory tract (URT) specimens (e.g., nasopharyngeal swabs) are lower than in lower respiratory tract (LRT) specimens such as tracheal aspirates and bronchoalveolar lavage fluid (BAL)\textsuperscript{82}, likely contributing to inefficient interhuman transmissibility. LRT excretion of MERS-CoV RNA could be detected beyond 1 month of illness in the majority of cases, suggesting that prolonged shedding could be a source for spread in outbreaks\textsuperscript{85}.

**Diagnostics**

As LRT specimens such as BAL, sputum and tracheal aspirates contain the highest viral loads\textsuperscript{29, 82, 84}, these should be collected whenever possible. A case of MERS may be confirmed by detection of viral nucleic acid or by serology. The presence of viral nucleic acid can be confirmed either by a positive rRT-PCR result on at least two specific genomic targets or by a single positive target with sequencing of a second positive PCR product\textsuperscript{86}. Currently available rRT-PCR tests include an assay targeting RNA upstream of the E gene (upE) and assays targeting open reading frames 1b (\textit{ORF} 1b) and 1a (\textit{ORF} 1a). The assay for the upE target is highly sensitive and is recommended for screening, whereas the \textit{ORF} 1a assay is of equal sensitivity. The \textit{ORF} 1b assay is relatively less sensitive than the \textit{ORF} 1a assay but is useful for confirmation. These rRT-PCR assays have not shown cross-reactivity with other respiratory viruses including human coronaviruses. Two target sites on the MERS-CoV genome suitable for sequencing to aid confirmation are in the RNA-dependent RNA polymerase (\textit{RdRp}) (present in \textit{ORF} 1b) and (\textit{N}) genes (Figure 2)\textsuperscript{86}.

In MERS cases confirmed by PCR, serial samplings for PCR testing from the URT and LRT plus other body compartments (e.g., serum, urine and stool) are strongly recommended in order to advance understanding of viral replication kinetics and to guide infection control measures. Respiratory samples should be collected at least every 2-4 days to confirm viral clearance after two consecutive negative results are obtained.

For confirmation of infection by antibody detection, paired serum samples should be collected 14-21 days apart with the first being taken during the first week of illness. A positive screening (ELISA, IFA) assay should be confirmed followed by a confirmatory (neutralization) assay. Single samples may also be of value for identifying probable cases and should be collected at least 14 days after the onset of symptoms\textsuperscript{52, 54, 87}. Serological results must be carefully interpreted because results may be confounded by cross-reactivity against other CoV\textsuperscript{88}.

**Treatment**

There is no specific drug treatment for MERS-CoV and supportive therapy remains the mainstay of management. Evidence-based recommendations for therapy were recently formulated and provide a basis for rational decision-making in clinical settings\textsuperscript{89}(Table 2). MERS-CoV is readily inhibited by type I interferons (IFN-\(\alpha\) and especially IFN-\(\beta\)) in
cultured cells, in combination with ribavirin reduced lung injury and modestly reduced lung titers) when administered to rhesus macaques within 8 hrs of virus inoculation. This combination was administered to severely ill patients with MERS with improvement in survival observed at 14 but not 28 days, possibly reflecting administration in the advanced stage of the disease. Several agents have shown inhibitory effects against MERS-CoV in cell cultures, including cyclosporin A, and mycophenolic acid. Other compounds (chloroquine, chlorpromazine, loperamide, and lopinavir) inhibit MERS-CoV replication in the low-micromolar range (EC50 values 3-8 μM) in vitro, although whether any of these will be useful in patients is unknown. MERS-CoV-specific peptide fusion inhibitors, which function similarly to the HIV drug, enfuvirtide, diminish virus replication in cultured cells, providing a novel approach to MERS therapy.

Human monoclonal neutralizing antibodies and convalescent sera from MERS recovered patients may be useful for therapy in MERS-CoV infection if delivered in a timely fashion. In support of this, an exploratory post-hoc meta-analysis of studies of SARS and severe influenza showed a significant reduction in the pooled odds of mortality following antibody treatment vs placebo or no therapy (OR 0.25; 95% CI 0.14 to 0.45).

Systemic corticosteroids have been used empirically in some patients with MERS-CoV infections to dampen immunopathologic host responses, although no survival benefits were observed. Steroids should be used cautiously, if at all, because their usage was associated with worsened outcomes in patients infected with SARS-CoV during the 2002-2003 epidemic. More animal model data and carefully conducted clinical and virological studies of priority therapies like convalescent plasma and interferons (ideally in randomized clinical trials if sufficient numbers of patients are available) are needed. At present, clinical management of patients with severe infection due to MERS-CoV largely relies on meticulous intensive care support and prevention of complications.

Prevention

Recommendations for prevention of MERS-CoV are available at the WHO, CDC and MOH websites.

Healthcare workers

The main infection prevention and control measures for managing MERS patients include droplet precautions (wearing a surgical mask within 1 m of the patient) and contact precautions (wearing gown and gloves on entering the room and removing them on leaving). Droplet precautions should be added to the standard precautions when providing care to all patients with signs of acute respiratory infection. In addition, eye protection should be undertaken when health care workers care for probable or confirmed cases of MERS-CoV infection. Public Health England, CDC, and MOH recommendations for management of known or suspected MERS-CoV infection include the use of Personal Protective Equipment (PPE) such as gowns, gloves, eye protection (goggles or face shield) and respiratory protection equivalent to a fit-tested NIOSH-certified disposable N95 filtering face piece respirator. MERS patients should be placed in negative pressure rooms or in rooms in which room exhaust is filtered through high-efficiency particulate air (HEPA).
filters. Airborne precautions with at least 6 air changes per hour should be applied in treatment rooms when performing aerosol-generating procedures. These recently updated WHO and MOH recommendations are evidence-based and have proven to be effective in hospitals in affected countries.

General public and camel owners

Camels infected with MERS-CoV may develop rhinitis or not show any signs of infection and may shed MERS-CoV through nasal and eye discharge and faeces. MERS-CoV may also be found in raw milk from infected camels. MERS-CoV is stable in camel breast milk for extended periods of time; thus pasteurization or cooking is recommended for destroying the virus. Raw urine should not be used for medicinal purposes. Because signs of disease are nonspecific, it is not possible to know whether an animal in a farm, market, race track or slaughterhouse is excreting MERS-CoV without virological testing. It is prudent for camel farm workers, slaughterhouse workers, market workers, veterinarians and those handling camels at racing facilities to practice good personal hygiene, including frequent hand washing after touching animals, avoiding touching eyes, nose or mouth with hands, and avoiding contact with sick animals. Consideration should also be given to wearing protective gowns and gloves while handling animals, especially if camels have signs of upper respiratory tract disease.

Pilgrims

The Saudi government issues updated health guidelines for pilgrims. Although MERS-CoV was not the cause of severe community acquired pneumonia in any of the 38 hospitalized pilgrims investigated during the 2013 Hajj, good infectious disease surveillance and control measures are essential to prevent major outbreak of MERS during mass gathering activities.

Unanswered questions and conclusions

Nearly three years since its first discovery, several important questions about MERS-CoV epidemiology, routes of transmission, pathogenesis, and treatment remain unanswered. While a zoonotic source of the virus is most likely, the route of transmission could be through either direct or indirect contact, or consumption of a contaminated food or food product. Whilst several studies have demonstrated the possible links between human cases of MERS-CoV and transmission from camels, several gaps about the origins, geographical distribution, exact mode of transmission of MERS-CoV and relationships between MERS-CoV and MERS-CoV-like infections in bats, other animals, camels, and humans remain. The intermittent sporadic nature of many cases of MERS has hindered in-depth case controlled studies and investigation of rates of secondary transmission, including determining the role of subclinical infection in human-to-human transmission. Also unclear are the natural history, pathogenesis, host susceptibility factors, MERS-CoV virulence, viral kinetics, optimal periods of infectiousness, underlying mechanisms of protective immunity, and factors governing treatment outcome. Absence of this knowledge base is hindering the development of drug treatment, adjunctive therapies, specific diagnostics, biomarkers, and vaccines.
Available data indicate that MERS-CoV has not yet fully adapted to infecting humans, and human-to-human transmission is not sufficiently efficient for pandemic potential. More information about how long the virus has infected humans, which could be obtained from analyses of pre-2012 human serum samples (if available), would aid in risk assessment of the likelihood of further adaptation to humans. Further genomic studies of MERS-CoV will provide valuable insights into the understanding of the molecular characteristics, mutation rates of MERS-CoV, and virus transmission dynamics, defining factors critical for species specificity and virulence, and enabling discovery of new drug targets, novel drugs, diagnostics and vaccines. Post mortem and histological studies have not been available and even limited autopsy or surgical specimens would help advance the scientific knowledge base. The availability of an animal model of severe MERS-CoV infection and disease will be important for understanding pathogenesis, natural history and immune responses and for developing effective therapies. There is some urgency to these future studies because the genetic mutability of coronaviruses is well established and MERS-CoV continues to circulate in countries on the Arabian peninsula. While interhuman transmission is still inefficient, it is important that healthcare authorities, governments and the research community be well prepared for the eventuality of outgrowth of virus with increased capacity for transmission.

**Search Strategy and Selection criteria**

We searched MEDLINE, EMBASE and GOOGLE SCHOLAR ((January 1st 2010 to February 15th 2014). We used the search terms “Middle East Respiratory Syndrome” or “MERS-CoV” in combination with the terms “Coronavirus”, or “Middle East”, or “Epidemiology”, or “Virology” or “Clinical features” or “Aetiology” or “Diagnostic tests”, or “Diagnosis” or “Management” or “Prevention” or “Vaccines” or “nosocomial” or “hospital” or “camels”. We also searched websites of global and national public health agencies such as US-CDC, UK-PHE, Europe-ECDC and the MOH portal. We selected publications in the English language literature published in past 30 months, and included references from WHO, CDC, ECDC websites and those on CIDRAP and ProMed (Supplementary Figure 1). We also searched the reference lists of articles identified by this search strategy and selected those we judged relevant to provide readers with more details and more references than this Seminar can accommodate.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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103. WHO. Update on MERS-CoV transmission from animals to humans, and interim recommendations for at-risk groups. 2014. cited 16Feb 2015; Available from: http://www.who.int/csr/disease/coronavirus_infections/MERS_CoV_RA_20140613.pdf?ua=1


Figure 1. Global MERS cases
A. Distribution of confirmed cases of MERS-CoV reported as of 30 January 2015, by date and place of probable infection (n=990). B. Number of MERS cases and deaths, by country of reporting as of 30 January 2015 (n=990). Map indicates countries in which MERS patients were identified. Also shown is the number of death/total cases for each country. Source of data: WHO (http://www.who.int/csr/don/archive/disease/coronavirus_infections/en/); Promed Mail (International Society for Infectious Diseases) (http://www.promedmail.org/).
Figure 2. MERS-CoV virion, replication strategy and genomic structure

**Upper panels**: Left—Electron micrograph of MERS-CoV virions in large single membrane vesicles and in the periphery of the cell. Virion spikes are visible (see also inset) as is the electron-dense core consisting of the RNA genome encapsidated in the N protein. Electron micrograph provided by Montserrat Barcena, Ronald Limpens & Eric Snijder (Leiden University Medical Center, The Netherlands). **Middle**—Schematic representation of MERS-CoV, showing viral RNA structural proteins spike (S), envelope (E), matrix (M) and nucleocapsid (N). **Right**: Human airway cells infected with MERS-CoV and examined for viral antigen by indirect immunofluorescence assay (IFA) using an anti-MERS-CoV N antibody. Green—MERS-CoV+ cells. Blue—nuclear stain. A similar IFA is used for the serological diagnosis of MERS. Figure provided by Christine Wohlford-Lenane (University of Iowa, Iowa City, IA).

**Central panel**: To enter host cells, MERS-CoV attaches to dipeptidyl peptidase 4 (DPP4). Protease cleavage of the S protein is required for virus-cell fusion and release of genomic RNA into the cytoplasm. Viral RNA transcription and replication occurs on double membrane vesicles, which are derived from the endoplasmic reticulum. Transcription of the sevensubgenomic mRNAs occurs via negative strand subgenomic RNA intermediates. Subgenomic RNAs are 3' co-terminally nested and are joined to a common leader encoded at the 5' end of the genome. Viral RNA is transported to the ERGIC, the site of assembly. Viral RNA encapsidated in the N protein buds into vesicles lined with the S, M and E proteins. Vesicles are then transported to the cell surface prior to release. **Lower panel**: The MERS-CoV genome consists of 11 open reading frames that code for the virus replication machinery (ORF 1a and ORF 1b) and the major structural proteins: spike (S), envelope (E), matrix (M) and nucleocapsid (N). ORF 1b, produced by a
−1 base pair frameshift from ORF 1a, encodes the RNA-dependent RNA polymerase (nsp12), helicase (nsp13), N7-methyltransferase (nsp14), a 3′-5′ exonuclease important for RNA proofreading (nsp14), 2′-O-methyltransferase (nsp 16), and an endonuclease specific for U and found only in nidoviruses(nsp 15). MERS-CoV also encodes five accessory proteins in the 3′ end of the genome (3a/b, 4, 5, 8b), which share no homology with proteins from host cells or other viruses, including coronaviruses. 4a and 4b are interferon antagonists\textsuperscript{111, 112} but functions of the other accessory proteins are unknown.
MERS-CoV may have originally spread from bats to camels and other, as yet unidentified, intermediate hosts. Virus has circulated in camel populations in Africa and the Arabian peninsula for at least 20 years. In 2012, MERS-CoV spread to infect human populations, with the camel considered the most likely source for the virus. Several possible routes of spread from camels to humans are illustrated in the figure. MERS-CoV is believed to be transmitted among humans by droplet, contact and perhaps airborne spread. MERS is manifested in humans in a variety of ways, ranging from asymptomatic to fulminant infections. Patients with underlying disease such as diabetes or kidney or liver disease or who are immunocompromised develop more severe disease and have a higher mortality rate after infection.

*Figure 3. Ecology and transmission of MERS-CoV*

MERS-CoV may have originally spread from bats to camels and other, as yet unidentified, intermediate hosts. Virus has circulated in camel populations in Africa and the Arabian peninsula for at least 20 years. In 2012, MERS-CoV spread to infect human populations, with the camel considered the most likely source for the virus. Several possible routes of spread from camels to humans are illustrated in the figure. MERS-CoV is believed to be transmitted among humans by droplet, contact and perhaps airborne spread. MERS is manifested in humans in a variety of ways, ranging from asymptomatic to fulminant infections. Patients with underlying disease such as diabetes or kidney or liver disease or who are immunocompromised develop more severe disease and have a higher mortality rate after infection.
Table 1
Comparison of clinical and laboratory features between MERS and SARS patients

<table>
<thead>
<tr>
<th></th>
<th>MERS-CoV\textsuperscript{7-10, 13, 29, 78, 81}</th>
<th>SARS-CoV\textsuperscript{79, 114-118}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Date of first case report</strong></td>
<td>April 2012 (Zarqa, Jordan) June 2012 (Jeddah, KSA)</td>
<td>Nov 2002 (Guangdong, China)</td>
</tr>
<tr>
<td><strong>Incubation period</strong></td>
<td>Mean: 5.2 days (95% CI: 1.9-14.7) Range: 2-13 days</td>
<td>Mean: 4.6 days (95% CI: 3.8-5.8) Range: 2-14 days</td>
</tr>
<tr>
<td><strong>Serial interval</strong></td>
<td>7.6 days</td>
<td>8.4 days</td>
</tr>
<tr>
<td><strong>Basic reproduction number</strong></td>
<td>&lt;1</td>
<td>2.3</td>
</tr>
<tr>
<td><strong>Age group</strong></td>
<td>Adults (98%) Children (2%)</td>
<td>Adults (93%) Children (5-7%)</td>
</tr>
<tr>
<td><strong>Age (years):</strong></td>
<td>Range: 1-94; Median: 50</td>
<td>Range: 1-91; Mean: 39.9</td>
</tr>
<tr>
<td><strong>Gender (M,F)</strong></td>
<td>M: 64.5%, F: 35.5%</td>
<td>M: 43%, F: 57%</td>
</tr>
<tr>
<td><strong>Case fatality rate (CFR)</strong></td>
<td>overall 40%</td>
<td>9.6%</td>
</tr>
<tr>
<td><strong>CFR in patients with co-morbidities</strong></td>
<td>13.3%</td>
<td>1-2%</td>
</tr>
<tr>
<td><strong>Disease progression</strong></td>
<td>Median 7 days Ventilatory support</td>
<td>Mean 11 days</td>
</tr>
<tr>
<td><strong>Time from onset to death</strong></td>
<td>Median 11.5 days</td>
<td>Mean 23.7 days</td>
</tr>
<tr>
<td><strong>Presenting symptoms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever (&gt;38^\circ C)</td>
<td>98%</td>
<td>99-100%</td>
</tr>
<tr>
<td>Chills / rigors</td>
<td>87%</td>
<td>15-73%</td>
</tr>
<tr>
<td>Cough</td>
<td>83% (dry) 56% (productive)</td>
<td>62-100% (29-75%) 4-92%</td>
</tr>
<tr>
<td>Haemoptysis</td>
<td>17%</td>
<td>0-1%</td>
</tr>
<tr>
<td>Headache</td>
<td>11%</td>
<td>20-56%</td>
</tr>
<tr>
<td>Myalgia</td>
<td>32%</td>
<td>45-61%</td>
</tr>
<tr>
<td>Symptom</td>
<td>MERS-CoV (^{7,10, 13, 29, 78, 81})</td>
<td>SARS-CoV (^{79, 114-118})</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------------------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Malaise</td>
<td>38%</td>
<td>31-45%</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>72%</td>
<td>40-42%</td>
</tr>
<tr>
<td>Nausea</td>
<td>21%</td>
<td>20-35%</td>
</tr>
<tr>
<td>Vomiting</td>
<td>21%</td>
<td>20-35%</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>26%</td>
<td>20-25%</td>
</tr>
<tr>
<td>Sore throat</td>
<td>14%</td>
<td>13-25%</td>
</tr>
<tr>
<td>Rhinorrhoea</td>
<td>6%</td>
<td>2-24%</td>
</tr>
<tr>
<td>Co-morbidities</td>
<td>76%</td>
<td>10-30%</td>
</tr>
<tr>
<td><strong>Laboratory results</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXR abnormalities</td>
<td>90-100%</td>
<td>94-100%</td>
</tr>
<tr>
<td>Leukopenia ((&lt; 4.0 \times 10^{9}/L))</td>
<td>14%</td>
<td>25-35%</td>
</tr>
<tr>
<td>Lymphopenia ((&lt; 1.5 \times 10^{9}/L))</td>
<td>32%</td>
<td>68-85%</td>
</tr>
<tr>
<td>Thrombocytopenia (&lt;140 \times 10^{9}/L))</td>
<td>36%</td>
<td>40-45%</td>
</tr>
<tr>
<td>Elevated LDH</td>
<td>48%</td>
<td>50-71%</td>
</tr>
<tr>
<td>Elevated ALT</td>
<td>11%</td>
<td>20-30%</td>
</tr>
<tr>
<td>Elevated AST</td>
<td>14%</td>
<td>20-30%</td>
</tr>
<tr>
<td><strong>Risk factors associated with poor outcome (severe disease or death)</strong></td>
<td>An immunocompromised state, comorbid illness (such as obesity, diabetes, cardiac disease and lung disease), concomitant infections, low albumin, age ≥65 yrs.</td>
<td>Advanced age, male gender, high initial or peak LDH, high neutrophil count on presentation, diabetes mellitus or other comorbid conditions, low CD4 and CD8 lymphocyte counts at presentation.</td>
</tr>
</tbody>
</table>
Table 2
Potentially useful antiviral agents for Middle East respiratory syndrome coronavirus (MERS-CoV) infection

| **Neutralising antibody***: convalescent plasma, polyclonal human immunoglobulin from transgenic cows, equine F(ab')2 antibody fragments, camel antibodies, anti-S monoclonal antibodies |
| **Interferons***: Interferon alfa, Interferon beta |
| ± **Repurposed drugs**: ribavirin monotherapy# (±interferon), HIV protease inhibitors (lopinavir*, nelfinavir), cyclophilin inhibitors (ciclosporin, alisporivir), chloroquine (active in vitro), mycophenolic acid, nitazoxanide. |
| **Recombinant human mannose-binding lectin** |
| **siRNA to key MERS-CoV genes** |

* Treatment benefits likely to exceed risks
# Risks likely to exceed benefits