MERS coronavirus outbreak: Implications for emerging viral infections

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PII: S0732-8893(18)30502-9
DOI: doi:10.1016/j.diagmicrobio.2018.10.011
Reference: DMB 14697

To appear in: *Diagnostic Microbiology & Infectious Disease*

Received date: 21 July 2018
Revised date: 28 September 2018
Accepted date: 11 October 2018

This is the author's manuscript of the article published in final edited form as:

Al-Omari, A., Rabaan, A. A., Salih, S., Al-Tawfiq, J. A., & Memish, Z. A. (2018). MERS coronavirus outbreak: Implications for emerging viral infections. Diagnostic Microbiology and Infectious Disease. https://doi.org/10.1016/j.diagmicrobio.2018.10.011

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(MERS-CoV). There have been several MERS-CoV hospital outbreaks in KSA, continuing to the present day, and the disease has a mortality rate in excess of 35%. Since 2012, the World Health Organization have been informed of 2,220 laboratory-confirmed cases resulting in at least 790 deaths. Cases have since arisen in 27 countries, including an outbreak in the Republic of Korea in 2015 in which 36 people died, but more than 80% of cases have occurred in Saudi Arabia.. Human-to-human transmission of MERS-CoV, particularly in healthcare settings, initially caused a 'media panic', however human-to-human transmission appears to require close contact and thus far the virus has not achieved epidemic potential. Zoonotic transmission is of significant importance and evidence is growing implicating the dromedary camel as the major animal host in spread of disease to humans. MERS-CoV is now included on the WHO list of priority blueprint diseases for which there which is an urgent need for accelerated research and development as they have the potential to cause a public health emergency while there is an absence of efficacious drugs and/or vaccines. In this review we highlight epidemiological, clinical, and infection control aspects of MERS-CoV as informed by the Saudi experience. Attention is given to recommended treatments and progress towards vaccine development.

Key words: coronavirus, MERS, respiratory, infection, transmission, Saudi Arabia, Middle East

Introduction

Middle East Respiratory Syndrome (MERS) arises from infection with the MERS-coronavirus (MERS-CoV), a betacoronavirus. Since the first confirmed case in June 2012, the World Health Organization (WHO) have been informed of 2,220 laboratory-confirmed cases resulting in at

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least 790 deaths [1, 2]. Although cases have arisen in 27 countries to date, including a major outbreak in the Republic of Korea in 2015, the overwhelming burden of infection has occurred in the Middle East and most particularly in the Kingdom of Saudi Arabia (KSA), where more than 80% of cases have occurred according to WHO estimates [2-6]. In this review, we consider current knowledge of MERS-CoV virology, molecular biology, immunology, epidemiology, diagnosis, transmission, therapy and vaccinology with special reference to the impact on the Middle East and KSA in particular.

Epidemiology

The first confirmed case of Middle East Respiratory Syndrome (MERS) was in June 2012. A previously healthy 60-year old Saudi male was hospitalised on 10th June 2012 in Bisha in the Kingdom of Saudi Arabia (KSA) with acute community-acquired pneumonia and was subsequently transferred to a private hospital in Jeddah on 13th June 2012, where he died on 24th June due to respiratory and renal failure [1]. Indirect immunofluorescence assays on day 1 sputum samples were negative for influenza A and B, parainfluenza 1 to 3, respiratory syncytial virus and adenovirus, however cytopathic changes in LLC-MK2 and Vero cells inoculated with the patient's sputum indicated the likelihood of viral replication [1]. PCR testing was negative for adenovirus, enterovirus, metapneumovirus, herpesviruses and paramyxoviruses but positive for detection of coronaviruses [1]. Sequencing of the PCR products confirmed the identification of a new virus belonging in lineage C of the betacoronavirus genus and initially named human coronavirus EMC (HCoV-EMC) [1]. In September 2012, the same virus was identified in a 49-year-old man who had been transferred from a hospital in Qatar to London with an unexplained,

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severe respiratory illness which required intubation and ventilation [7]. Importantly, this man had a history of travel in KSA, where he had experienced a mild undiagnosed respiratory illness in August 2012 [7]. The first cluster of human cases was retrospectively confirmed from a group of thirteen people who had become ill with an unexplained respiratory illness in a public hospital in Zarqa city in Jordan in April 2012 [8].

Since then, most outbreaks have occurred in KSA. These include a cluster of 25 cases in Al-Hasa between April 1st and May 23rd 2013 [9], 255 laboratory-confirmed cases in Jeddah between January 1st and May 16th, 2014 [10,11], 45 cases in King Fahad Medical City in Riyadh between March 29th and May 21st, 2014, with contemporaneous outbreaks in other Riyadh hospitals between March and April 2014 [12,13], and 130 cases at King Abulaziz Medical City in Riyadh during late June–late August 2015 [14]. An exception was the major outbreak that occurred in in the Republic of Korea between 20 May and 27 July 2015 [3-5]. This outbreak encompassed 186 MERS-CoV cases, and resulted in 36 deaths [3,4,15]. However, in common with cases that have arisen in other countries outside the Middle East, the Korean outbreak began with a man with a preceding travel history to Middle Eastern countries. According to reports made to WHO and the Centers of Disease Control and Prevention (CDC), laboratory-confirmed cases of MERS have occurred in Middle Eastern countries inlcuding KSA, Bahrain, Iran, Jordan, Kuwait, Lebanon, Oman, Qatar, United Arab Emirates (UAE), and Yemen, as well as in countries outside the Middle East including Algeria, Austria, China, Egypt, France, Germany, Greece, Italy, Malaysia, Netherlands, Philippines, Republic of Korea, Thailand, Tunisia, Turkey, United Kingdom (UK), and United States of America (USA), but associated with individuals with a travel history in the Middle East.

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MERS-CoV is now included on the WHO list of priority blueprint diseases for which there which is an urgent need for accelerated research and development as they have the potential to cause a public health emergency while there is an absence of efficacious drugs and/or vaccines [16]. Cases continue to arise in KSA and exact a high mortality rate, including 20 cases from eleven areas of the country reported to WHO by the National IHR Focal Point between December 2017 and 17 January 2018, resulting in nine deaths [17]. Frequent small outbreaks include two clusters of cases in the Al Jawf Region of KSA, i.e. a cluster of thirteen cases in a hospital between 2nd and 11th August 2017, among them eight healthcare workers (HCWs), and seven cases in Dawmet Aljandal City between 24th and 31st August 2017 [18,19]. In three clusters in three Riyadh hospitals in June 2017, two of which were related, 49 individuals were infected of whom ten died [20].

Clearly, MERS-CoV is a serious public health issue in KSA. Extended outbreaks of the disease have been focused on healthcare facilities, with transmission apparently dependent on close human-to-human contact [9-14]. The emergence of this disease has therefore had a profound impact on infection control and prevention procedures in KSA as outbreaks in healthcare facilities have been associated with defective or inadequate infection prevention and control measures [21-24].

Infection prevention and control measures in Saudi Arabia

Public health authorities in KSA worked with WHO in identifying shortcomings in infection and control procedures in healthcare facilities which contributed to MERS-CoV transmission [2]. Problems which were identified included emergency room overcrowding and neglect of basic

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infection and prevention control measures such as handwashing [2]. The KSA Ministry of Health updated guidelines for infection prevention and control in line with WHO recommendations [22,23]. The Ministry of Health now specifies that "Standard Precautions" should be adhered to in all patient interactions within hospitals, and that these should be further supplemented with the specific precautions for suspected or confirmed MERS-CoV cases [25]. Infection prevention and control measures include comphrensive basic procedures such as hand hygiene, including application of 'my five moments for hand hygiene'[26], respiratory precautions, contacts control, and use of personal protective equipment (PPE), which comprises surgical or correctly fitted and sealed N95 mask, gloves and gown, and goggles/face shield where indicated, and prevention of overcrowding in emergency rooms. More advanced precautions for care of patients with acute respiratory infections include use of effective triage, droplet and airborne precautions, safe patient transport and continuous training and education of healthcare workers. Frequent and thorough cleaning of MERS patient rooms with special attention to frequently touched surfaces, preferably by designated, well-trained housekeeping staff and with a clearly defined scope for cleaning of patient-care equipment, is also recommended [25]. Ministry of Health guidelines should also be followed for cleaning and disinfection after MERS patient discharge, handling of textiles, use of disposable dishes and eating utensils for MERS-CoV patients and diposal of medical waste [25]. Detailed guidelines are included on management of contacts of MERS-CoV patients, including household contacts, healthcare workers and patients; contact monitoring for fourteen days after date of exposure is recommended [25]. Home isolation procedures and duration of isolation precautions should be based on laboratory testing if available to assure absence of viral shedding; appropriate duration of isolation is an area that is still being researched [25]. Handling of bodies in the mortuary, as well as guidelines for extracorporeal

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membrane oxygenation (ECMO), which is available in designated MERS-CoV centres in Riyadh, Jeddah and Dammam, but which is of uncertain benefit for MERS-CoV treatment, are also detailed [25].

Implementation of these infection prevention and control guidelines for MERS-CoV in line with most up-to-date case definition and surveillance guidance have resulted in a decline in cases in KSA [25]. However, diligence in needed in maintaining surveillance standards and furthering understanding of transmission patterns within KSA and elsewhere. Comparison of epidemiology of KSA outbreaks to that of the Republic of Korea 2015 outbreak suggests that while there are similarities in terms of mean age of infected individuals (51 and 54 y respectively) and the higher risk of infection or death for older males (≥70 y), nevertheless there is evidence that transmission patterns and risk factors are different in KSA [26]. While in Korea the transmission pattern was almost exclusively nosocomial, in KSA zoonotic transmission, human-to-human transmission and unknown pathways were all present in addition to nosocomial infection [26]. In some 59.9% of cases in KSA outbreaks, exposure risk was unknown [26]. Thus in addition to the infection prevention and control guidelines for healthcare facilities, WHO has also issued guidance on potential zoonotic transmission in the community, in particular with respect to dromedary camels which are recognised as a major MERS-CoV host reservoir and animal source for human infection [6]. In KSA it is recommended that people visiting places where dromedary camels are present should practice general hygiene measures and avoid contact with sick animals. Furthermore, consumption of raw or uncooked meat, milk or urine from dromedaries is discouraged, with pasteurization, cooking, or other heat treatments recommended for rendering these products fit for consumption [6]. Immunocompromised people and other vulnerable groups such as people with diabetes, renal failure or chronic lung disease are advised to avoid contact

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with dromedaries in general and not to consume camel food products that have not been pasteurised or adequately cooked [6]. Recent studies, including those based on serological evidence, support the role of dromedary camels as important zoonotic sources of human MERS-CoV infection. MERS-CoV antibodies are present in more than 90% of dromedary camels tested in the Middle East and in many African countries [27-34]. Dromedary camel exposure within two weeks of illness onset has been identified as a significant risk factor in a study examining MERS-CoV infection cases documented between May and November 2014 in KSA [35]. Changes in dromedary camel production and farming practices, including intensification and location close to cities, may have contributed to zoonotic transmission in KSA [36]. Thus, in KSA the emergence of MERS-CoV has had an impact on the agricultural, animal husbandry, food production and veterinary fields, as well as infection and prevention control procedures in healthcare settings [37]. For example, the association between the calving season and MERS-CoV infection in dromedary camels and the highesr risk of MERS-CoV infection in calves compared to adult cows, has led to suggestions that weaning of calves could be delayed to reduce the opportunity for human exposure to calves [28, 29, 37]. Furthermore, there is a need to increase understanding of the implications in terms of MERS-CoV transmission and spread, as well as viral exchange, amplification and dissemination, of the economically important bidirectional movement of camels between African countries and the Middle East, including KSA [37].

Meanwhile, when a case of MERS-CoV is suspected, effective identification is achieved by molecular methods. The currently WHO-recommended methods used in KSA are based on polymerase chain reaction (PCR) targeting of a number of MERS-CoV genes, which has been made possible by development in understanding of MERS-CoV classification and genomics.

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General virology

Classification

In the 1960s, the first human respiratory illness-causing coronaviruses, (HCoVs) 229E and HCoV-OC43, were discovered [38, 39]. In 2003, a new CoV named Severe Acute Respiratory Syndrome (SARS)-CoV SARS was involved in a series of international outbreaks causing close to 800 deaths [40-42]. The NL63 and HKU1 human coronaviruses were discovered in 2004, both of which also cause human respiratory illness [43, 44]. MERS-CoV was first isolated in September 2012, and initially named human coronavirus EMC [1].The coronavirus study group later renamed this novel virus as the Middle East respiratory syndrome coronavirus (MERS-CoV), reflecting its origin [45].

Coronaviruses (CoVs) are members of the *Coronavirinae* subfamily of the *Coronaviridae* family. CoVs infect humans as well as other species. The subfamily is comprised of four genera, alpha CoVs, beta CoVs, gamma CoVs, and delta CoVs (Figure 1) CoVs are enveloped single-stranded, positive-sense RNA viruses with genomes of 25 to 32 kilobases (kb). HCoV-NL63 and HCoV-229E are alphaCoVs, while SARS-CoV, MERS-CoV, HCoV-HKU1 and HCoV-OC43 are betacoronoaviruses (Figure 1). The betacoronavirses can be further subdivided into four lineages. MERS-CoV is unique among CoVs infecting humans in belonging to lineage C (lineage 3) of the beta CoVs (Figure 1) [46-48].

Bats are potentially the main MERS-CoV mammalian reservoir, as with other coronaviruses [49]. Closely related lineage 3 viruses include the bat viruses NeoCoV, isolated from a

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Neoromicia zuluensis bat in South Africa, and the prototypic lineage c betacoronaviruses, *Tylonycteris* bat virus HKU4 and *Pipistrellus* bat HKU5 virus (Figure 1) [50-52]. Studies on the phylogeny of lineage C betacoronaviruses suggest that evolution of MERS-CoV in camels occurred prior to that in humans and that there was exchange of genetic elements among ancestral viruses either in bats, or within the camel genetic 'mixing vessel', leading to MERS-CoV emergence [51]. Other potentially important mammalian hosts are members of the *Eulipotyphla* taxon, the closest sister taxon to bats which includes hedgehogs [47]. EriCoV, another lineage C virus which is closely related to both MERS-CoV and the bat lineage C coronaviruses, was found to be present in approximately 59% of European hedgehog (*Erinaceus europaeus*) fecal samples in a study in Germany [47].

Genomics

The MERS-CoV has a genome of 30119 nucleotides comprising seven predicted open reading frames (ORFs) (1a, 1b, 3, 4a, 4b, 5, 8b) and four structural genes encoding the spike (S), nucleocapsid (N), membrane (M) and envelope (E) proteins (Figure 2) [53-55]. The overlapping ORF1a and 1b are located at the 5' end of the single stranded positive RNA alongside a 278 nucleotide un-translated region (UTR) (Figure 2). ORF1a and ORF1b comprise the majority of the MERS-CoV genome and are translated into polyproteins pp1a and pp1ab, which are then cleaved by viral proteases to give sixteen non-structural proteins termed nsp1 to nsp16 (Figure 2). These form the replication-transcription complex (RTC) of the virus. Individual nsp proteins have different roles in viral replication. For example, nsp3 has a papain-like protease (PLpro) activity which mediates the initial processing of pp1a [55-57]. Nsp3 also works with nsp4 and nsp6 to anchor the viral RTC to intracellular membranes and form a reticulovesicular

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membranous network where the viral RNA can replicate. Meanwhile nsp5 also has a protease activity, 3C-like protease (3CLpro), which also mediates pp1a and pp1ab cleavage into nsp 1-16. Nsp7 to nsp11 medate primer-making activity and regulate nsp12, which is the main viral RNA-dependent RNA polymerase (RdRp) [55-57]. Nsp13 to 16 are involved in viral RNA modification [55-57].

The genes for the S, E, M and N proteins are downstream of ORF1 (Figure 2). The S protein is vital in MERS-CoV transmission and host cell infection, determining tropism of the virus and host cell entry. The S protein is a trimeric, envelope protein which can be cleaved by host proteases into S1 (N-terminal) and S2 (C-terminal) subunits [58]. The S1 subunit contains a receptor binding domain (RBD), which mediates binding of S protein to the host cell human dipeptidyl peptidase 4 (DPP4; CD26) receptor [59, 60]. Once the MERS-CoV binds to DPP4 via the S1 RBD, endocytosis occurs. Cleavage at the S1/S2 junction then occurs, mediated by host proteases including the serine protease TMPRSS2, the endosomal cathepsin L, and furin protease [61-65], followed by viral fusion with the host cell membrane mediated by the S2 subunit. The S2 subunit contains a fusion peptide, two heptad repeat domains HR1 and HR2, and a transmembrane (TM) domain [66]. Fusion is facilitated by rearrangement of S2 into a six-helix bundle (6HB) fusion core, centred on a trimer of the HR1 and HR2 dimer. This folding of H1/H2 allows exposure of the fusion peptide and insertion into host cell membrane, and hence fusion [61, 66].

DPP4 receptor

The MERS-CoV S protein DPP4 receptor is widely expressed in human cells including lower respiratory tract non ciliated bronchial epithelium, kidney epithelial cells, small intestine cells, T

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lymphocytes and macrophages [67-70]. There is limited expression of DPP4 in the upper respiratory tract epithelium in humans when compared to dromedary camels, which may contribute to the limited replication of MERS-CoV in the human upper respiratory tract and to restriction of human-to-human transmission [68]. Infection of macrophages by lentiviral particles pseudotyped with MERS-CoV S protein resulted in attenuation of macrophage responses via expression of IRAK-M, a negative regulator of Toll-like receptor (TLR) signaling, and of the transcriptional repressor PPARγ [69]. Use of the DPP4 inhibitor sitagliptin or DPP4-siRNA reduced the effects of MERS-CoV S protein on IRAK-M, PPARγ and IL-10, indicating that the suppression of macrophage immune responses by MERS-CoV is mediated via DPP4 [69]. Mathematical modelling suggests that reducing the rate of DPP4 expression would reduce MERS-CoV spread [70]. Indeed, levels of DPP4 mRNA and protein are higher in lung tissues of smokers and individuals with chronic obstructive pulmonary disease (COPD) compared to never-smokers [71]; both smoking and COPD are associated with increased susceptibility to MERS-CoV infection. Host species restriction of MERS-CoV infection has been linked to thirteen DPP4 residues which are key in interacting with the S protein RBD [58, 72, 73]. Phylogenetic analyses have shown that these residues are either conserved or differ by only one or two residues in DPP4 of species that are permissive either *in vitro* or *in vivo*, including camel, macaque, marmoset, goat, pig, civet, and horse [58], but to have multiple variations in non-permissive species including mouse, hamster and ferret [73].

Other host cell mediators may also be involved along with DPP4 in MERS-CoV S protein binding and viral infection. In a recent virus overlay protein binding assay (VOPBA) study, the carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) was identified as a another MERS-CoV cell surface binding target which interacts with the S protein in cell culture

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[74]. While over-expression of CEACAM5 could not independently support MERS-CoV entry into non-permissive cells, it did enhance viral attachment, while in permissive cells CEACAM5 over-expression enhanced viral entry in conjunction with DPP4 [74]. MERS-CoV has also been shown to bind with high specificity but low affinity to sialic acid (Sia) in a hemagglutination assay with human erythrocytes and intact virus [75]. The S1 domain or its S1A subdomain expressed on nanoparticles could bind Sia-dependently to human erythrocytes or mucin, while Sia depletion on the surface of Calu-3 human airway cells reduced MERS-CoV viral entry [75]. Thus in addition to DPP4 expression, Sia may also contribute to MERS-CoV host range and tissue tropism.

Pathogenesis and immunity

Infection routes

The human respiratory tract is the primary target for infection by MERS-CoV [76, 77]. DPP4-expressing bronchial epithelial cells, bronchiolar epithelial cells, alveolar epithelial cells and the endothelial cells of pulmonary vessels have all been found to be infected by the virus in *ex vivo* human lung tissue [54, 59, 68, 70, 71, 76-78]. The human intestinal tract has been recently proposed to be an alternative route for MERS-CoV infection [79]. Human primary intestinal epithelial cells, small intestine explants, and intestinal organoids have all been shown to be susceptible to MERS-CoV infection and replication, while enteric MERS-CoV has been identified in clinical patient stool samples [79]. In DPP4-transgenic mice, direct intragastri inoculation with MERS-CoV resulted in lethal infection while histology demonstrated the presence of enteric infection in all inoculated mice, with development of sequential respiratory

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infection [79]. MERS-CoV can target both the innate and adaptive human immune responses in a number of direct and indirect ways. A feature of MERS-CoV infection spread is the occurrence of nosocomial outbreaks. In a recent outbreak which occurred in May/June, 2017, there were 44 reported MERS-CoV cases from three simultaneous clusters in three different healthcare facilities in Riyadh; eleven cases were fatal [81]. This outbreak highlights the need to develop rapid point-of-care testing to enable emergency room healthcare staff to rapidly identify MERS-CoV cases as the outbreak was the result of delay in diagnosis of MERS-CoV in a patient who presented with acute renal failure and who directly exposed one hundred twenty contacts including healthcare workers and other patients during fourteen hours spent in the open area of the emergency department and two hemodialysis sessions [81].Hospital outbreaks, the fact that up to 50% of MERS-CoV cases in Saudi Arabia have been classified as due to human-to-human transmission through contact with asymptomatic or symptomatic individuals and the difficulty inherent in distinguishing the clinical features of MERS-CoV infection from other respiratory tract infections further highlights the importance of specific point-of-care testing and high degree of clinical awareness among clinical staff in Saudi Arabia [82].

Innate immune response: interferon

Detection of positive-stranded RNA viruses such as MERS-CoV by the host innate immune system depends on recognition of pathogen-associated molecular patterns (PAMPs) by host pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) [80]. An important PAMP relevant to MERS-CoV is viral double-stranded (ds)-RNA. The host cell innate immune response to ds-RNA involves induction of type I interferon (IFN) expression via the RIG-1-like

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helicases including Rig-1 and MDA-5, as well as other activities including activation of protein kinase R (PKR), which reduces translation in the infected host cell, and activation of the 2',5'-oligoadenylate synthetase (OAS)/RNaseL pathway, which can degrade viral RNA [80, 81]. Importantly, while MERS-CoV is significantly more susceptible to type I interferon (IFN)-mediated innate immune responses than SARS-CoV, it also has strategies for evading these responses. In common with other coronaviruses, the MERS-CoV nsp3 PLpro removes ubiquitin (Ub) (deubiquitination; DUB), and interferon-stimulated gene 15 (ISG15) (deISGylation) from host cell proteins, which in turn blocks production of IFN-β and hence reduces type-1 interferon responses in cell line studies [82, 83]. MERS-CoV nsp15, which contains an endonuclease (EndoU) activity, has also been recently shown in primary cell lines and in macrophages to reduce early innate immune responses by inhibition of MDA-5, PKR and OAS responses and IFN activation [84]. Nsp16, a viral 2'O-methyltransferase (2'O-MTase), has also been recently implicated in viral pathogenesis and type I- IFN inhibition in both primary human airway cell cultures and *in vivo* mouse models [85].

Meanwhile non-structural protein NS3, NS4a, NS4b and NS5, as well as the structural M protein, have been implicated in IFN antagonism and inhibition of the innate immune response in cell culture studies [86-93]. Lack of homology between MERS-CoV and SARS-CoV in their accessory ORF-3, 4a, 4b and 5 genes highlights the fact that immune defence mechanisms may differ between the viruses. Deletion of MERS-CoV ORF-3 to 5 has been shown both *in vitro* and *in vivo* mouse models to impact on viral replication and pathogenesis via dysregulation of host cell responses, including increased activation of the type-1 IFN pathway and induction of inflammatory responses [86]. ORF5 has been shown to partially modulate the inflammation-associated NF-κB transcription factor [86]. The ORF4b-encoded NS4b protein has been shown

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in cell culture studies to inhibit IFN- and NF-κB- mediated signaling, IFN-β production and the (OAS)/RNaseL pathway [87-89]. Presence of NS4b in MERS-CoVinfected cells results in tethering of NF-κB in the cytoplasm while NS4a is located in the nucleus [90]. However in the absence of NS4b, or in the presence of mutant NS4b lacking a a nuclear localization signal (NLS), NF-κB can translocate to the nucleus and induce pro-inflammatory cytokine expression [90]. NS4b-induced NF-κB translocation inhibition appears to be mediated by its binding to karyopherin-α4 (KPNA4), a protein essential for NF-κB nuclear translocation [90]. Binding of NS4b to KPNA4 during infection inhibited its interaction with the NF-κB-p65 subunit. NS4a is potentially particularly potent in IFN-inhibition as it targets both IFN-β production and signaling via interferon-sensitive response element (ISRE) promoter elements [91]. NS4a-mediated inhibition of IFN production has been linked *in vitro* to its binding to the host cell ds-RNA-binding protein, interferon-inducible double-stranded RNA-dependent protein kinase activator A (PACT), which is a critical innate immune mediator responsible for activation of Rig-1 and MDA-5 and hence type 1-IFN in response to coronavirus infection [92]. This is linked to NS4a-mediated inhibition of the PKR-induced stress response, as PACT is a PKR-associated protein [93]. NS4a is a ds-RNA binding protein and hence can effectively mask the viral ds-RNA PAMP from the host innate immune response [94].

Innate immune response: cellular targeting

MERS-CoV virus infects and replicates in human macrophages -including alveolar macrophages- and can induce pro-inflammatory and chemotactic cytokines and chemokines expression from the infected macrophages [95, 96]. Binding and infection of MERS-CoV is

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supported by expression of DPP4 receptor on alveolar macrophages [97]. Levels of DPP4 are higher on alveolar macrophages, as well as on alveolar epithelial cells, in individuals with pre-existing pulmonary disease such as cystic fibrosis or chronic obstructive pulmonary disease, which could predispose them to MERS-CoV morbidity and mortality [97]. In human monocyte-derived macrophages (MDMs), MERS-CoV productive infection did not induce expression of antiviral IFN-α or IFN-β, but induced similar levels of interleukin (IL)-6 and tumour necrosis factor (TNF)-α to SARS-CoV, and significantly higher levels of other proinflammatory cytokines including IL-12 and IFN-γ, and chemokines including IP-10/CXCL-10, MCP-1/CCL-2, MIP-1α/CCL-3, RANTES/CCL-5, and IL-8 [96]. This could contribute to the level of pulmonary inflammation and tissue damage associated with MERS-CoV induced progressive pneumonia. On the other hand, recent studies in differentiated THP-1 macrophages infected with lentiviral particles pseudotyped with MERS-CoV S protein suggested that macrophage responses including IL-6 and TNF-α production were reduced, while LPS-induced production of the immunosuppressive IL-10 was increased [98]. This increase in IL-10 production was mediated by DPP4 binding and activation of IRAK-M, a negative regulator of TLR signaling and the transcriptional repressor PPARγ [98]. These results suggest that MERS-CoV may employ IRAK-M and PPARγ to evade destruction by macrophages.

In vitro studies on antigen-presenting cells (APCs) have shown that human plasmacytoid dendritic cells (pDCs) could be infected by MERS-CoV and that unlike B cells, macrophages, or monocyte-derived dendritic cells (MDDCs) they secreted type I- and type III- IFNs upon MERS-CoV infection [99]. This was accompanied by initial steps of viral infection and replication, evidenced by increased N protein RNA in infected cells, but not by productive replication or

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viral amplification [99]. Recent studies suggested that while mature MDDCs did not seem to be permissive to MERS-CoV infection, immature MDDCs were permissive but, unlike with macrophages, infection *in vitro* did not result in up-regulation of proinflammatory cytokine and chemokine production [100]. As dendritic cells enter peripheral tissues and carry antigens to lymphoid tissues, it has been suggested that they may contribute to MERS-CoV dissemination by acting as vehicles, possibly explaining the isolation of MERS-CoV from specimens other than respiratory tract samples such as blood, stool, and urine from MERS-CoV infected patients [101, 102].

Adaptive immune response

In one cell culture study, MERS-CoV but not SARS-CoV could efficiently infect human primary T cells, including cells from peripheral blood, spleen and tonsils [103]. CD4 T cells appeared to be more susceptible than CD8 T cells, and infection resulted in DPP4 receptor down-regulation and in T cell apoptosis by both extrinsic and intrinsic pathways [103]. Spleen and tonsil cells were apparently vulnerable to a higher degree of infection and apoptosis than peripheral blood cells [103]. Infection of common marmosets with MERS-CoV resulted in dissemination of virus to the spleen and infection of T cells *in vivo* [103]. Results of a recent study on a transgenic mouse model expressing human DPP4 (hDPP4) suggested that depletion of CD8 T cells could actually protect from MERS-CoV-induced pathology and symptoms, whereas depletion of macrophages exacerbated the pathology and symptoms [104]. Meanwhile recent *in vitro* studies suggested that, in common with H5N1-VN1203 influenza virus, MERS-CoV can attempt to evade the adaptive immune response by down-regulation of antigen-presentation gene

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expression, mediated by epigenetic mechanisms [105]. Down-regulated genes in the human airway epithelial cell line Calu3 included HLA-A, -B, or –C, whose expression was increased in the presence of SARS-CoV infection, as well as transcription factors (CTIIA) and genes expressing elments of the antigen processing machinery (TAP2 and PDIA3). HLA-A, -B, or –C peptides were also decreased by MERS-CoV infection, although H5N1-VN1203 reduced only HLA-A and-C peptides. In the case of MERS-CoV the major epigenetic mechanism appeared to be DNA methylation whereas H5N1-VN1203 employed a number of mechanisms [105]. Results from use of mutant viruses suggested that both host and viral processes were involved in the antigen presentation down-regulation, although this conclusion awaits definitive data [105]. In terms of humoral responses to MERS-CoV, the S protein has been shown to be the most immunogenic MERS-CoV antigen and is central to neutralizing antibody and T cell responses to MERS-CoV [106]. As a result, the S protein is the target of a number of proposed MERS-CoV vaccines, which we have recently extensively reviewed [107] and which are considered in more detail below.

Case definition

In the light of the pathogenicity of MERS-CoV, its ability to potentially evade the immune system, and its high mortality rate, it is vital that accurate case definition criteria are established and updated as knowledge of the virus expands. This is of particular concern in KSA, which remains the site of the greatest number of cases. The WHO and the CDC regularly update case definitions in order to help healthcare professionals in recognition and classification of cases. Case definitions categorize patients into either confirmed or probable cases.

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Confirmed Case

Both WHO and the CDC define a confirmed case as a patient with a laboratory confirmation of MERS-CoV regardless of clinical presentation [108-110]. Laboratory confirmation as currently defined can be via detection of viral nucleic acid or serology. The bases for WHO and CDC definitions are shown in Table 1. Viral nucleic acid confirmation can be either by positive results for nucleic acid amplification assays (NAAT), for example reverse transcription polymerase chain reaction (RT-PCR) directed against a minimum of two specific genomic targets (either upstream of the E protein gene (upE) and ORF1a, ORF1b or N gene), or against a single positive target with sequencing of a second target, preferably the RNA-dependent RNA polymerase (RdRp; nsp12) or N genes [108-113]. In the USA, an Emergency Use Authorization (EUA) was issued by the FDA to authorize the use of the WHO-approved RealStar® MERS-CoV RT-PCR Kit, as there is currently no FDA-cleared/approved test available for MERS-CoV testing in the USA [110, 112,115,116]. For serology, WHO case confirmation requires demonstration of sero-conversion in two samples, ideally taken at least fourteen days apart, by a screening test including enzyme-linked immunosorbent assay (ELISA) or immunofluorescence assay (IFA) and a neutralization assay for confirmation [108, 109]. For the CDC, a two-phase approach is also adopted, involving one screening test (ELISA) and two confirmatory tests (IFA, microneutralization) to detect MERS-CoV antibodies (Table 1) [110]. The CDC specifies that serology tests are for surveillance or investigational purposes rather than for diagnosis.

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Probable case

There are different possible definitions of probable cases according to WHO criteria, all of which involve a febrile patient with respiratory disease, either with evidence of pulmonary parenchymal disease (e.g. pneumonia or Acute Respiratory Distress Syndrome (ARDS)); or of any severity, along with other criteria shown in Table 1, including residence or recent travel in the Middle East, or a direct epidemiological link to a laboratory-confirmed case [108]. The CDC criteria for a probable case or person under investigation (PUI) are also shown in Table 1, and again involve patients who are febrile and/or have evidence of respiratory illness (acute or otherwise), along with criteria including recent travel, or being in a healthcare facility, in or near the Arabian Peninsula, or close contact with a laboratory-confirmed case [110].

Diagnosis

Detection of viral RNA

Several assays for detection of MERS-CoV RNA have been developed using real-time PCR. Corman and colleagues introduced assays that target the region upstream of the E protein (upE), ORF1b, and ORF1a (Figure 2) [112-114]. The high sensitivity of RT-PCR (upE) and RT-PCR (ORF1a) compared to ORF1b rendered them valuable options for screening of MERS-CoV RNA [109, 112-114]. The CDC validated a suggested alternate testing strategy based on screening of one N gene signature sequence (N2) combined with upE testing for enhanced sensitivity, and a second N gene signature (N3) for confirmation of positive tests [109, 115]. Corman and colleagues ultimately developed the upE and ORF1a real-time PCRs into the RealStar® MERS-

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CoV RT-PCR Kit, which was clinically validated using samples of a German MERS-CoV case and respiratory samples from other respiratory disease patients [113]. The RealStar® MERS-CoV RT-PCR Kit has been WHO approved and granted an FDA Emergency Use Authorization (EUA) in the United States [108, 116]. The same study group introduced RT –PCR assays for sequencing in RdRp gene (RdRpSeq assay) and in the N gene (NSeq assay) now recommended by the WHO as confirmatory tests [114].

Although the RealStar® MERS-CoV RT-PCR Kit is the only upE and ORF1a-detecting kit approved by WHO and the Conformité Européenne (CE), and permitted FDA Emergency Use Authorization (EUA), several MERS-CoV RNA detection kits have been developed. For example, in a recent study six commercially available real-time RT-PCR MERS-CoV RNA detection kits based on upE and ORF1a were analysed and clinically validated on nasopharyngeal swabs taken during the 2015 outbreak in Korea [117]. Results suggested that sensitivity and specifity of all of these assay systems would be sufficient for confirmation of MERS-CoV infection, although use of appropriate internal controls would be important in specimens where PCR inhibition is an issue [117]. In another recent study, a MERS-CoV r-gene ® 32 rRT-PCR assay 33 (bioMérieux, France), targeting the S protein gene, was shown to have comparable accuracy to the WHO recommended in-house rRT-PCR assay targeting upE and ORF1a in a set of 130 respiratory samples [118]. Loop-mediated isothermal amplification RT PCR assays (RT-LAMP) have also been developed for field use given their rapid results with high sensitivity profiles. They require minimal instrumentation, thus they can also be used for portable point-of-care testing [119]. Other assays targeting small RNA molecules (leader sequences) have good sensitivity profiles [120].

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Respiratory specimens -nasopharyngeal swabs, sputum, tracheal aspirate and bronchoalveolar lavage (BAL) are commonly used for detection of viral RNA. Results of tests on patients from KSA and elsewhere comparing the viral load and genomic fraction yield among respiratory specimens obtained from different sites have shown that lower respiratory samples (e.g. tracheal aspirate and bronchoalveolar lavage) yield significantly higher viral loads and genomic fractions compared with upper respiratory tract samples [121-123]. In the context of MERS-CoV testing in RSA and elsewhere, this means that WHO recommends that lower respiratory tract specimens should be collected whenever possible [109]. However, a case series from KSA also showed that there is value in collecting and testing upper respiratory tract specimens such as nasopharyngeal/oropharyngeal swabs so long as nasopharyngeal swabs are taken from the nasopharynx following WHO guidelines, not just from the nostril, and that nasopharyngeal and oropharyngeal swabs are placed in the same tube [109, 124, 125]. Thus WHO recommends that when it is possible, both upper and lower respiratory tract specimens should be collected, while speciments from sites outside the respiratory tract should not be used for routine diagnostic testing [109]. WHO further recommends that samples should be collected for symptomatic patients for NAAT testing on presentation, followed by repeat sequential sampling every two-four days, until results are negative on two sequential samples to confirm viral clearance [109].

Antigen detecting tests

For diagnosis of MERS-CoV in camels, which is highly relevant in the KSA context, molecular testing based on NAAT is not always a feasible option largely due to the expense and impracticality of carrying out large numbers of tests on animal herds in a timely manner.

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Recognition of the need for a relatively affordable test for use in diagnosis in camels which would also be sensitive and specific led to the development of an immunochromatographic assay (ICA) for the rapid and direct qualitative detection of MERS-CoV antigen [126]. The test was based on use of monoclonal antibodies for detection of N protein at room temperature and was 93.9% and 100% sensitive and specific respectively in relation to UpE and ORF1a real-time RT-PCR-based detection in a study on 571 camel nasal swabs [126]. Another N protein antigen-detection test, this time capture enzyme-linked immunosorbent assay (ELISA) based on two N protein-specific monoclonal antibodies (MAbs) has also been developed and shown to be 100% specific in testing of a series of 129 nasopharyngeal aspirates known to be positive for various respiratory viruses [127]. Such a sensitive and specific ELISA test would be feasible for MERS-CoV detection both in clinical samples, in particular for point-of-care testing, and in dromedaries and other animals, and may have particular utility in field studies in KSA and elsewhere in the Middle East and in mass gathering contexts such as Hajj [127, 128]. The relative affordability and lower resource-intensiveness would give it an advantage over RT-PCR based methods in these types of contexts. These antigen-detection tests require further refinement as they have not yet been completely validated for use in human samples and are usually not as sensitive as NAAT, which has limited their use to date [128].

Detecting human immune response

Several serological assays have been developed for detection of anti MERS-CoV antibodies, notably against N protein or S protein. While NAAT-based testing is the gold standard for MERS-CoV diagnosis, serological assays have some advantages such as a less restricted time

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frame for antibody versus viral RNA detection, easier application in the field during an outbreak situation, and more economical application in animal testing [129-131]. However, potential pitfalls of serological testing were exposed during the SARS-CoV outbreak, including the possibility of cross-reactivity to antigens from other coronaviruses [129]. A recent assessment of the utility of ELISA-based detection of MERS-CoV S1 IgG compared to viral RNA detection was carried out on nasopharyngeal

swab specimens from 174 patients in a hospital in Riyadh, between January 2016 and December 2016, during which a MERS-CoV

outbreak occurred [132]. While MERS-CoV RNA was detected in thirty patient samples, only six samples were positive by serological testing, including four who were recently MERS-CoV RNA-positive and two who were MERS-CoV RNA-negative. This lack of correlation between NAAT and serological results suggested that MERS-CoV-IgG testing may not be appropriate for diagnosis of acute infection, estimation of outbreak prevalence, or determination of disease severity [132]. Nevertheless, serological testing remains one of the approved methods for MERS-CoV case confirmation by both WHO and CDC [108-110]. One recent validation study suggested that combination of indirect MERS-CoV N and S ELISAs in combination with confirmation by microneutralization assay can improve overall detection sensitivity and specificity [130]. Another recent innovation suggests the possibility of using competitive ELISA rather than IgG/IgM ELISAs that rely on a species-specific secondary antibody [131]. In this case, labeled monoclonal antibodies (MAb) against MERS-CoV S protein were developed and used to compete with test serum antibodies for target epitopes, allowing detection of antibodies in a species-independent manner [131]. The competitive ELISA successfully detected MERS-

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CoV-specific antibodies in sera from infected rats and rabbits immunized with MERS-CoV S protein, and the test was also validated on sera from 66 Ethiopian dromedary camels in compariton to a neutralization test, giving sensitivity and specificity of 98% and 100%, respectively. These results suggest that competitive ELISA might be a useful serological test in epidemiological investigations in KSA and elsewhere in the Middle East [131]. WHO recommends that for serology testing in symptomatic patients, paired samples should be collected within the first week of illness and the second ideally three to four weeks later [109].

Clinical manifestations of MERS-CoV

Incubation period

Variable incubation periods for MERS-CoV have been calculated in studies from different countries [124, 133, 134]. A median of 5.2 days (95% CI 1.9–14.7 days) (range 2-13 days) was reported in one study of 47 laboratory confirmed MERS-CoV cases in KSA [124]. Investigators in France reported a longer incubation period of between nine and twelve days [133]. Early during the 2015 Korean outbreak, the median incubation period of MER-CoV was found to be 6.3 days [134]. Accommodating the range of these observations, it is currently recommended that people who have contact with confirmed cases must be evaluated for a full fourteen days from day of contact for any symptoms or signs suggestive of MERS-CoV.

Clinical features

The clinical spectrum of MERS-CoV infection ranges from mild respiratory illness to severe disease with severe acute respiratory distress syndrome, septic shock and multi-organ failure

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[135, 136]. Most reported cases do run a severe clinical course. Fever and cough are the predominant symptoms in symptomatic cases. Early in the history of MERS-CoV, analyzing the clinical presentation among 47 confirmed cases in KSA showed fever with temperature above 38^0C in almost 98% of the patients [124]. Fever was also found to be a predictive factor for progression of pneumonia in a study following up the clinical course of five confirmed MERS-CoV cases during the Korean outbreak .The progression of pneumonia appeared to slow or even stop after fever subsided [137].

Cough was present in 83% of infected individuals in the KSA study of 47 cases, while gastrointestinal (GI) symptoms including abdominal pain, vomiting and diarrhea were also reported in a significant number of patients included in this study [124]. GI symptoms were also reported in 12.9 % of the 186 cases involved in the South Korean MERS-CoV outbreak [138]. Arabi et al. reported the clinical manifestation in twelve cases from two hospitals in KSA, showing that symptoms could be attributed to the lower respiratory tract [139]. Upper respiratory tract symptoms, such as rhinorrhea and sore throat, were found to be uncommon [139]. Renal complications are well known to occur in MERS-CoV infection. The first ever reported case suffered from acute kidney injury [1]. Proteinuria, hematuria and acute kidney injury (AKI) were noted in a retrospective study of thirty MERS-CoV cases in South Korea, in which diabetes, AKI, and the application of a continuous renal replacement therapy (CRRT) were observed to be risk factors for MERS-CoV-related mortality [140]. Seizures, DIC, and rhabdomyolysis were also reported as complications related to MERS-CoV infection in a study of seventy patients in a single centre in KSA [141].

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About 75% of confirmed MERS-CoV infections occur in patients with comorbid disease. Frequent comorbid conditions seen in patients with MERS-CoV infection are diabetes mellitus, obesity, chronic kidney disease, cardiac diseases, and hypertension, as well as respiratory diseases including asthma and COPD [124, 138-146 149]. Disease severity and mortality risk is impacted by comorbidities and age. For example, in one study age > 50 years and diabetes were significantly associated with mortality and all patients in this series requiring renal replacement therapy died [141]. Age > 65 years was significantly associated with mortality in another single centre study in KSA [142]. A study analyzing publicly available data from KSA reported that pre-existing lung disease appeared not to be a significant risk factor for severity and mortality, however this study did not use multivariate risk modelling [143, 144]. Other case-control and retrospective observational studies from both KSA and Korea have suggested that smoking and/or comorbid respiratory diseases are significant risk factors for MERS-CoV-related mortality [138, 145-148]. Higher levels of DPP4 mRNA and protein in lung tissues of smokers and COPD patients compared to never-smokers may predispose these individuals to MERS-CoV infection [71]. Systematic review and meta-analysis has shown that obesity is present in 16% of MERS-CoV cases and may influence disease severity as with other respiratory conditions [149]. Asymptomatic MERS-CoV infection also occurs in household contacts, healthcare workers and people who have contact with dromedary camel [135, 136].

Children

Although older age has been confirmed as a risk factor for MERS-CoV infection and mortality, it is not only a disease of adults but also occurs in children, albeit rarely [149-151]. 80.6% of the

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31 paediatric cases reported between June 2012 and April 2016 were from KSA, with a mean age of 9.8 ± 5.4 years, and they were most commonly infected due to household contacts [149]. Mortality is lower in children than in adults and is commonly associated with underlying comorbid conditions In one study from KSA, MERS-CoV was detected in eleven pediatric patients ranging in age from 2 to 16 years [150]. While nine of the eleven were asymptomatic and were detected during a contacts investigation on older patients, two symptomatic patients had underlying conditions and one died [150]. Meanwhile a nine- month old infant with infantile nephrotic syndrome being treated with prednisolone died in the PICU of a Riyadh hospital as result of MERS-CoV infection and his clinical course was complicated by acute renal failure [151].

Pregnancy

Information is limited on the impact of MERS-CoV in pregnancy, but in common with other severe respiratory viral infections the impact appears to be severe both maternally and perinatally. In one study on five pregnant women in KSA infected with MERS-CoV, all five needed ICU admission [152]. While two recovered and went on to deliver healthy infants, one of the mothers died due to multiple organ failure related to her infection after delivering a healthy infant at 38 weeks gestation, another died due to complications of her infection a few weeks after her infant was surgically delivered at 24 weeks and died after 4 hours of life, and one infant was stillborn at 34 weeks [152]. One case of a second trimester stillbirth during a MERS-CoV outbreak in Jordan was attributed to MERS-CoV on the basis of maternal exposure history and serological testing [153]. In another case a woman at 32 weesk gestation died due to MERS-

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CoV-related complications including ARDS and septic shock after delivering a healthy infant by caesarean section [154].

Laboratory and radiological manifestation

In a study of 47 cases of MERS-CoV infection in KSA, 14% had leukopenia, 34% had lymphopenia and 11% had lymphocytosis, while thrombocytopenia was present in 36% of cases [124]. Lymphocytopenia and thrombocytopenia have also been detected in other studies, including among members of a KSA MERS-CoV family cluster [135]. Impaired liver function findings are a feature of MERS-CoV infection, including the 47-case study which revealed raised concentrations of lactate dehydrogenase (49% of patients), alanine aminotransferase (11% of patients) and aspartate amino transferase (15% of patients), although other liver function tests were normal [124] and in a retrospective study of 29 confirmed cases of MERS-CoV infections from March to May 2014 at two hospitals in the Al-Madinah region of KSA, in which elevated liver enzymes were observed in 50% of cases [155]. Elevation of urea and creatinine levels indicating renal impairment has also been widely observed, including in case series from KSA [139, 155]. Animal studies on human DPP4 (hDPP4)-expressing transgenic mouse models infected with MERS-CoV, while not entirely reflective of disease in humans, have shown multi-organ damage, including to liver and kidney as well as brain and spleen [156]. However, other studies on a hDPP4 transgenic mouse models have suggested that while infection with 10 LD50 of MERS-CoV resulted in persistent inflammatory infiltrates in the lungs and brain stems two and four days post-infection respectively, and focal infiltrates in the liver, there was no definite pathology in other organs [157]. Recently, post-mortem histopathological findings on a 33-year-

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old male T lymphoma patient who contracted MERS-CoV were reported [158]. Histopathological examination of tissue needle biopsies from multiple sites inlcuding brain, heart, lung, liver, kidney and skeletal muscle showed evidence of virally induced pulmonary and extrapulmonary pathological changes. These included necrotising pneumonia, pulmonary diffuse alveolar damage, acute kidney injury, hepatitis and myositis with muscle atrophic changes, however there were no notable findings for brain and heart. For the first time, ultrastructural viral particles were shown in renal cells, as well in pneumocytes, pulmonary macrophages and macrophages infiltrating the skeletal muscles [158]. A wide range of radiological features have been shown on chest X-rays of MERS-CoV infected patients including ground glass opacification, consolidation (either patchy or confluent), reticular opacities, nodular opacities and reticulo-nodular infiltrates [124,159,160]. Use of serial chest radiographs can be used to classify disease progression into four types ranging from type 1, in which initial radiographic deterioration is followed by improvement, all the way up to type 4, where there is progressive radiographic deterioration [159]. Importantly, in a study of 55 adult patients with acute MERS-CoV infection, chest radiographic score was shown to be an independent predictor of mortality, with mean chest radiographic score significantly higher in patients who died than in those who survived. Pneumothorax, bilateral pleural effusion and type 4 radiographic progression were all significantly higher in patients who died [160]. Bilateral pleural effusion has also been identified as an independent predictor of short-term mortality for community-acquired pneumonia but not SARS [161, 162]. Similar to the radiographic findings, the more sensitive computed tomography (CT) scans also showed ground glass opacity (53% of patients), or consolidation (20% of patients), or both together (33% of patients), as well as pleural effusion (33%) and interlobular

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thickening (26%) within a week of infection [159, 160]. As disease progressed, bronchial abnormalities and organizing pneumonia emerged on CT scans [159, 160].

Source and Transmission

Bats

As mentioned above, it has been assumed that bats are the likely main MERS-CoV mammalian source reservoir, as with other coronaviruses, because sequences related to the MERS-CoV were found in samples taken from different bat species (Figure 1) [49, 163]. The HKU4 bat coronavirus RBD in the S protein shares high sequence identity to MERS-CoV and pseudotyped viruses embedding HKU4 S protein can bind human DPP4 and enter cells *in vitro* [164]. HKU4 S protein binds human DPP4 with only low affinity, however introduction of two mutations, N762A and S746R, into the bat S gene enabled HKU4 to bind with higher affinity and more efficiently enter human cells [65]. These mutations are part of human protease motifs in the S1/S2 junction in MERS-CoV and thus facilitate S protein cleavage and human cell infection and may have been instrumental in transmission of MERS-CoV from bats to humans [65]. However, positing that bats are a direct source of MERS-CoV human infections is difficult given the infrequent contact of human with bats. In a study in KSA, it was found that samples from only one bat found near the home of a MERS-CoV infected patient among 823 samples collected from different bat species had total nucleotide identity with MERS-CoV sequence obtained from the patient [163].

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Camels

There is growing evidence that dromedary camels act as the source of MERS-CoV. Dromedary camels' sera from different parts of the world –especially from the Middle East and broad areas of Africa, including Nigeria, Tunisia, Egypt and Ethiopia – have tested positive for anti-MERS-CoV antibodies [165-172]. Serological studies on camels in Africa and the Middle East within the last 30 years suggest that MERS-CoV was circulating among camels for decades before it was first documented in human beings in 2012 [167,170]. All Canary Islands dromedary camels which have positive serological evidence of MERS-CoV infection were originally imported from Africa twenty years ago or more [173]. However, there are lower than expected levels of MERS-CoV human infection in Africa, which suggests there may be under-reporting of human cases, possibly related to limited resources for testing. Extension of sero-surveys among the human population would help in furthering understanding of the extent of levels of MERS-CoV infection in Africa. In one study use of ELISA, IFA and ppNT showed that there was evidence for unrecorded cases of human MERS-CoV in Kenya, similar to previous reports in KSA [169,174].

There is some genetic evidence to suggest transmission of MERS-CoV occurs from camels to humans. During one outbreak in Qatar, MERS-CoV sequences obtained from nasopharyngeal swabs from two infected human cases residing on a farm and from three seropositive camels within the same farm were found to be identical [175]. In another case in Jeddah in KSA, a shared unique single nucleotide polymorphism (SNP) signature was found in both a MERS CoV patient and a MERS-CoV-carrying dromedary camel for which he had been caring [176, 177]. Comparison of the sequence of the full genome of the MERS-CoV variant associated with the

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Korean outbreak showed 99.96 – 99.98% similarity with the full genome of CoVs obtained from a camel in Riyadh, Saudi Arabia [178]. In this study RT-PCR testing was carried out on nasal swab samples from 1309 camels. Coronaviruses were identified in 25.3% of samples and three different lineages of coronaviruses, including MERS-CoV, betacoronavirus 1 (betacoronavirus, group A); and human CoV 229E (alphacoronavirus) were found to be circulating among dromedary camels [178]. The study showed camels aged less than one year have the highest rate of infection with coronaviruses compared to older camels [178]. The identification of camels as the probable natural zoonotic source for human infection with MERS-CoV has economic implications for countries of the Middle East, including KSA, given the importance of the camel trade between the Middle East and Africa [179].

Other animals

There was no evidence of MERS CoV upon testing of other animals such as sheep, goats, cattle, or water buffalo, although results of one study suggests alpaca may be a possible viral reservoir [180-182]. Detection of MERS-CoV in this New World camelid raises the possibility of zoonotic spread of MERS CoV to areas where alpacas are farmed, including South America and the United States [182].

Human-to-human transmission

Strong evidence of human to human transmission was obtained from epidemiological and genomic studies investigating clustering of cases in hospitals and among household contacts [9,

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34, 135]. Investigating a hospital outbreak in the city of Al-Ahsa in the Eastern Province of KSA revealed that all isolates of MERS-CoV infecting the 23 patients were from one monophyletic lineage and 91.3% of cases occurred as a result of person-to-person contact [9]. Human-to-human transmission was also responsible for most of the MERS-CoV cases reported during the outbreak that occurred in Jeddah in 2014 [10]. The majority of cases were attributable to contact with a health care facility, other patients, or both, highlighting the role of healthcare facilities in human-to-human transmission that also arose in subsequent outbreaks, including hospital outbreaks in Riyadh and the 2015 outbreak in Korea [10-15]. As explained above, healthcare facility human-to-human transmission has been associated with defective or inadequate infection prevention and control measures [20-25]. The infection tends to be milder in secondary cases, in which a patient is infected as a result of close contact with a primary source, and can even be asymptomatic. The number of cases who get infected from confirmed cases is low; the rate of transmission among household contacts has been calculated to be around 5% in one study done in KSA in 2014 [183] .However, epidemiological analysis of the Korean hospital outbreak in 2015 showed that the fatality rate was not significantly different between primary cases and subsequent generations [184]. This outbreak highlighted the danger posed by a combination of circumstances including a primary source travelling from the Middle East, infection among secondary and tertiary contacts due to movement of infected individuals between healthcare facilities, and inadequate infection prevention and control measures [3-5].

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Epidemic potential

From the data available to date, MERS-CoV has failed to demonstrate the potential to result in an epidemic. A study based on Bayesian analysis was carried out to estimate the basic MERS-CoV reproduction number (R0), which represents the number

of secondary cases for each index case in a fully susceptible population [185]. Epidemic potential is achieved when R0 is above 1, . R0 for MERS-CoV was estimated to be between 0·60 and 0·69, however these calculations were based on data obtained in June 2013 in advance of many of the important outbreaks and so may be underestimated [185]. There is in any case no room for complacency, as the potential is always present for viral mutations that could increase zoonotic or human-to-human transmissibility. Thus development of effective directed therapies remains a top priority.

Vaccination and therapy

Current and potential treatments

In 2015, Public Health England (PHE) and the WHO International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC-WHO) published a position paper on MERS-CoV therapies [186]. They concluded that there was a positive benefit.versus risk balance for convalescent plasma, lopinavir/ritonavir, interferons and monoclonal/polyclonal antibodies, but a negative balance for ribavirin monotherapy or corticosteroids [186]. It was deemed that there was insufficient information available for interferon/ribavirin combination therapy, nitazoxanide and chloroquine [186]. Currently, no specific evidence-based therapy or vaccine for MERS-CoV

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is available. We have recently extensively reviewed candidate MERS-CoV therapies and vaccines [107]. Table 2 shows a summary of current and proposed therapies and vaccines, including targets, advantages and disadvantages, updated to include some potential agents that have emerged since the publication of our review [107, 197, 206, 208, 211, 212, 219, 233]. Development of a targeted anti- MERS-CoV therapy and availability of effective vaccines would require coordinated efforts to carry out properly controlled and organised clinical trials. This would be of particular importance for KSA, given the relatively major impact of MERS-CoV there; availability of reliable directed therapies and the possibility of either a prophylactic vaccine programme or a vaccine that could be rapidly available in the event of a major outbreak would be a major advantage in effectively tackling this disease.

The S protein and its binding to DPP4 is the target of many proposed direct MERS-CoV therapies, including a large number of antibodies which target the interaction either from the viral or the host side [187-198]. Monoclonal antibodies against the RBD of the S1 region have particularly strong neutralising capacity, although full-length S or S1 targeting antibodies may have greater potential in a vaccine context given their larger number of target epitopes and the reduced chance of escape mutations [187-198]. A fusion product in which truncated RBD (residues 377-588) was joined to the Fc portion of human IgG could bind human DPP4 and inhibit MERS-CoV infection *in vitro* in cell culture and *in vivo* in infected mice (Table 2) [199]. *In vivo* studies on mice have also indicated that intranasal administration of this fusion product induced comparable sustained IgG humoral responses to subcutaneous injection, and superior cellular immune responses and local mucosal responses in lungs [200, 201]. Use of an adjuvant, particularly MF59 or AddaVax, significantly improved both the humoral and T cell responses in subcutaneously immunized mice [202]. Recently, a high-yield CHO cell line capable of large-

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scale production of this S1 RBD-Fc fusion product was described, strengthening the possibility of sustainable manufacture and human testing for this potential vaccine antigen [203]. Another recent study showed that five recombinant RBDs incorporating mutations which arose in different MERS-CoV outbreaks or in camel strains could induce neutralizing antibody responses against several MERS-CoV pseudoviruses [204].

A particularly promising antibody candidate for MERS-CoV therapy is the human antibody LCA60 , as it targets both the N-terminal domain (NTD) and the RBD of S1 (Table 2) [195]. LCA60 was isolated from B cells of a MERS-CoV-infected human donor, and has been used to establish a stable CHO cell line from which clinical grade antibody is reliably available [195]; this type of ready availability would be of particular benefit in KSA for outbreak situations. It had both prophylactic and therapeutic activities against MERS-CoV infection in two transgenic mouse models, Ad5/hDPP4 and type I interferon receptor (IFNAR)- KO [195]. Another human anti-RBD antibody, 3B11-N, has shown promising results in a non-human primate model, i.e. rhesus monkeys infected with MERS-CoV, in which it prophylactically reduced lung pathology [196]. Recently a suite of potent MERS-CoV-neutralizing anti-S protein antibodies were derived from B cells of an infected patient, specifically from the first imported case in China [197]. Two of the antibodies in particular, MERS-GD27 and MERS-GD33, had potent and synergistic neutralizing *in vitro* activity against both pseudotyped and live MERS-CoV (Table 2) [197]. The two antibodies targeted different epitopes, with the MERS-GD27 epitope almost entirely overlapping the receptor binding site [197]. Thus there is a wide range of S protein directed antibodies and fusion products available for potential passive immunization strategies, but thus far they have not entered human clinical trials. Availability of monoclonal antibodies may be of particular use in outbreak situations, which continue to arise in KSA. Other potential S protein-

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targeting vaccine candidates include nanoparticles expressing full-length S protein [205] and active immunization strategies using vectors including modified vaccinia, adenoviruses or measles viruses or plasmids expressing full-length S protein as potential vaccine candidates, discussed in more detail in the next section [206-214].

Antiviral peptides that target the HR2 regions of the S protein and hence virus-host cell fusion have also been shown to have potential therapeutic activities in cell culture and transgenic animal studies [215-218]. The HR2 peptide HR2P, covering residues 1251–1286, reduced viral replication and fusion *in vitro* [215] while its analogue, HR2P-M2, blocked fusion even more potently *in vitro,* and inhibited pseudovirus infection by blocking 6HB bundle formation [216-218]. Convenient intranasal administration of HR2P-M2 *in vivo* to Ad5/hDPP4 transgenic mice protected them from MERS-CoV infection, which was enhanced by co-administration of IFN-β [217]. Recently a synthetic protein named MERS-five-helix bundle (MERS-5HB) was derived from the 6HB bundle involved in MERS-CoV fusion and was shown to bind strongly to HR2P and to effectively inhibit pseudotyped MERS-CoV fusion and entry in *in vitro* studies (Table 2) [219]. This represents another potentially useful directed MERS-CoV therapeutic candidate.

At present, combined antiviral therapies tend to be used in patients who develop respiratory illness, based on experience with SARS-CoV therapy, for example pegylated interferon (IFN)-α, ribavirin, and/or lopinavir/ritonavir [141, 224-232]. While *in vitro* and animal studies suggested their potential efficacy, *in vivo* and clinical evidence is less well-established [141, 220-232]. Clinical studies have been mainly confined to case studies and case series, and retrospective analyses [224-232]. Thus there is a need for properly controlled clinical trials of IFN combination therapy in MERS-CoV, preferably early in the illness when it seems to be most

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effective,. These types of therapies function essentially by challenging the immune evasion tactics employed by the virus [141, 220-232]. Recently, results of a study using *in vitro* and human *ex vivo* explant cultures suggested that a combination of IFN-α and cyclosporine had a synergistic effect on reduction of MERS-CoV replication, based on immunomodulation and induction of IFN-stimulated gene expression, suggesting that clinical trials may be warranted (Table 2) [233]. Corticosteroid treatment is also commonly used in treatment of critically ill MERS-CoV patients, despite the findings of the ISARIC-WHO group that its risks may outweigh benefits [186]. A recent marginal structural modelling study was carried out on data from 309 critically ill ICU patients with MERS-CoV, of whom 151 received corticosteroids, from fourteen KSA health facilities between September 2012 and October 2015 [234]. The results indicated that corticosteroid therapy was not associated with significantly different mortality outcomes when time-varying confounding effects such as worsening condition of the patient were considered, but that it was associated with delayed clearance of viral RNA. These findings suggest that bias in determining potentially harmful effects of therapies can emerge in observational studies if only the baseline characteristics rather than time-variant characteristics of the patients are considered and further highlight the need for properly controlled clinical trial data.

Another useful tactic would be to make use of therapies that have already been clinically approved for other purposes and for which there is a sound scientific rationale for possible use in MERS-CoV therapy. An example would be camostat, which is an inhibitor of TMPRSS2 [62, 235]. Camostat has been shown to block infection, viral spread and pathogenesis in a pathogenic mouse model of SARS-CoV and would be likely to have a similar inhibitory effect on MERS-CoV [235]. Camostat is already used clinically for treatment of chronic pancreatitis, and is thus a

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potentially safe and effective therapeutic option. Another TMPRSS2 inhibitor, nafamostat, has also been identified *in vitro* as a potent inhibitor of MERS-CoV S protein-mediated host-viral membrane fusion and is also already in clinical use as an FDA-approved anticoagulant [236]. In a screen of FDA-approved drugs, an inhibitor of cathepsin L called teicoplanin has been shown to block cytoplasmic entry of MERS-CoV, SARS-CoV and Ebola pseudoviruses [63, 237]. Teicoplanin is in current clinical use as an antibiotic for serious Gram-positive bacterial infections and its derivatives, including dalbavancin, oritavancin, and telavancin, also block cytoplasmic viral entry. While these therapies all target host proteases, another possibility is targeting of viral proteases. The nsp3 encoded PL(pro) activity, which mediates the initial processing of pp1a (Figure 2) [55-57], can be inhibited *in vitro* by the SARS-CoV PL(pro) inhibitors, 6-mercaptopurine (6MP) and 6-thioguanine (6TG) and by a commercial compound F2124–0890 (Life Chemicals) [238, 239]. The main MERS-CoV protease Mpro/3CLpro, encoded by nsp5 (Figure 2) can be targeted *in vitro* and *in vivo* by lopinavir, a protease inhibitor with activity against the SARS-CoV Mpro and which emerged in a screen of a library of 348 FDA-approved drugs as one of four compounds that inhibited MERS-CoV viral activity in a low micromolar range [240-242]. However, lopinavir clinical efficacy has not been convincingly established in MERS-CoV treatment as it has generally been used clinically in combination with IFN and data is only available from case studies and series. In marmosets infected with MERS-CoV, it gave favourable clinical outcomes and reduced mortality in combination with ritonavir [240].

Early treatment (within four-five days of symptoms onset) with convalescent plasma (or hyperimmune IV immunoglobulin (HVIG) from plasma of convalescent donors) has been associated with decreased viral load and reduced mortality for influenza and SARS-CoV

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infection, although the quality of studies for SARS-CoV has been uneven and there have been few adequate clinical trials [243-246]. The PHE and ISARIC-WHO position paper identified convalescent plasma as a potential treatment for MERS-CoV infection, however no clinical trial have yet been completed [186]. A clinical trial in KSA on safety and efficacy of convalescent plasma treatment for critically ill MERS-CoV patients was initiated in May 2014 and is still listed as active but not recruiting [247; ClinicalTrials.gov Identifier: NCT02190799]. It was due to report in June 2017 but in common with many convalescent plasma trials it has been affected by logistical and technical issues, such as availability both of sufficient donors and sufficient levels of MERS-CoV antibodies in the plasma that is collected [247, 248]. Clinical data is sparse on use of convalescent plasma in treatment of MERS-CoV and is confined to two case reports in which its role in patient recovery was unclear [249, 250]. Use in marmosets infected with MERS-CoV in a recent study indicated that while convalescent plasma treatment reduced signs of clinical disease, including reduced respiratory tract viral load, it did not induce a decrease in gross pathology [251]. Thus while convalescent plasma is a possible candidate MERS-CoV therapy, technical and logistical difficulties with its collection and preparation and uncertainty over the extent of its protective effects may undermine its potential usefulness.

Vaccines

Studies from KSA have suggested that while patients who survived MERS-CoV produced anti-MERS-CoV IgG and neutralizing antibodies, these antibody levels only weakly inversely correlated with lower respiratory tract (LRT) viral load and would be insufficient to eliminate LRT virus [252]. T cell responses to MERS-CoV infection are not yet well-understood, but in a recent study on 21 survivors of MERS-CoV in KSA, both CD4 and CD8 T cell responses

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developed in all of them [253]. MERS-CoV specific neutralizing antibody responses along with memory CD4 T cell but not CD8 T cell responses were shown to correlate with disease severity, while virus-specific CD8 T cell responses were observed in all MERS-CoV survivors, even when serological responses were not observed [253]. Robust CD8 T cell responses might therefore be important in early clearance of viral infection and hence antibody and CD4 T cell responses may not develop so strongly. Measurement of T cell responses along with antibodies may also give a more accurate estimate of disease prevalence. *In vitro* studies have shown that MERS-CoV infection down-regulates MHC and antigen presentation molecules via a methylation-based mechanism, which could have implications for both T cell and humoral adaptive immune responses [Menachery et al., 2018- old ref 105]. The combination of the apparent inadequacy of the humoral adaptive immune response to clear MERS-CoV and the high mortality rate associated with the disease point up the importance of vaccine development, particularly for the Middle East and KSA in particular. Induction of both antibody and T cell responses would be an important feature of a useful vaccine. WHO have issued guidelines on proposed MERS-CoV vaccines; they will consider prospective vaccines on a case-by-case basis [254]. WHO distinguished between vaccine types to be aimed at three different defined target populations, all of which have direct relevance in the Middle East. The three types are: dromedary camel vaccines designed to prevent camel-camel and camel-human transmission; prophylactic human vaccines for individuals who may be at long-term risk, for example healthcare workers and people working with potentially infected animals; and finally human vaccines which would be suitable for use in outbreaks [254]. WHO have defined preferred and minimally acceptable criteria for each vaccine type. These WHO guidelines are particularly welcome in the context of the difficulties that have prevailed in defining populations who should

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be targeted in a MERS-CoV vaccination program and/or in randomized clinical trials, especially given the current relatively low incidence of disease in humans, and the difficulties in developing suitable small animal models, depending on transduced or transgenic human DPP4-expressing mouse models [255].

As with therapy development, the S protein is the focus of many candidate vaccines [205-214]. Vectors including modified vaccinia virus Ankara (MVA), ad5 or ad41-type adenoviruses, measles virus, chimeric vesicular stomatitis virus (VSV) and chimpanzee adenovirus (ChAdOx1) have been successfully used to express MERS-CoV S protein and induce neutralizing antibodies in mice and in other animal models including camels and rhesus monkeys (Table 2) [205-214]. These virus vectors have the advantage of good safety profiles in humans. Production of candidate vaccines with potential for veterinary use in dromedary camels in order to reduce cross-species transmission is a welcome development in keeping with the WHO guidelines. These include an S protein-expressing MVA-based vaccine (MVA-MERS-S) which can a strong neutralizing antibody and cytotoxic T lymphocyte response and reduction of viral replication in transduced mice and induce mucosal immunity in MERS-CoV-infected dromedary camels [207, 208]. These viruses are due to enter human clinical trials soon as a candidate prophylactic MERS-CoV vaccine. Another potential vaccine due to be evaluated in camels and to enter human clinical trials is a ChAdOx1 MERS vaccine (Table 2) [212]. In mouse studies, a single dose of ChAdOx1 MERS with the leader sequence of the human tissue plasminogen activator gene (tPA) induced an equivalent humoral response to two doses of an MVA-based vaccine [212]. Another potentially efficient prophylactic vaccination strategy recently tested in mice involved heterologous prime–boost vaccination regimens using Ad5/MERS in combination with S protein nanoparticles [206]. Heterologous prime-boost elicited both anti-MERS-CoV

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neutralizing antibodies and simultaneous Th1 and Th2 responses, while homologous prime–boost regimens did not induce simultaneous Th1 and Th2 responses. Homologous Ad5/MERS also did not induce neutralizing antibody responses, while immunization schedules involving Ad5/MERS did induce Th1 cell activation and those including only S protein nanoparticles did not. Thus overall, heterologous prime–boost schedules gave superior results and are likely to induce more effective and sustained immune responses against MERS-CoV [206]. This type of vaccine would again be in keeping with WHO guidelines. A DNA-plasmid-based vaccine called GLS-5300 which encodes MERS-CoV S protein and was co-developed by Inovio, GeneOne Life Science Inc. and the Walter Reed Army Institute of Research, is meanwhile the first potential MERS-CoV vaccine to be tested in clinical trials in humans [213]. A phase I clinical trial in healthy volunteers is ongoing to evaluate its safety and its ability to generate sustained humoral and cellular immune responses over a one year period [213]. Pre-clinical trials were performed in mice, camels, and macaques, in which the vaccine induced robust immune responses which were effective in preventing viral infection [214].

Conclusions

Since its initial description in 2012, MERS-CoV has exacted a high mortality rate particularly in KSA. While epidemic potential has not been evident thus far, the potential exists for viral mutation that could increase zoonotic and/or human-to-human transmission. Outbreaks have tended to occur in healthcare facility settings and infection rates in KSA have been reduced by stringent efforts to improve infection control and prevention standards. However, the inclusion by WHO of MERS-CoV on its list of priority blueprint diseases is a timely reminder of the

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urgent need for accelerated research and development as this disease has the potential to cause a public health emergency and there are currently no directly efficacious drugs and/or vaccines available [16]. The virus clinical spectrum varies from asymptomatic, to mild-moderate disease and potential for severe disease with a high case fatality rate. The impact of asymptomatic cases, including healthcare workers, on transmission is not yet fully understood. Multiple studies have suggested that dromedary camels are the likely main zoonotic source of MERS-CoV infection in humans, and this has major implications for the valuable camel trade between the Middle East and Africa. The apparently lower than expected numbers of human cases in Africa may be attributable to inadequacies in surveillance systems that should be addressed. The role of other animals such as bats and hedgehogs also needs further clarification, and the possible emergence of alpacas as a potential zoonotic source deserves attention. While NAAT detection systems are highly sensitive and specific, further attention is needed to the most effective and feasible detection systems that can be employed in the field. A major ongoing issue is the lack of any accepted specific treatment for MERS-CoV infection. Current treatment guidelines are too much based on experience with SARS-CoV therapy, despite numerous key differences between these coronaviruses, and there is an urgent need to move from *in vitro* and *in vivo* models and clinical case studies to properly managed randomized control trials on some of the numerous direct therapeutic and vaccine candidates that have been identified. Further clarification of issues such as duration of isolation of patients with MERS-CoV infection is also needed. Thus in our view, priorities include further clarification of transmission modes, for example the role of asymptomatic individuals in disease spread, ongoing vigilance in monitoring possible cross-species transmission, the ongoing need for well-validated human and animal sera panels, and the need to add some urgency to the clinical response progress, including advancement of possible

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direct therapies to human clinical trials. While progress has undoubtedly been made in our understanding of MERS-CoV, much remains to be done to reduce the impact of this disease, particularly in KSA, and to ensure that any future outbreaks can be effectively contained.

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Compliance with ethics guidelines

This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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Case definition	WHO	CDC
Confirmed	**Nucleic acid testing** RT-PCR: two specific genomic targets • upE31 • ORF1a, ORF1b or N gene OR RT-PCR: one specific genomic target and: Sequencing of a second target: nsp12 or M gene **Serology** Screening test: • ELISA • IFA Confirmation test: • Neutralization	**Nucleic acid testing** RT-PCR: two specific genomic targets • upE31 • ORF1a, ORF1b or N gene OR RT-PCR: one specific genomic target and: Sequencing of a second target: nsp12 or M gene **Serology** Screening test: • ELISA Confirmation test: • IFA • Microneutralization Surveillance, investigation Not diagnosis
Probable (WHO) **or** **Patient under investigation (PUI) (CDC)**	1. Febrile acute respiratory illness with clinical, radiological, or histopathological evidence of pulmonary parenchymal disease AND Direct epidemiologic link with a laboratory-confirmed MERS-CoV case AND Testing for MERS-CoV is unavailable, negative on a single inadequate specimen or inconclusive 2. Febrile acute respiratory illness with clinical, radiological, or histopathological evidence of pulmonary parenchymal disease that cannot be explained fully by any other etiology AND Resides or travelled in the Middle East, or in countries where MERS-CoV is known to be circulating in dromedary camels or where human infections have recently occurred AND Testing for MERS-CoV is inconclusive 3. Acute febrile respiratory illness of any severity AND Direct epidemiologic link with a confirmed MERS-CoV case AND Testing for MERS-CoV is inconclusive	1. Fever AND pneumonia or acute respiratory distress syndrome AND EITHER: history of travel from countries in or near the Arabian Peninsula within 14 days before symptom onset, OR close contact with a symptomatic traveler who developed fever and acute respiratory illness (not necessarily pneumonia) within 14 days after traveling from countries in or near the Arabian Peninsula, OR a member of a cluster of patients with severe acute respiratory illness of unknown etiology in which MERS-CoV is being evaluated, in consultation with state and local health departments 2. Fever AND symptoms of respiratory illness (not necessarily pneumonia) AND being in a healthcare facility (as a patient, worker, or visitor) within 14 days before symptom onset in a country or territory in or near the Arabian Peninsula in which recent healthcare-associated cases of MERS have been identified. 3. Fever OR symptoms of respiratory illness (not necessarily pneumonia) AND close contact with a confirmed MERS case while the case was ill.

Table 1: WHO and CDC case definitions for MERS-CoV

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Therapeutic target	Type of therapy	Therapy/ Vaccine name	Study type	Advantages	Disadvantages	Reference
S1/DPP4 binding	Antibody (mouse): S1 RBD	Mersmab	*In vitro*			187
	Antibody (human): S1 RBD	m336, m337, m338	*In vitro* *In vivo* (Mouse, rabbit- m336)			188 - 190
	Antibody (human): S1 RBD	MERS-4, MERS-27	*In vitro*			191, 192
	Antibody (mouse-humanized): S1 RBD	4C2	*In vitro* *In vivo* (Mouse)	Prophylactic and therapeutic		193
	Antibody (mouse-humanized): S1 RBD	hMS-1	*In vitro* *In vivo* (Mouse)			194
	Antibody (human): S1 RBD	LCA60	*In vitro* *In vivo* (Mouse)	Targets both NTD and RBD; stable CHO cell line; prophylactic and therapeutic		195
	Antibody (human): S1 RBD	3B11-N	*In vitro* *In vivo* (rhesus monkeys)	Prophylactic		196
	Antibody (human): S1 RBD	MERS-GD27 MERS-GD33	*In vitro*	Synergistic effect; Different epitopes; MERS-GD27 overlaps receptor binding site		197
	Antibody (human- anti-DPP4)	2F9, 1F7, YS110	*In vitro*			198
	RBD-IgG fusion vaccine candidate	RBD s377-588- Fc IgG fusion	*In vitro* *In vivo* (Mouse)	Humoral response in mice; potential intranasal administration; improved by adjuvant; divergent strains/ escape mutants; CHO cell line		199 - 204
Nanoparticles vehicle (vaccine	Full-length S protein proprietary		*In vitro* *In vivo* (Mouse)	Use of adjuvants improves	Stable expression of abundant	205

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candidate)	nanoparticles			humoral response	full-length S protein difficult	
Nanoparticles and virus vehicle (vaccine candidate)	Full-length S protein: Ad5/MERS and S protein nanoparticles	Heterologous prime-boost:	*In vivo* (Mouse)	T cell and neutralizing antibody responses; potentially prophylactic		206
Virus vehicle (vaccine candidate)	MVA expressing full-length S protein	MVA-MERS-S	*In vitro* *In vivo* (Mouse, camel)	T cell and neutralizing antibody responses; entering human clinical trials; potential for veterinary use-		207, 208
	ad5 or ad41 adenovirus expressing full-length S		*In vitro* *In vivo* (Mouse)	T cell and neutralizing antibody responses		209
	Measles virus expressing full-length S		*In vitro* *In vivo* (Mouse)	T cell and neutralizing antibody responses		210
	Chimeric vesicular stomatitis virus (VSV) expressing full-length S		*In vitro* *In vivo* (Rhesus monkeys)	T cell and neutralizing antibody responses		211
	Chimpanzee adenovirus (ChAdOx1) expressing full-length S		*In vitro* *In vivo* (mouse)	T cell and neutralizing antibody responses; entering human clinical trials; potential for veterinary use		212
	Plasmid vaccine	GLS-5300	*In vitro* *In vivo* (Mouse, camels, and macaques) Human clinical trials	T cell and neutralizing antibody responses; in phase I clinical trial		213, 214
Viral S2-host membrane fusion	Anti-HR2 viral peptide	HR2P	*In vitro*			215
	Anti-HR2 viral peptide	HR2P-M2	*In vitro* *In vivo* (Mouse)	Blocks 6HB bundle formation; enhances IFN-β effect; potential		216 - 218

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				intranasal treatments		
	Three HR1 and two HR2 protein	MERS-5HB	*In vitro*	Inhibits fusion and entry		219
Immune evasion response	IFN-α2b and ribavirin		*In vitro* *In vivo* (Macaque)	Combination therapy-reduced dose of each; non-human primate model; ten different gene pathways		220 - 222
	IFN-β1b and lopinavir		*In vitro* *In vivo* (Marmoset)	Combination therapy-reduced dose of each		223
	IFN combination therapy (ribavirin and/or lopinavir		Case studies (human)		Only prophylactic or early use; insufficient evidence of clinical efficacy as yet	224 - 227
	IFN combination therapy (ribavirin)		Retrospective cohort studies (human)	Probable benefit of early use in less vulnerable patients; safety and efficacy established for other viral illnesses	Only prophylactic or early use; insufficient evidence of clinical efficacy as yet	141, 228 - 232
	IFN combination therapy (cyclosporine)		*In vitro* Human *ex-vivo* explant	Synergistic effect; safety and efficacy established for other viral illnesses		233
S protein host proteases	TMPRSS2 inhibitor	Camostat	*In vivo*-mouse, SARS-CoV	Already in clinical use		235
	TMPRSS2 inhibitor	Nafamostat	Split-protein-based cell-cell fusion assay	Already in clinical use		236
	Cathepsin L inhibitor	Teicoplanin dalbavancin oritavancin telavancin	High-throughput screening	Already in clinical use		237
Viral proteases	PL(pro) inhibitor	6-mercaptopurine (6MP) 6-thioguanine (6TG)	*In vitro*	Potential for more MERS-specific agents		238

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	PL(pro) inhibitor	F2124–0890	*In vitro*		May lose potency in physiological reducing environments	239
	Mpro	Lopinavir	*In vitro* *In vivo* (marmosets)	High activity at low micromolar range *in vitro*; better outcomes, in marmosets	Clinical efficacy not fully established in humans	240 - 242

Table 2: Summary of potential MERS-CoV therapies and vaccines

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Highlights

- 2,220 laboratory-confirmed cases of MERS-CoV resulting in at least 790 deaths since 2012.
- MERS-CoV is on the WHO list of priority blueprint diseases
- Zoonotic and human-to-human transmission modes need further clarification
- No specific therapy has yet been approved
- There is a need for well-controlled clinical trials on potential direct therapies

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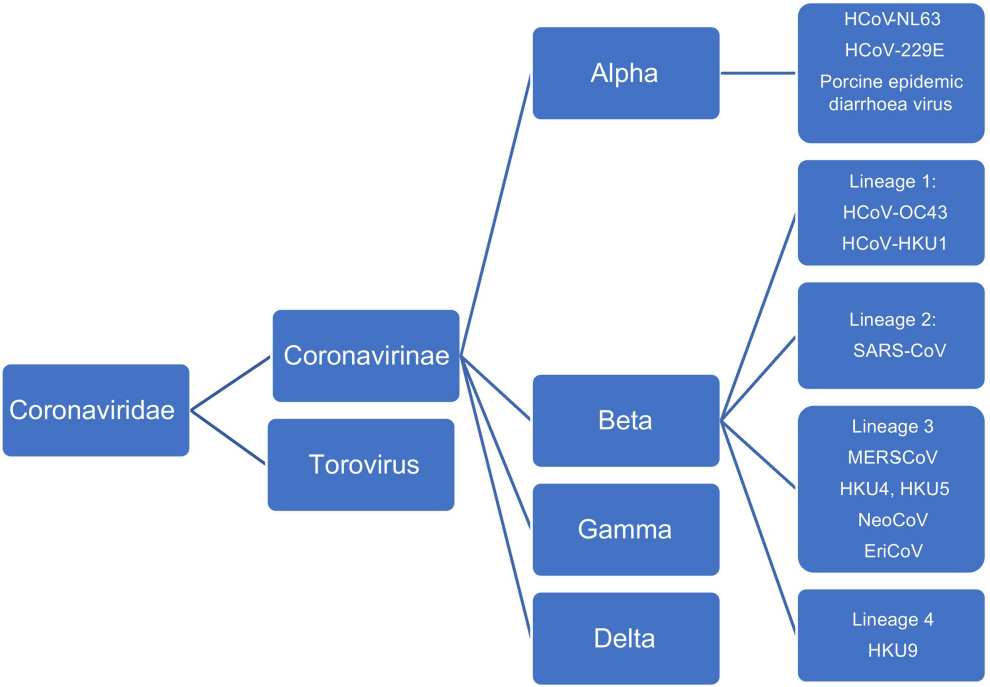
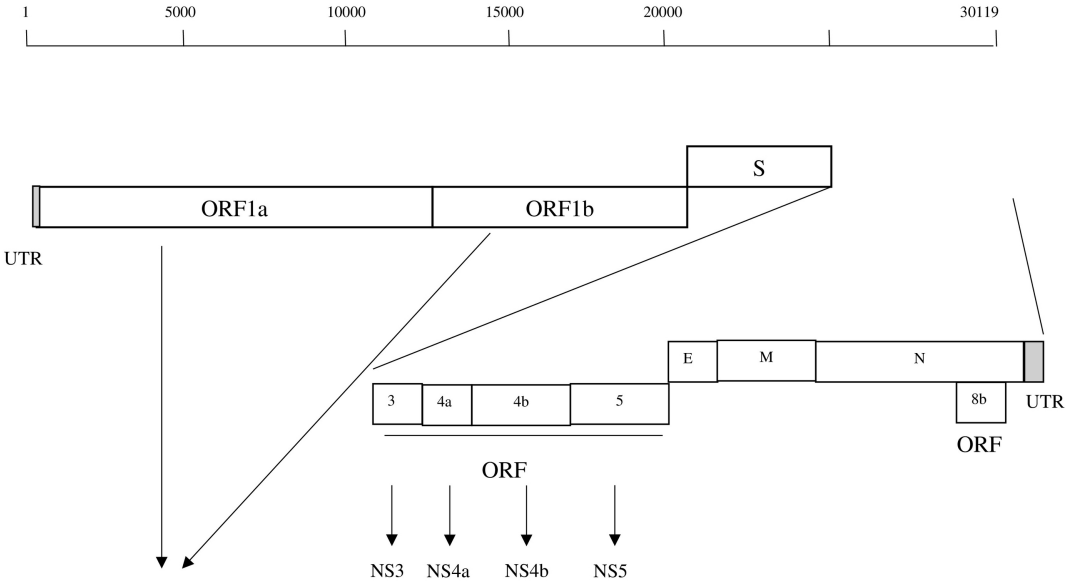

Coronaviridae
Coronavirinae
Torovirus
Alpha
Beta
Gamma
Delta
HCoV-NL63
HCoV-229E
Porcine epidemic diarrhoea virus
Lineage 1:
HCoV-OC43
HCoV-HKU1
Lineage 2:
SARS-CoV
Lineage 3
MERSCoV
HKU4, HKU5
NeoCoV
EriCoV
Lineage 4
HKU9

Figure 1



pp1a pp1ab

Cleavage by viral proteases

nsp1
nsp2
nsp3 (PL(pro))
nsp4
nsp5 (Mpro/3CLpro)
nsp6
nsp7
nsp8
nsp9
nsp10
nsp11

Primer-making activity

nsp12 regulation

nsp12 RNA-dependent RNA polymerase

nsp13
nsp14
nsp15
nsp16

RNA modification

Figure 2