Title: Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing severe disease

Running title: Middle East respiratory syndrome coronavirus

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SUMMARY

The source of the SARS epidemic was traced to wildlife market civets and ultimately to bats. Subsequent hunting for novel coronaviruses (CoVs) led to the discovery of two additional human and over 40 animal CoVs, including the prototype lineage C betacoronaviruses, *Tylonycteris* bat CoV HKU4 and *Pipistrellus* bat CoV HKU5, which are phylogenetically closely related to the Middle East respiratory syndrome coronavirus that has affected >900 patients with >35% fatality since its emergence in 2012. All primary cases of MERS are epidemiologically linked to the Middle East. Some had contacted camels which shed virus and/or had positive serology. Most secondary cases are related to healthcare-associated clusters. The disease is especially severe in elderly men with comorbidities. Clinical severity may be related to MERS-CoV's ability to infect a broad range of cells with DPP4 expression, evade host innate immune response, and induce cytokine dysregulation. Reverse transcription-PCR on respiratory and/or extrapulmonary specimens rapidly establishes diagnosis. Supportive treatment with extracorporeal membrane oxygenation and dialysis is often required in patients with organ failure. Antivirals with potent *in-vitro* activities include neutralizing monoclonal antibodies, antiviral peptides, interferons, mycophenolic acid, and lopinavir. They should be evaluated in better animal models before clinical trials. Developing camel MERS-CoV vaccine and implementing appropriate infection control measures may control the expanding epidemic.

INTRODUCTION: FROM SARS TO MERS

Frequent mixing of different animal species in markets in densely populated areas and human intrusions into the natural habitats of animals have facilitated the emergence of novel viruses. Examples with specific geographical origins include severe acute respiratory syndrome coronavirus (SARS-CoV) and avian influenza A/H7N9 and H5N1 in China, Nipah virus in Malaysia and Bangladesh, and Ebola and Marburg viruses in Africa (1-8). The Middle East is a region encompassing the majority of Western Asia and Egypt that contains 18 countries with various ethnic groups. It is one of the busiest politicoeconomic centers in the world with many unique religious and cultural practices such as the annual Hajj along with a reliance on camels for food, business, and travel in both rural and urban areas. These distinct regional characteristics have provided favorable conditions for new and rapidly mutating viruses to emerge. Similar to the first decade of the new millennium during which the world witnessed the devastating outbreak of SARS caused by SARS-CoV, the beginning of the second decade was plagued by the emergence of another novel CoV, Middle East respiratory syndrome coronavirus, that has caused an outbreak of severe respiratory disease in the Middle East with secondary spread to Europe, Africa, Asia, and North America since 2012 (3, 9). MERS-CoV is similar to SARS-CoV in being a CoV that is likely to have originated from animal reservoirs and crossed interspecies barriers to infect humans (1). The disease, Middle East respiratory syndrome (MERS), was initially called a "SARS-like" illness at the beginning of the epidemic as both are human CoV infections that manifest as severe lower respiratory tract infection with extrapulmonary involvement and high case-fatality rates (10, 11), whereas the other four CoVs that cause human infections, namely human coronavirus (HCoV)-OC43, HCoV-229E, HCoV-HKU1, and HCoV-NL63, mainly cause mild, self-limiting upper respiratory tract infections such as the common cold (10). MERS-CoV,

like SARS-CoV, is considered by the global health community as a potential pandemic agent since person-to-person transmission occurs and effective therapeutic options are limited. However, unlike the SARS epidemic, which rapidly died off after the intermediate amplifying hosts were identified and segregated from humans by closure of wild animal markets in Southern China, the MERS epidemic has persisted for more than two years with no signs of abatement (3, 12). Detailed analysis of the epidemiological, virological, and clinical aspects of MERS and SARS reveals important differences between the two diseases, and identifies unique aspects of MERS-CoV that may help to explain the evolution of the MERS epidemic. A summary of the key differences between the MERS and SARS epidemics is provided in Table 1. In this article, we review the biology of MERS-CoV in relation to its epidemiology, clinical manifestations, pathogenesis, laboratory diagnosis, therapeutic options, immunization, and infection control, and identify key research priorities that are important for the control of this evolving epidemic.

TAXONOMY, NOMENCLATURE, AND GENERAL VIROLOGY

MERS-CoV belongs to lineage C of the genus *Betacoronavirus* (βCoV) in the family *Coronaviridae* under the order *Nidovirales* (Fig. 1A). Prior to the discovery of MERS-CoV, the only known lineage C βCoVs were two bat coronaviruses that are phylogenetically closely related to MERS-CoV, namely *Tylonycteris* bat CoV HKU4 (Ty-BatCoV-HKU4) and *Pipistrellus* bat CoV HKU5 (Pi-BatCoV-HKU5) discovered in *Tylonycteris pachypus* and *Pipistrellus abramus* respectively in Hong Kong in 2006 (Fig. 1B) (13-15). MERS-CoV is the first lineage C βCoV and the sixth CoV known to cause human infection. It was designated as a novel lineage C βCoV based on the International Committee on Taxonomy of Viruses (ICTV) criteria for CoV species identification using rooted phylogeny. Calculation of pairwise evolutionary distances for

seven replicase domains showed that MERS-CoV had an amino acid sequence identity of <90% when compared to all other known CoVs at the time when MERS-CoV was discovered (16). Before the virus was formally named MERS-CoV by the Coronavirus Study Group of ICTV, it was also known by other names including "novel coronavirus", "human coronavirus EMC", "human betacoronavirus 2c EMC", "human betacoronavirus 2c England-Qatar", "human betacoronavirus 2C Jordan-N3", and "betacoronavirus England 1", which represented the places where the first complete viral genome was sequenced (Erasmus Medical Center, Rotterdam, the Netherlands) or where the first laboratory-confirmed cases were identified or managed (Jordan, Qatar, England) (9, 17-20). Similar to other CoVs, MERS-CoV is an enveloped positive-sense single-stranded RNA virus (16). Its single-stranded RNA genome has a size of approximately 30 kb, G+C content of 41%, and contains 5'-capped, polyadenylated, polycistronic RNA (16, 20, 21). The genome arrangement of 5'-replicase-structural proteins (spike-envelope-membrane-nucleocapsid)-poly(A)-3' [ie: 5'-ORF1a/b-S-E-M-N-poly(A)-3'] is similar to that of other βCoVs, and unambiguously distinguishes MERS-CoV from lineage A βCoVs, which universally contain the characteristic hemagglutinin-esterase (HE) gene (16, 20-22). Many of these genes and their encoded proteins are useful diagnostic, therapeutic, or vaccination targets (Fig. 2). There are 10 complete, functional open reading frames (ORFs) expressed from a nested set of seven subgenomic mRNAs carrying a 67-nt common leader sequence in the genome, eight transcription-regulatory sequences, and two terminal untranslated regions (16, 20, 21). The putative roles and functions of the ORFs and their encoded proteins are derived by analogy to other CoVs (Table 2). Proteolytic cleavage of the large replicase polyprotein pp1a/b encoded by the partially overlapping 5'-terminal ORF1a/b within the 5' two-thirds of the genome produces 16 putative non-structural proteins (nsp), including two viral cysteine proteases, namely nsp3

(papain-like protease) and nsp5 (chymotrypsin-like, 3C-like, or main protease), nsp12 (RNA-dependent RNA polyemerase; RdRp), nsp13 (helicase), and other nsps which are likely involved in the transcription and replication of the virus (16, 20, 21). The membrane anchored trimeric S protein is a major immunogenic antigen involved in virus attachment and entry into host cell, and has an essential role in determining virus virulence, protective immunity, tissue tropism, and host range (23). The other canonical structural proteins, namely E, M, and N proteins, are encoded by ORF6, -7, and -8 respectively, and are involved in the assembly of the virion. The M protein, as well as the papain-like protease and accessory proteins 4a, 4b, and 5, exhibit *in vitro* interferon antagonist activities that may modulate *in vivo* replication efficiency and pathogenesis (24-28).

VIRAL REPLICATION CYCLE

The replication cycle of MERS-CoV consists of numerous essential steps that can be efficiently inhibited by antiviral agents *in vitro* (Fig. 3). CoVs are so named because of their characteristic solar corona (*corona soli*) or "crown-like" appearance observed under electron microscopy, which represents the peplomers formed by trimers of S protein radiating from the virus lipid envelope. The MERS-CoV S protein is a class I fusion protein composed of the amino N-terminal receptor-binding S1 and carboxyl C-terminal membrane fusion S2 subunits (Fig. 2). The S1/S2 junction is the location of a protease cleavage site which is required to activate membrane fusion, virus entry, and syncytia formation. The S1 subunit consists of the C-domain, which contains the receptor binding domain (RBD), and an N-domain (29). The RBD of MERS-CoV has been mapped by different groups to a 200 to 300-residue region spanning residues 358 to 588, 367 to 588, 367 to 606, 377 to 588, or 377 to 662 (29-36). Among these RBD-containing fragments, the one that encompasses residues 377 to 588 appears to be the most stable and

neutralizing fragment in structural analysis and virus neutralization assays (36). Neutralizing monoclonal antibodies against the RBD potently inhibit virus entry into host cells and receptor-dependent syncytia formation in cell culture, and vaccines containing the RBD induce high levels of neutralizing antibodies in mice and rabbits (31, 34, 36-43). The S2 subunit contains the heptad repeat 1 and 2 (HR1 and HR2) domains, a transmembrane domain, and an intracellular domain that form the stalk region of S protein which facilitates fusion of the viral and cell membranes necessary for virus entry (44, 45). The binding of the S1 subunit to the cellular receptor triggers conformational changes in the S2 subunit which inserts its fusion peptide into the target cell membrane to form a six-helix bundle fusion core between the HR1 and HR2 domains that approximates the viral and cell membranes for fusion. This fusion process can be inhibited by HR2-based antiviral peptide fusion inhibitors which prevent the interaction between the HR1 and HR2 domains (44, 45).

The key functional receptor of the host cell attached to by the MERS-CoV S protein is dipeptidyl peptidase-4 (DPP4), which is also known as adenosine deaminase complexing protein 2 or CD26 (46). MERS-CoV is the first coronavirus that has been identified to use DPP4 as a functional receptor for entry into host cells (1, 46). DPP4 is a multifunctional 766-amino-acid-long type II transmembrane glycoprotein presented as a homo-dimer on the cell surface which is involved in the cleavage of dipeptides (46, 47). It has important roles in glucose metabolism and various immunological functions including T cell activation, chemotaxis modulation, cell adhesion, and apoptosis (46, 47). In humans, it is abundantly expressed on the epithelial and endothelial cells of most organs including lung, kidney, small intestine, liver, and prostate, as well as immune cells, and exists as a soluble form in the circulation (46-48). This broad tissue expression of DPP4 may partially explain the extrapulmonary manifestations seen in MERS.

190 Adenosine deaminase, which is a natural competitive antagonist, and some anti-DPP4
191 monoclonal antibodies exhibit inhibitory effects on *in vitro* MERS-CoV infection (49, 50).
192 The energetically unfavorable membrane fusion reaction in endosomal cell entry is
193 overcome by low pH and the pH-dependent endosomal cysteine protease cathepsins, and can be
194 blocked by lysosomotropic agents such as ammonium chloride, bafilomycin A, and cathepsin
195 inhibitors in a cell type-dependent manner (23, 51). Additionally, various host proteases, such as
196 transmembrane protease serine protease-2 (TMPRSS2), trypsin, chymotrypsin, elastase,
197 thermolysin, endoproteinase Lys-C, and human airway trypsin-like protease, cleave the S protein
198 into the S1 and S2 subunits to activate the MERS-CoV S protein for endosomal-independent host
199 cell entry at the plasma membrane (23, 51-53). Inhibitors of TMPRSS2 can abrogate this
200 proteolytic cleavage and partially block cell entry (23, 51, 52). In some cell lines, MERS-CoV
201 demonstrates the ability to utilize both the cathepsin-mediated endosomal and the TMPRSS2-
202 mediated plasma membrane pathways to enter host cells (51, 52).
203 In addition to these cellular proteases, furin has recently been identified as another
204 protease that has essential roles in the MERS-CoV S protein cleavage activation (54). Furin and
205 furin-like proprotein convertases are broadly expressed serine endoproteases that cleave the
206 multibasic motifs R*X*(R/K/*X*)R and processes proproteins into their biologically active forms
207 (55). Proprotein convertases including furin have been implicated in the processing of fusion
208 proteins and therefore cell entry of various viruses including human immunodeficiency virus,
209 avian influenza A/H5N1 virus, Marburg virus, Ebola virus, and flaviviruses (55-57). The MERS-
210 CoV S protein contains two cleavage sites for furin at S1/S2 ($_{748}$RSVR$_{751}$) and S2' ($_{884}$RSAR$_{887}$)
211 and exhibits an unusual two-step furin-mediated activation process (Fig. 2) (54). Furin cleaves
212 the S1/S2 site during S protein biosynthesis and the S2' site during virus entry into host cell (54).

213 Furin inhibitors such as dec-RVKR-CMK block MERS-CoV entry and cell-cell fusion (54).
214 Treatment of MERS-CoV infection with a combination of inhibitors of the different cellular
215 proteases utilized by MERS-CoV for S activation should be further evaluated in *in vivo* settings.
216 After cell entry, MERS-CoV disassembles to release the inner parts of the virion
217 including the nucleocapsid and viral RNA into the cytoplasm for translation of the viral 1a and
218 1b polyproteins and replication of genomic RNA (Fig. 3). The characteristic replication
219 structures of CoVs including double-membrane vesicles and convoluted membranes are formed
220 by the attachment of the hydrophobic domains of the MERS-CoV replication machinery to the
221 limiting membrane of autophagosomes (58). These structures can be observed at the perinuclear
222 region of the infected cells under electron microscopy (58). The viral papain-like protease and
223 3C-like protease co-translationally cleave the large replicase polyproteins pp1a and pp1b
224 encoded by ORF1a/b into nsp1 to nsp16 (16, 59, 60). These nsps form the replication-
225 transcription complex where transcription of the full length positive genomic RNA yields a full
226 length negative strand template for synthesis of new genomic RNAs as well as a series of
227 overlapping subgenomic negative strand templates for synthesis of subgenomic 3' co-terminal
228 mRNAs that will be translated to make viral structural and accessory proteins (58). The relative
229 abundance of the subgenomic mRNAs of MERS-CoV is similar to those of other CoVs, with the
230 smallest mRNA, which encodes the N protein, being the most abundant (58). After adequate
231 viral genomic RNA and structural proteins have been cumulated, the N protein assembles with
232 the genomic RNA in the cytoplasm to form the helical nucleocapsid. The nucleocapsid then
233 acquires its envelope by budding through intracellular membranes between the endoplasmic
234 reticulum and Golgi apparatus. The S, E, and M proteins are transported to the budding
235 compartment where the nucleocapsid probably interacts with M protein to generate the basic

236 structure and complexes with the S and E proteins to induce viral budding and release from the
237 Golgi apparatus (61). The viral replication cycle is completed when the assembled virion is
238 released through exocytosis to the extracellular compartment.
239
240 **SEQUENCE OF EVENTS IN THE MERS EPIDEMIC**
241 On 23 September 2012, the World Health Organization (WHO) reported two cases of acute
242 respiratory syndrome with renal failure associated with a novel CoV in two patients from the
243 Middle East (Table 3). The viral strains obtained from the respiratory tract specimens of these
244 two epidemiologically-unlinked patients shared 99.5% nucleotide identity with each other, with
245 only one nucleotide mismatch in partial replicase gene sequencing (18). In the following week,
246 the WHO and other collaborative healthcare authorities rapidly responded to the outbreak by
247 providing a unified interim case definition, making the complete genome sequence publicly
248 available in GenBank, and establishing a laboratory diagnostic protocol for real-time reverse
249 transcription (RT)-PCR using the upE (upstream of E gene) and ORF1b assays (16, 62). With
250 these important tools, sporadic cases were increasingly detected in the Middle East over the
251 subsequent six months, including two retrospectively diagnosed cases that occurred in a
252 healthcare-associated cluster of severe respiratory disease in Zarqa, Jordan, in April 2012 (19,
253 63-66). Additional cases were also reported in Europe and Africa among patients with recent
254 travel to the Arabian Peninsula and their close hospital and household contacts (18, 67-74). The
255 fear of person-to-person transmission was further heightened by the occurrence of a large-scale
256 outbreak involving over 20 patients in four interrelated hospitals in Al-Hasa, the Kingdom of
257 Saudi Arabia (KSA), from April to May 2013 (75).
258 In view of the significant epidemiological link of all the reported cases to the region, the

ICTV formally named the novel virus MERS-CoV on 15 May 2013 (17). However, the epidemic was not contained within the Middle East as its name implied, and the number of patients and countries involved continued to escalate over the following years (76-81). In particular, there was a sudden surge of over 400 cases in KSA and the United Arab Emirates (UAE) within just two months from mid-March to May 2014 as a result of both an increased number of primary cases possibly related to the weaning season of dromedary camels, a probable zoonotic source of MERS-CoV, and an amplification of the number of secondary cases by several healthcare-associated outbreaks in the region during the same period (82, http://www.who.int/csr/disease/coronavirus_infections/MERS_CoV_Update_09_May_2014.pdf) . As of 17 December 2014, the WHO has reported a total of 938 laboratory-confirmed cases of MERS including 343 deaths. The affected countries with primary cases include KSA, Qatar, Jordan, UAE, Oman, Kuwait, Egypt, Yemen, Lebanon, and Iran in the Middle East. The countries with imported cases include the United Kingdom, Germany, France, Italy, Greece, the Netherlands, Austria, and Turkey in Europe, Tunisia and Algeria in Africa, Malaysia and the Philippines in Asia, and the United States in North America.

EPIDEMIOLOGY

Among the first 699 laboratory-confirmed cases of MERS, 63.5% were male and the median age was 47 years, with a range of 9 months to 94 years (http://www.who.int/csr/disease/coronavirus_infections/MERS-CoV_summary_update_20140611.pdf). The persistence of the epidemic is postulated to be related to repeated animal-to-human transmissions from at least one type of animal reservoir that is in frequent contact with residents in the region, which are amplified by non-sustained person-

to-person transmission in multiple large-scale healthcare-associated outbreaks and limited household clusters (67, 68, 70, 71, 73-75, 83, 84, http://www.who.int/csr/disease/coronavirus_infections/MERS-CoV_summary_update_20140611.pdf). Human infection has been linked to the contacts with dromedary camels (*Camelus dromedarius*) or other humans infected with MERS-CoV, but alternative sources of infection are possible as many patients did not have epidemiological link to infected camels or humans. All primary MERS cases were epidemiologically linked to the Middle East and all secondary cases in other countries were linked to primary cases imported from the Middle East. The incubation period is estimated to be 5.2 days, with a range of 1.9 to 14.7 days, and 95% of infected patients have symptom onset by day 12.4 (63, 75). The serial interval, representing the time between the case's symptom onset and the contact's symptom onset, is estimated to be 7.6 days with a range of 2.5 to 23.1 days, and is less than 19.4 days in 95% of the cases (63, 75). The rate of secondary transmission among household contacts of MERS patients is estimated to be about 4% (85).

Risk Factors for Severe Disease

Among the first 536 laboratory-confirmed cases reported by the WHO, 62% were severe cases that required hospitalization (77). Severe cases requiring hospitalization were more commonly seen among primary cases which mainly consist of older patients with comorbidities. The secondary cases were mostly younger patients and healthcare workers without comorbidities, but severe nosocomial infection among patients sharing contaminated equipment with improper barrier controls have also been reported (75, http://www.who.int/csr/disease/coronavirus_infections/MERS-

CoV_summary_update_20140611.pdf) (Table 4). In a clinical cohort from KSA with 47 severe cases requiring hospitalization, the patients' median age was 56 years. There was a male predominance with a male to female ratio of 3.3 to 1 (63). About 96% of the patients had comorbidities, with the most common being diabetes mellitus (68%), chronic renal disease (49%), hypertension (34%), chronic cardiac disease (28%), and chronic pulmonary disease (26%). Smoking and obesity were also reported in 23% and 17% of the patients respectively. The predominance of older males with comorbidities among severe cases was also reported in other case series at variable rates, depending on the size and setting of the studies (63, 66, 75, 80, 86-89). Furthermore, age of over 50 years, male sex, and the presence of multiple comorbidities were associated with a higher fatality rate (63, 87, 90). Some of these conditions are highly prevalent among residents in the Middle East, for example, diabetes mellitus in nearly 63% of persons at or older than 50 years in KSA (91). Their relative risk of developing severe MERS requires further evaluation in large-scale case-control studies. Patients who develop complications such as acute respiratory distress syndrome requiring hospitalization and/or intensive care are also at risk of fatal outcome (87).

Seroepidemiology

The interim WHO case definition used early in the epidemic was criticized for being focused on identifying severe cases which might have over-estimated the clinical severity and significance of MERS (92). This was supported by the increasing number of asymptomatic and mild cases identified in subsequent enhanced surveillance among contacts of MERS patients in various clusters. It was thus suggested that the genuine epidemiology of MERS-CoV might be more similar to that of HCoV-HKU1 rather than SARS-CoV in that the infection is prevalent in the

general population, but only manifests severely in the elderly and immunocompromised (93-96). However, seroepidemiological studies conducted so far have refuted this hypothesis as there is little evidence of past infection among the general population in the Middle East. Serum anti-MERS-CoV antibodies were not detected in archived serum samples of 2400 control in- or out-patients without MERS in KSA, suggesting that MERS-CoV was unlikely to be circulating in the general population during the preceding two years (9, 90). Similarly, serum neutralizing anti-MERS-CoV antibodies were not detected among 158 children hospitalized for lower respiratory tract infections and 110 adult male blood donors in KSA between May 2010 and December 2012 (97). Even among 226 slaughterhouse workers who had contact with various livestock species that might serve as zoonotic sources of MERS-CoV, neutralizing anti-MERS-CoV antibodies were not detected in serum samples collected in October 2012 (98). Additional region-wide seroepidemiological studies that include large collections of archived samples from earlier timepoints may determine the true prevalence and clinical severity of MERS among residents in the affected areas.

Animal Surveillance

Given the sudden emergence of MERS-CoV without definite serological evidence of past exposure in the general population, a novel episode of interspecies transmission of the virus was postulated. An intense hunt for animal reservoirs of MERS-CoV was sparked by the early recognition of the close phylogenetic relationship between MERS-CoV and the prototype lineage C βCoVs, Ty-BatCoV-HKU4 and Pi-BatCoV-HKU5, which suggested the possibility of MERS-CoV being a zoonotic agent (9, 13, 14, 21, 99). Subsequent functional studies showed that Ty-BatCoV-HKU4 also utilizes DPP4 as a functional receptor for cell entry in pseudotyped virus

assay (100, 101). These findings concur with the existing notion that bats are the likely gene sources of most αCoVs and βCoVs including SARS-CoV (1, 15, 102-107). Recent reports also show a high nonsynonymous (d_N) to synonymous (d_S) nucleotide substitutions per site ratio in the bat DPP4-encoding genes (108). This adaptive evolution on the bat DPP4 is suggestive of long-term interactions between bats and MERS-CoV-related viruses (108). In addition to Ty-BatCoV-HKU4 and Pi-BatCoVHKU5 which are found in bats in Hong Kong and Southern China, other lineage C βCoVs closely related to MERS-CoV were also identified in different bat species in the Middle East, Africa, Europe, and Central America after the MERS epidemic started (Table 5). The virus that is most closely related to MERS-CoV phylogenetically was a βCoV detected in the fecal pellet of a *Taphozous perforatus* bat caught in Bisha, KSA, near the home of a patient with laboratory-confirmed MERS, which shared 100% nucleotide identity with MERS-CoV by partial RdRp gene sequencing (109). However, this study was limited by the short length of the gene fragment analyzed (182 nucleotides) and its detection in only one of 29 (3.4%) *T. perforatus* bats caught at the same location. Furthermore, no live virus was isolated from any of these bats. Subsequent studies identified a closely related virus, NeoCoV, in the feces of a *Neoromicia capensis* bat in South Africa which had a complete genome sequence sharing 85.6% nucleotide identity with those of MERS-CoV from infected humans and dromedary camels (110, 111). Based on the estimated evolutionary rate of MERS-CoV, the most recent common ancestor between NeoCoV and human MERS-CoV strains was proposed to exist in bats more than 44 years ago (112). As the same lineage of CoVs are usually found and originate from closely related bat species, the likelihood of MERS-CoV originating from both *T. perforatus* (superfamily *Emballonuroidea*) and vespertilionid bats (*Neoromicia capensis*, *Pipistrellus* sp., and *Tylonycteris pachypus* in the superfamily *Vespertilionoidea*), which belong

374 to two distantly related superfamilies of insectivorous bats, is low (20, 110, 111). Interestingly,
375 European hedgehogs (*Erinaceus europaeus*) belonging to the order *Eulipotyphla*, which is
376 closely related to bats phylogenetically, also carry high concentrations of a MERS-CoV-related
377 lineage C βCoV, *Erinaceus* CoV, in their feces and intestines (113). Further surveillance and full
378 virus genome sequencing involving a larger population of different bat and bat-related species is
379 required to confirm these preliminary findings.

380 Besides the possibility of direct interspecies transmission of SARS-CoV from bats to
381 humans, it is postulated that intermediate amplifying animal hosts such as civets and raccoon
382 dogs might also have been important in the transmission of SARS. Therefore, specific
383 intermediate animal hosts of MERS-CoV with frequent contact with infected humans were
384 sought since the early phase of the MERS epidemic (3, 114, 115). In *in vitro* studies, MERS-CoV
385 can replicate efficiently not only in a variety of bat cell lines, but also in cell lines originating
386 from other animal species including camelid, goat, pig, rabbit, and civet (116-118) (Table 6). The
387 host range is mainly determined by the binding of the MERS-CoV S protein to the host receptor
388 DPP4, which is relatively conserved among mammalian species (30, 48, 49, 119, 120). The first
389 *in vivo* evidence to support the presence of an intermediate animal reservoir of MERS-CoV
390 emerged when high-titer of serum neutralizing IgG against the MERS-CoV S1 RBD were
391 detected in dromedary camels (121). All 50 Omani dromedary camels were seropositive as
392 compared to less than 10% of the Spanish dromedary camels and none of the other common
393 livestock species in the study. This suggested that widespread circulation of MERS-CoV or a
394 closely related virus was present among dromedary camels in this Middle Eastern country.
395 Numerous seroepidemiological studies also demonstrated serological evidence of MERS-CoV
396 infection in dromedary camels in other Middle Eastern countries including KSA, Qatar, UAE,

and Jordan, and also in African countries including Egypt, Kenya, Nigeria, Ethiopia, Tunisia, Somalia, and Sudan where most of the camels found in the Middle East have originated from (Table 5). Serological evidence of infection among camels was detected in archived specimens collected in as early as 1992 and 1983 in KSA and eastern Africa respectively, and was especially prevalent in areas of high dromedary population density (122-133). These findings suggested that unrecognized primary human cases of MERS might also be present outside the Middle East. On the other hand, studies in Qatar and several other countries showed that anti-MERS-CoV antibodies were not detected in the sera of other livestock species tested including goats, sheep, cows, water buffaloes, swine, and wild birds (http://www.who.int/csr/disease/coronavirus_infections/MERS_CoV_RA_20140613.pdf). Furthermore, it was also shown that the percent seropositivity of neutralizing anti-MERS-CoV antibodies was much lower in juvenile than adult dromedary camels, suggesting that acutely infected juvenile dromedary camels without neutralizing antibodies might be a more important source for transmission to humans than adult dromedary camels (123, 127).

The significance of camels as the major source of animal-to-human transmission required further virological studies on the pattern of viral shedding in camels and their relationship to laboratory-confirmed human cases (Fig. 4). An investigation of a disease outbreak in dromedary camels in Qatar demonstrated MERS-CoV in nasal swabs, but not rectal swabs or fecal samples, of three of 14 (21.4%) camels by RT-PCR sequencing (133). The nucleotide sequences of a 940-nucleotide ORF1a fragment and a 4.2 kb concatenated gene fragment of these camel strains were very similar to those of two epidemiologically-linked human strains. This study, however, was not able to conclusively establish the direction of transmission or exclude the presence of a third source of infection. Subsequently, the detection of MERS-CoV in dromedary camels was

reported in a number of studies conducted in different areas in the Middle East, which provided further insights into the viral shedding kinetics in camels (123, 128, 129, 131, 134). In agreement with the lower frequency of neutralizing anti-MERS-CoV antibodies in juvenile camels, the rate of detection of MERS-CoV RNA in the nasal and/or rectal swabs of juvenile camels was higher than those of adult camels (123). These findings may partially explain the absence of serum neutralizing anti-MERS-CoV antibodies among camel abattoir workers who have predominantly contacted adult camels (135, 136). These serological surveys should be confirmed by virus neutralization assays. Nevertheless, infected adult camels might still be a source of human infection. Similar to HCoVs and other respiratory viruses that can cause repeated infections in humans over a lifetime, MERS-CoV shedding could be observed in camels with pre-existing serum antibodies, suggesting that prior infection and passively acquired maternal antibodies might not provide complete protection from MERS-CoV infection and/or re-infection in camels (129). The fact that the majority of amino acid residues critical for receptor binding are identical between most human and camel strains further supports the potential of the dromedary MERS-CoVs to infect humans despite differences in clinical manifestations of infected humans and camels (129, 131). The higher positivity rate of MERS-CoV RNA in nasal swabs than in rectal swabs or fecal samples, and the isolation of MERS-CoV from cultures of nasal swabs but not rectal swabs of camels in Vero E6 cells correlated with the predominantly upper respiratory tract symptoms in acutely infected symptomatic camels (129, 137). Together with the genetic stability of MERS-CoV in camels, these serological and virological data from animal surveillance support the hypothesis that MERS-CoV has likely originated from bats in Africa and then crossed species barriers to infect camels in the greater Horn of Africa many years ago. Infected camels were then transported to the Middle East where they transmitted the virus to non-immune humans to cause

443 the epidemic (111).

444 The strongest evidence of direct cross-species transmission of MERS-CoV from camels
445 to humans was provided in a study reporting the isolation of the virus from a dromedary camel
446 which had a complete genome sequence identical to that of a human strain from a patient who
447 developed MERS after close contact with sick camels that had rhinorrhea (138). Serological tests
448 showed seropositivity in the camels but not in the patient before the human infection occurred
449 (138). The air sample collected from the camel barn on the same day when a sick camel tested
450 positive for MERS-CoV, but not on the subsequent two days, was also positive for MERS-CoV
451 RNA by RT-PCR (139). This suggests that the virus may persist in the air surrounding infected
452 animals or humans for less than 24 hours, although viral infectivity is uncertain because the virus
453 was not culturable from the air sample. Another similar study also reported a human case of
454 MERS that developed after the patient had contact with sick camels with respiratory symptoms
455 (128). Comparison of eight RT-PCR fragments, constituting 15% of the virus genomes derived
456 from the infected camel and from an epidemiologically-linked patient, showed nearly 100%
457 nucleotide identity (128). The genomes of both the camel and human strains of MERS-CoV
458 contained unique nucleotide polymorphism signatures not found in any other known MERS-CoV
459 sequences and therefore supported direct cross-species transmission (128). Preliminary results
460 from an ongoing investigation in Qatar showed that people working closely with camels,
461 including farm workers, slaughterhouse workers, and veterinarians, may be at higher risk of
462 developing MERS than those who do not have regular contact with camels
463 (http://www.who.int/csr/disease/coronavirus_infections/MERS_CoV_RA_20140613.pdf).
464 Notably, while these studies support camel-to-human transmission, a bidirectional mode of
465 transmission cannot be completely excluded at this stage.

In spite of these examples that support the hypothesis of direct camel-to-human cross-species transmission of MERS-CoV, a number of important questions remain unresolved at this stage. Firstly, it is uncertain whether camels are intermediate amplification hosts or the natural reservoirs of MERS-CoV. Although bats are postulated to be the natural host of most βCoVs including MERS-CoV, the detection of anti-MERS-CoV antibodies in archived sera of camels dating back to more than 28 years ago in eastern Africa and more than 20 years ago in KSA, the high genetic stability of MERS-CoV in camels, and the high sequence nucleotide identities between camel and human strains of MERS-CoV suggest that the virus was well adapated and circulating in camels for a long time (123, 129). The reason why human infection has not been reported until 2012 remains elusive. Notably, a different novel lineage A βCoV, named dromedary camel CoV UAE-HKU23, has also been discovered in the fecal samples of dromedary camels in Dubai, UAE recently (140). Further surveillance studies may provide novel insights into the role of this unique camelid species, which also have heavy-chain antibodies as humoral defense, in the emergence of novel CoVs (141). Another unresolved question is whether an alternative source may be present but undetected at this stage. It is noteworthy that a significant proportion of laboratory-confirmed human cases did not have a clear history of contact with camels (83, 142). Evaluation of other animal species endemic in the region using validated serological and virological assays should be conducted. Finally, the route of transmission of MERS-CoV from camels to humans remains unknown at this stage. Droplet transmission appears likely as evidenced by the high viral loads in the nasal and conjunctival swabs of camels and the surrounding air samples. However, viral shedding in nasal secretions is usually short-lasting during acute infection, which may limit viral transmission by this route (129). Direct contact with other infected bodily fluids including blood and feces is also possible,

but viral shedding in these samples is also transient in acute infection (129). Food-borne transmission through ingestion of infected unpasteurized camel milk, in which MERS-CoV can survive for at least 48 hours at 4°C or 22°C, has also been suggested. But it has yet to be definitively proven that camels actively shed MERS-CoV in their milk as contamination by feces, nasal secretions, or calf saliva containing the virus cannot be completely excluded (143). The presence of neutralizing antibody in milk may also limit the virus' infectivity *in vivo* (144). In human MERS cases without direct exposure to camels, contact with environments contaminated with infected camel secretions and aerosol transmission are other possibilities that warrant further investigations (139, 145).

Molecular Epidemiology

Detailed analysis of the molecular evolution and spatiotemporal distribution of genomes of human and animal strains of MERS-CoV provides useful information for detecting viral adaptation to animal-to-human and person-to-person transmissions, identifying zoonotic and other sources of human infections, and assessing the pandemic potential of the virus. Comparative analysis of 65 complete or near-complete genomes of human MERS-CoV strains identified early in the epidemic from June 2012 to September 2013 estimated the evolutionary rate of the coding regions of the viral genome to be 1.12×10^{-3} (95% confidence interval, 8.76×10^{-4} to 1.37×10^{-3}) substitutions per site per year (146). The time to the most recent common ancestor (TMRCA) of MERS-CoV was estimated to be March 2012 (95% confidence interval, December 2011 to June 2012) (112, 146). Compared with the genome of one of the earliest human MERS-CoV strains, the genomes of the MERS-CoV strains obtained from patients diagnosed between October 2012 and June 2013 showed various nucleotide changes in the last

512 third of the genomes, which represent potential amino acid changes in the accessory proteins and
513 the S protein encoded at nucleotide positions 21,000-25,500 (112). Specifically, codon 1020 at
514 the HR1 domain of the S gene was identified to be under strong episodic selection among
515 different geographical lineages with either a histidine or arginine at this position (112, 146).
516 Although the amino acid variations are not predicted to change the alpha helical structure of this
517 region, the histidine and arginine provide an endosomal protonated residue and a potential
518 endosomal protease cleavage site respectively that may affect the S protein membrane fusion
519 activity (146). Codon 158 at the N-terminal domain and codon 509 at the RBD of the S gene are
520 also noted to be under weaker positive selection (146). As mutations in the RBD of the S protein
521 of CoVs may be associated with changes in the transmissibility across and within species, the
522 phenotypic changes associated with these genomic variations should be ascertained (3, 29, 147-
523 149).

524 In addition to the results of animal surveillance studies and investigations of human
525 MERS outbreaks, genomic analysis also supports the hypothesis that MERS-CoV is transmitted
526 from both animal-to-human and person-to-person. Among genomes of sporadic human MERS
527 cases, numerous distinct phylogenetic clades and genotypes exist, which likely represent separate
528 instances of incursions from animals to human (112). Indeed, at least four clades of MERS-CoV
529 were identified in KSA, with three of them apparently no longer widely circulating during May
530 to September 2013 (146). In a large healthcare-associated outbreak in Al-Hasa, person-to-person
531 transmissions were supported by genomic analysis in at least 8 of 13 patients (75, 112). Two
532 phylogenetically distinct MERS-CoV strains were detected in a family cluster in Riyadh, KSA,
533 in October 2012, suggesting that at least two distinct lineages of MERS-CoV were circulating in
534 Riyadh during this time period and that human clusters might involve multiple sources with more

than one virus lineage (112). The genomic diversity of MERS-CoV detected in patients from the same locality and the geographical dispersion of MERS-CoV lineages in the Middle East suggest the presence of multiple mobile infection sources such as animal reservoirs, infected animal products, and/or infected patients in the epidemic regions (146). This hypothesis fits well with the evidence of MERS-CoV infection in dromedary camels, which are an important vehicle for transportation of goods and travelers, as well as food source in the Middle East. Notably, quasispecies of MERS-CoV within single samples have been detected in samples from dromedary camels but not humans or Vero cell isolates from the same animal (137). Further studies using next-generation high throughput sequencing are required to confirm the presence of quasispecies and clonal virus populations within individual human cases, which may help identify specific genotypes that can pass the bottleneck selection to cause cross-species transmission from camels to humans, and help to explain the relative rarity of human cases despite the widespread circulation of MERS-CoV in dromedary camels for prolonged periods in the Middle East and North Africa (137).

Mathematical Modeling

Mathematical modeling has been widely used to predict the spread and pandemic potential of emerging viruses. Although the interval for data accumulation may diminish the predictive value of mathematical modeling and its impact on epidemiological control or policy setting, these studies provide a preliminary estimate of the pandemic potential of emerging viruses if enough data are included in the calculations. Three real-time predictions of the spread of MERS-CoV have been conducted for the current epidemic and have estimated the basic reproduction number (R_0), the number of secondary cases per index case in a fully susceptible population, to be 0.30-

558 0.77 (150), 0.60-0.69 (90), or 0.8-1.3 (151), as compared to about 0.8 for pre-epidemic SARS-
559 CoV. These estimates imply the occurrence of a subcritical epidemic in the Middle East, which is
560 unlikely to sustain person-to-person transmission of MERS-CoV, especially when infection
561 control measures are implemented (150). The estimated daily rate of MERS-CoV introductions
562 into the human population in the Middle East is 0.12-0.85 and the expected yearly incidence of
563 MERS introduction was estimated to be between 160 and 320 cases per year (90, 150). Clearly,
564 these estimations are at most only modestly accurate for a number of reasons. Firstly, these
565 studies were conducted early in the epidemic when the total number of laboratory-confirmed
566 cases was only less than one-eighth of that reported by the WHO as of 17 December 2014 (90,
567 150, 151). This low number limited the accuracy of the predictions as sufficient caseload is
568 required to calculate the basic parameters for estimation of the worst- and best-case scenarios to
569 gauge the magnitude of the epidemic. The omission of large clusters may underestimate the R_0
570 (90). Secondly, most of the cases reported in the early period of the epidemic were biased
571 towards including more severe cases. The increasingly recognized number of asymptomatic or
572 mildly symptomatic cases identified through enhanced surveillance programmes may further
573 underestimate the R_0 (90). Finally, the R_0 may also be affected by community demographics,
574 contact structure, large gatherings such as the Hajj, and exportation of patients from the
575 relatively less populated Middle East to densely populated areas such as Southeast Asia (78, 90).
576 Updated mathematical modeling using the latest available epidemiological and virological data
577 may increase the accuracy of these estimates.
578
579 **CLINICAL MANIFESTATIONS**
580 The early reports of MERS have focused on severe cases which typically presented as acute

pneumonia with rapid respiratory deterioration and extrapulmonary manifestations (Table 7). Few clinical and radiological features can reliably differentiate MERS from acute pneumonia caused by other microbial agents (80). The common presenting symptoms of MERS are non-specific, and include feverishness, chills, rigors, sore throat, non-productive cough, and dyspnea. Other symptoms of respiratory tract infections including rhinorrhea, sputum production, wheezing, chest pain, myalgia, headache, and malaise may also be present. Rapid clinical deterioration with development of respiratory failure usually occurs within a few days after these initial symptoms (80). Physical signs at the time of deterioration may include high fever, tachypnea, tachycardia, and hypotension. Diffuse crepitations may be present on chest auscultation, but they may be disproportionately mild compared with radiological findings (68).

Chest radiograph abnormalities are found in nearly all severe cases and often progress from a mild unilateral focal lesion to extensive multifocal or bilateral involvement especially of the lower lobes as the patient deteriorates (63). The radiological changes are non-specific and indistinguishable from other viral pneumonias associated with acute respiratory distress syndrome (ARDS), and include air-space opacities, segmental, lobar or patchy consolidations, interstitial ground glass infiltrates, reticulonodular shadows, bronchial wall thickening, increased bronchovascular markings, and/or pleural and pericardial effusions (Table 7). Rarely, pneumonia may be an incidental finding in chest radiograph and precede the sudden deterioration in respiratory function in patients who are harboring a "walking pneumonia" with minimal respiratory tract symptoms (63, 68). The most common thoracic computerized tomography (CT) scan features are bilateral, predominantly basilar and subpleural airspace involvement, with extensive ground-glass opacities, and occasional septal thickening and pleural effusions (152). Tree-in-bud pattern, cavitation, and lymph node enlargement have not been reported. Fibrotic

changes including reticulation, traction bronchiectasis, subpleural bands, and architectural distortion may be found in thoracic CT scans performed three weeks after symptom onset. These different changes in thoracic CT scan throughout the course of disease are suggestive of organizing pneumonia and may mimic those seen in other viral pneumonias such as influenza (4, 8, 153-156).

Various extrapulmonary manifestations involving multiple body systems have been reported in MERS (Table 7). Acute renal impairment was the most striking feature in the early reports (9, 18). This finding was confirmed in subsequent sporadic reports and at least three case series that provided specific details on renal function, in which more than half of the patients developed acute renal impairment at a median time of around 11 days after symptom onset, with most requiring renal replacement therapy (88, 152, 157). This is unique among CoV infections of human. For SARS, only around 6.7% of patients developed acute renal impairment mainly due to hypoxic injury at a median duration of 20 days after symptom onset and 5% required renal replacement therapy (158, 159). The exceptionally high incidence of this distinctive manifestation of MERS is likely multi-factorial. These include the high prevalence of background chronic renal impairment among severe cases and the renal tropism of MERS-CoV (63, 116, 157). The presence of MERS-CoV RNA in urine also supports the possibility of direct renal involvement, but the exact incidence and prognostic significance of this finding is unknown at present (72).

As in humans infected with SARS-CoV and animals infected with other CoVs, patients infected with MERS-CoV may have enteric symptoms in addition to respiratory tract involvement (3, 160, 161). Gastrointestinal symptoms are found in more than a quarter of hospitalized cases in a large cohort in KSA (63). Diarrhea is the most common symptom and

627 occurs in 6.7% to 25.5% of severe cases. Nausea, vomiting, and abdominal pain may also occur.
628 The detection of viral RNA in fecal samples has been reported, but longitudinal studies on the
629 pattern of viral shedding are lacking (72). It remains to be determined whether cases of acute
630 abdomen presenting as ischemic bowel or negative findings on laparotomy result from hypoxic
631 damage or direct viral invasion of the gastrointestinal tract (88).

632 Other extrapulmonary features of MERS include hepatic dysfunction, pericarditis,
633 arrhythmias, and hypotension (66). Hematological abnormalities include leukopenia or
634 leukocytosis, usually accompanied by lymphopenia with normal neutrophil count, and
635 thrombocytopenia. Compared to other patients with pneumonia, patients with MERS are more
636 likely to have a normal leukocyte count on admission (80). Anemia, coagulopathy, and
637 disseminated intravascular coagulation have also been reported (64, 72, 162). Elevated levels of
638 serum transaminases, lactate dehydrogenase, potassium, creatine kinase, troponin, C-reactive
639 protein, and procalcitonin, and reduced levels of serum sodium and albumin are seen
640 occasionally.

641 Complications of MERS include bacterial, viral, and/or fungal co-infections, ventilator-
642 associated pneumonia, septic shock, delirium, and possibly stillbirth (9, 69, 71, 73) (Table 7).
643 Respiratory failure with ARDS and multiorgan dysfunction syndrome is not uncommon, and the
644 majority of such patients require admission to the intensive care unit at a median of 2 to 5 days
645 from symptom onset. The median time from symptom onset to invasive ventilation and/or
646 extracorporeal membrane oxygenation (ECMO) in these patients is 4.5 to 7 days, which is at
647 least 6 days earlier than that of SARS (63, 75, 88, 162, 163). The duration of stay in the intensive
648 care unit is often prolonged with a median of 30 days (range: 7 to 104 days). The case-fatality
649 rate is up to 25.0% to 76.5% in various cohorts (Table 7).

With enhanced surveillance of healthcare-associated and family contacts of MERS patients, an increasing number of asymptomatic and mild cases have been identified. Most of these patients are young, healthy female healthcare workers or children who do not have any comorbidities (65, 164). Among 402 patients identified in the recent clusters that occurred in KSA between 11 April 2014 and 9 June 2014, 109 (27.1%) were healthcare workers. Of note, though many were either asymptomatic or mildly symptomatic, more than one-third developed moderate to severe disease requiring hospitalization and nearly 4% died (http://www.who.int/csr/disease/coronavirus_infections/MERS-CoV_summary_update_20140611.pdf). Severe and even fatal cases have also been reported among infected children, especially in those who have underlying diseases such as cystic fibrosis and Down's syndrome with congenital heart disease and hypothyroidism (164). Therefore, even healthcare workers and children with MERS should be monitored closely for clinical deterioration.

HISTOPATHOLOGY AND PATHOGENESIS

The pathogenesis of MERS is under-studied and poorly understood. Serial sampling for characterization of the innate and adaptive immune responses is lacking in human cases of MERS. Due to religious and cultural reasons, post-mortem examination was seldom performed in Islamic patients who died of MERS and no post-mortem findings have been reported so far. Thus, the current understanding on the histopathology and pathogenesis of MERS is limited to findings in *in vitro*, *ex vivo*, and animal experiments.

Histological Changes

673 In rhesus macaques infected with MERS-CoV, macroscopic changes of acute pneumonia
674 including multifocal to coalescent bright red palpable nodules with congestion occurred
675 throughout the lower respiratory tract in necropsy lung tissues collected on day 3 post-infection
676 (165-167). On day 6 post-infection, these inflamed areas progressed into dark reddish purple
677 lesions. Microscopically, the changes resembled those seen in mild to severe acute interstitial
678 pneumonia, characterized by alveolar infiltration by small to moderate numbers of macrophages
679 and fewer neutrophils with occasional multinucleate syncytia, and thickening of alveolar septae
680 by edema fluid and fibrin on day 3 post-infection. Lesions similar to those described in early
681 bronchiolitis obliterans with organizing pneumonia consisting of aggregations of fibrin,
682 macrophages, and sloughed pulmonary epithelium that occluded small airways, and multifocal
683 perivascular infiltrates of inflammatory cells within and adjacent to the affected areas of lungs
684 were also reported. On day 6 post-infection, moderate to marked microscopic changes including
685 type II pneumocyte hyperplasia, alveolar edema, and hyaline membranes of fibrin were observed
686 (166). In situ hybridization and immunohistochemistry demonstrated viral RNA and antigen
687 respectively in type I and II pneumocytes, alveolar macrophages, and occasionally round
688 mononuclear cells and stellate cells within the cortex of the mediastinal lymph nodes, but not in
689 pulmonary endothelial cells, on both days 3 and 6 post-infection (166, 167). Infected cells were
690 not observed in the kidney, brain, heart, liver, spleen, and large intestine of the infected rhesus
691 macaques (167). Common marmosets infected with MERS-CoV showed similar but more severe
692 histological findings. In necropsied lungs of common marmosets euthanized on days 3 to 4 post-
693 infection, extensive transcriptional evidence of pulmonary fibrosis was present (168). In
694 immunosuppressed rhesus macaques using cyclophosphamide and dexamethasone with depleted
695 T and B cells and disrupted splenic and mesenteric lymph node architectures, MERS-CoV

696 replicated more efficiently and affected more tissues as compared to non-immunosuppressed
697 controls. Interestingly, the immunosuppressed animals had fewer histological changes associated
698 with infection despite having higher virus replication in the lungs, suggesting that
699 immunopathology might also play a key role in MERS (169).
700
701 **Innate Immune Response**
702 Immune evasion is an important strategy utilized by CoVs to overcome the innate immune
703 response for efficient replication in the host. MERS-CoV is capable of inhibiting recognition,
704 delaying interferon induction, and dampening interferon-stimulated genes (ISGs) expression in
705 polarized human bronchial epithelia (Calu-3) cells until peak viral titers have been reached (170).
706 While MERS-CoV triggers an activation of pattern recognition receptors that is similar to SARS-
707 CoV, their subsequent levels of interferon induction in Calu-3 cells are markedly different (171).
708 This may be related to the different structural and accessory proteins of the two viruses that act
709 as interferon antagonists. Instead of the papain-like protease, accessory proteins 3b and 6, nsp1,
710 M, and N proteins which are the major putative interferon antagonists of SARS-CoV, the papain-
711 like protease encoded by nsp3 of ORF1a/b, M protein encoded by ORF7, and accessory proteins
712 4a and 4b encoded by ORF4a and -4b respectively of MERS-CoV antagonize interferons *in vitro*
713 (3, 24, 25, 27, 28, 172). Among them, the MERS-CoV accesory protein 4a, a double-stranded
714 RNA-binding protein, exhibits potent antagonistic activity at multiple levels of the interferon
715 response including the prevention of interferon-β synthesis through the inhibition of interferon
716 promoter activation and interferon regulatory factor 3 (IRF3) function, and inhibition of the
717 interferon-stimulated response element (ISRE) promoter signaling pathway in human (HEK-
718 293T) and/or primate kidney (Vero) cells (24). Specifically, it inhibits PACT-induced activation

719 of retinoic acid-inducible gene 1 (RIG-I) and melanoma differentiation-associated protein 5
720 (MDA5), which are key cytosolic recognition receptors of virus-derived RNAs (25).
721 Furthermore, preliminary data show that MERS-CoV, but not SARS-CoV, may employ an
722 additional mechanism to antagonize ISG via altered histone modification which affects a diverse
723 spectrum of biological processes including gene regulation (170). With the attenuated interferon
724 response at the cellular level, the virus may then employ the deISGylating and deubiquitinating
725 activities of its papain-like protease to take over the host metabolic apparatus (28, 172, 173).
726 Efficient viral replication may follow and result in cell damage through direct virus-induced
727 cytolysis or immunopathology via dysregulated pro-inflammatory cytokine induction.

728 In addition to these *in vitro* data, the roles of the different branches of the innate immune
729 response have been assessed in a limited number of animal models and patients. MERS-CoV
730 infection is more severe in knockout C57BL/6 and BALB/c mice with impaired type I interferon
731 or Toll-like receptor signaling than those with impaired RIG-I-like receptor signaling, suggesting
732 that the former signaling pathways are more important for controlling the infection (174). The
733 depletion of natural killer cells, a major cellular component of the innate immune response, does
734 not significantly affect the clinical disease severity or viral clearance kinetics (174). In rhesus
735 macaques, the innate immune response occurs and resolves very rapidly after MERS-CoV
736 inoculation. A type I interferon response is observed on days 1 and 2 and disappears on day 3
737 after infection (166, 175). Robust but transient up-regulation of the expression levels and
738 elevated serum levels of proinflammatory cytokines and chemokines including interleukin-6 (IL-
739 6), chemokine (C-X-C motif) ligand 1 (CXCL1), and matrix metalloproteinase 9 (MMP9) are
740 associated with chemotaxis and activation of neutrophils as evidenced by increased numbers of
741 neutrophils in the blood and lungs of the infected animals (166). In humans who develop severe

MERS, significant differences are noted between the innate immune responses of fatal and non-fatal cases. Compared to a patient who survived, a patient who died from MERS induced lower expression levels of RIG-I and MDA-5, which led to decreased expression levels of IRF3 and IRF7 (176). This was associated with a major decrease in the amount of mRNA and protein of interferon-α in serum and bronchoalvelolar lavage. Additionally, the antigen presentation pathway was broadly down-regulated and affected types I and II major histocompatibility (MHC) genes which were associated with significantly lower expression levels of the key cytokines involved in the activation of lymphocytes into CD4+ Th1 cells, including IL-12 and interferon-γ (176, 177). Increased levels of IL-17A and IL-23 in the serum and bronchoalveolar lavage within the first week after symptom onset, and persistent uncontrolled secretion of the type-1 interferon-triggered CXCL10 and IL-10 beyond the first week after symptom onset, were noted in fatal MERS cases and might be associated with poor outcome as in SARS and other respiratory viral infections (176, 178-181). A poorly coordinated innate immune response with ineffective activation of the adaptive immune response that failed to clear MERS-CoV viremia appeared to be associated with fatal outcome (176, 182).

Adaptive Immune Response

Systematic study on the adaptive immune response to MERS in large cohorts of human cases is lacking. T-cell or combined T- and B-cell deficiencies, but not B-cell deficiency, were found to be associated with persistent infections and lack of virus clearance in C57BL/6 and BALB/c mice transduced with adenoviral vectors expressing human DPP4, highlighting the important role of T cells in acute clearance of MERS-CoV (174). In terms of antibody-mediated immunity which is essential for protection against subsequent challenge by the virus, the CD8 T-cell

765 response to the immunodominant epitopes located in the MERS-CoV S protein is shown to peak
766 at days 7 to 10 post-infection and exhibits only low level of cross-reactivity with the T-cell
767 response to SARS-CoV infection (174). In rhesus macaques infected with MERS-CoV, serum
768 neutralizing antibodies are detected on as early as day 7 post-infection, reaching a peak titer on
769 day 14 post-infection, and decreasing slightly in titer on day 28 post-infection. In patients with
770 MERS, high titers of serum neutralizing antibodies can be detected on day 12 and persist for at
771 least 13 months after symptom onset (66, 72, 81, 183). Both IgM and IgG against S and N
772 proteins are detectable in the sera of infected patients on day 16 after symptom onset, with the
773 titer of IgG being at least 10 times higher than that of IgM, suggesting that the initial IgM
774 antibody response is likely mounted before this time period (72). IgG titers peaked at three
775 weeks after symptom onset, while IgM titers remained elevated between two to five weeks after
776 symptom onset in a patient (184). Notably, serum anti-MERS-CoV antibodies were undetectable
777 in a patient who died on days 26 and 32 after symptom onset, suggesting that inadequate
778 antibody response may be associated with poor clinical outcome (66). The exact onset and
779 changes in titer of serum neutralizing anti-MERS-CoV antibodies should be further evaluated in
780 subsequent clinical cohorts consisting of patients with different severities and outcomes.
781 Moreover, given the *in vitro* observation that the viral fitness and evolution may be restricted by
782 the immunodominance of the anti-MERS-CoV-RBD neutralizing antibody response that blocks
783 binding to human DPP4, B cell-associated antibodyome studies from MERS patients should be
784 performed to further assess the role that immunoglobulin polymorphisms play in determining the
785 protective antibody repertoire and clinical outcomes (40).
786
787 **Organ-Specific Pathology and Systemic Virus Dissemination**

788 Although *in vitro* cell line studies and even e*x vivo* organ cultures may not completely represent
789 *in vivo* scenarios, they have provided insightful clues to explain the pathogenesis involved in the
790 pulmonary and extrapulmonary manifestations of MERS, before findings from animal models
791 and post-mortem examination are available (Table 6). The *in vitro* observation that MERS-CoV
792 replicates more efficiently in a variety of lower respiratory tract cell lines than in upper
793 respiratory tract cell lines, and the inability of the human bronchial epithelium to mount a timely
794 and adequate innate immune response against MERS-CoV infection in the absence of
795 professional cytokine-producing cells including dendritic cells and macrophages may partially
796 explain the high incidence of severe cases in MERS (116, 157, 171, 185-188). The finding in *ex*
797 *vivo* culture systems that MERS-CoV is capable of infecting most cell types of the human
798 alveolar compartment including non-ciliated and possibly ciliated epithelial cells, types I and II
799 pneumocytes, and endothelial cells of pulmonary vessels further supports the notion that all the
800 host cell factors necessary for viral replication are available in the human lung (187, 189-191).
801 Additionally, MERS-CoV can also infect pulmonary vascular endothelial cells and lung
802 macrophages, which corroborates with the clinical observation of systemic dissemination of the
803 virus with viremia in severe cases (191).

804 Besides lower respiratory tract cells, MERS-CoV also exhibits a peculiar tropism for
805 renal cells that is not seen in any other CoVs associated with human infections and not
806 explainable by the expression of their respective host cell receptors. Avian nephropathogenic
807 infectious bronchitis virus may cause lymphoplasmacytic interstitial nephritis, but rarely
808 pneumonia, in broiler chickens (192). MERS-CoV replicates efficiently to about 5 logs above the
809 baseline titer with abundant N protein expression and prominent cytopathic effects (CPE) within
810 72 hours after infection in human embryonic kidney cells (116). In primary kidney epithelial

811 cells and primary bronchial epithelial cells infected with either MERS-CoV or SARS-CoV,
812 pronounced CPE with rounding, detachment, and death of the majority of cells occur only in
813 primary kidney epithelial cells infected with MERS-CoV, although viral replication was
814 detectable with both viruses (157). The concentration of infectious MERS-CoV progeny in
815 primary kidney epithelial cells was almost 1000-fold higher than that in primary bronchial
816 epithelial cells (157). Together with the clinical observation that MERS-CoV RNA may be
817 detectable in the urine without viremia after almost 2 weeks of symptom onset, these *in vitro*
818 findings suggest that the kidney may be a potential site of autonomous virus replication (72,
819 157). Comparable findings are also observed in many bat and primate kidney cell lines, although
820 clinical disease in these animals is much milder than in humans and viral RNA is not detectable
821 in the kidneys of infected rhesus macaques (116, 117). As in the case of *ex vivo* lung cultures, it
822 would be important to elucidate the specific pathways involved in virus-host cell interactions
823 affecting different cell types such as podocytes in the renal cortex and others in the medulla
824 which are often involved in renal disease pathogenesis.

825 In view of the pronounced systemic inflammatory response with multi-organ involvement
826 and hematological abnormalities seen in patients with MERS, the specific roles of immune cells
827 in the pathogenesis of the disease have been investigated. Among the immune cells, human
828 histiocytes efficiently support viral replication with N protein expression *in vitro* on as early as
829 day 1 post-infection, while increased viral RNA levels without N protein expression are
830 detectable in human monocyte and T lymphocyte cell lines (116). Correspondingly, *ex vivo*
831 culture systems of human monocyte-derived dendritic cells and macrophages confirm that
832 MERS-CoV can productively infect both of these important professional antigen-presenting cell
833 types with high-level and persistent induction of immune cell-recruiting cytokines (191, 193).

This leads to recruitment and infiltration of a large number of immune cells into the infected lung tissues as is seen clinically. Moreover, the sequestration of lymphocytes at infected tissues resulting from the induction of CXCL10 and monocyte chemotactic protein 1 (MCP-1) may also explain marked peripheral lymphopenia that is commonly seen in MERS (191). Together with the wide distribution of DPP4 in different human cell types, the ability of MERS-CoV to hijack these professional antigen-presenting cells as vehicles for systemic dissemination to and induction of immunopathology at various organs may help to explain the unusually severe multi-organ involvement in MERS.

LABORATORY DIAGNOSIS

There are no pathognomonic clinical, biochemical, or radiological features that reliably differentiate MERS from other causes of acute community- or hospital-acquired pneumonia. Due to the lack of Biosafety Level 3 (BSL-3) containment, nucleic acid amplification assays are the most widely used method to provide laboratory confirmation of MERS with a short turn-around time using a unified testing protocol that was established early on in the epidemic. The WHO criteria for a laboratory-confirmed case include either a positive RT-PCR result for at least two different specific targets on the MERS-CoV genome, or one positive RT-PCR result for a specific target on the MERS-CoV genome and an additional different RT-PCR product sequenced, confirming identity to known sequences of MERS-CoV (Table 8) (http://www.who.int/csr/disease/coronavirus_infections/MERS_Lab_recos_16_Sept_2013.pdf?ua=1). Isolation of infectious MERS-CoV from respiratory tract specimens, and possibly also blood, urine, and fecal samples, also provides laboratory confirmation, but virus isolation has a longer turn-around time than nucleic acid amplification assays, requires experienced staff for

interpretation of CPE and confirmation of infection by RT-PCR or immunostaining. Serological assays for detection of specific neutralizing anti-MERS-CoV antibodies in paired sera, taken at the acute and convalescent phases 14 to 21 days apart, also provides evidence of infection, but none of the serological assays developed so far has been thoroughly validated or compared against each other. Furthermore, viral culture and neutralizing antibody detection assays using whole virus require BSL-3 containment, which is not widely available in standard clinical microbiology laboratories.

Specimen Collection

The ideal clinical specimen for laboratory diagnosis is one which can be readily obtained by non-invasive means and contains a large number of infected cells with high viral load. Although lower respiratory tract specimens including tracheal aspirate and bronchoalveolar lavage contain higher viral loads and genome yields than upper respiratory tract specimens and sputum, they require invasive procedures for collection and may not be easily obtainable in the early phase of illness (71, 72, 194). Therefore, upper respiratory tract specimens including nasopharyngeal aspirate or swabs, and oropharyngeal swabs are the most commonly collected specimens in suspected cases of MERS. Clinical specimens from extrapulmonary sites, especially urine, feces, blood, and/or tissues, may occasionally be positive and should also be collected if available, especially for their possible impact on infection control implementation (71, 72, 81, 176, 182). Notably, the diagnosis of MERS in a Tunisian patient was established by RT-PCR targeting the upE and N genes followed by nucleotide sequencing of RNA from a serum sample collected 10 days after symptom onset, whereas his mini-bronchoalveolar lavage tested negative (74). As for the optimal timing of specimen collection, there is a lack of data on the viral shedding kinetics of

MERS-CoV in infected humans over time. Analysis of a limited number of laboratory-confirmed MERS cases suggests that the pattern may be more similar to that of SARS than that of other HCoV infections (195). Thus, the viral load of MERS-CoV in nasopharyngeal specimens may also peak in the second week of illness rather than at symptom onset (163, 182, 196, 197). Repeated testing of upper and preferably lower respiratory tract specimens at different time points should be performed in suspected cases of MERS even when the first samples have tested negative (77, http://www.who.int/csr/disease/coronavirus_infections/MERS_Lab_recos_16_Sept_2013.pdf?ua=1). Virus shedding in the upper respiratory tract may be found in up to 30% of case contacts with minimal symptoms (198). Severe cases appear to have more prolonged virus shedding than mild cases (198). In critically ill patients who may have detectable MERS-CoV RNA in respiratory tract specimens and/or blood for more than three weeks, continued compliance with infection control measures is required during patient-care procedures as a precautionary measure despite the presence of serum neutralizing antibody (88, 176, 182, 184). Aerosol-generating procedures for specimen collection should be performed under strict compliance with droplet precautions along with additional measures including the wearing of a N95 respirator, eye shield, long-sleeved gown and gloves in an adequately ventilated room (http://www.who.int/csr/disease/coronavirus_infections/IPCnCoVguidance_06May13.pdf?ua=1). The specimens should be sent to the laboratory in viral transport medium as soon as possible after collection, or be stored at -80^{o}C if delay in transfer was expected (http://www.who.int/csr/disease/coronavirus_infections/MERS_Lab_recos_16_Sept_2013.pdf?ua=1).

Nucleic Acid Amplification Assays

With the successful isolation and propagation of MERS-CoV and sequencing of its complete genome early in the epidemic, specific primers and a standardized laboratory protocol were rapidly developed and evaluated (199). Several gene targets can be used for RT-PCR as screening and/or confirmatory testing for MERS-CoV (Table 8). The most widely adopted approach uses the upE assay as a screening test, followed by the ORF1a or the ORF1b assays as confirmation. If the ORF1a assay or the ORF1b assay is negative or equivocal despite a positive upE assay, further testing of other specific gene targets, including the N, RdRp, and/or S genes, followed by amplicon sequencing, should be performed. If further testing is not available, but the patient had a compatible epidemiological and clinical history, then the case is considered to be a probable case of MERS (http://www.who.int/csr/disease/coronavirus_infections/MERS_Lab_recos_16_Sept_2013.pdf?ua=1). Notably, assays targeting the abundant N gene may be more sensitive than those targeting the other genes, although direct comparison with the upE assay in human clinical specimens has not been performed (133). However, a 6-nt deletion was found in N gene of the strain from the second laboratory-confirmed patient when compared to the one obtained from the first patient, and therefore potential false-negative results due to mutations in this region may occur (62). For all positive cases, a second sample should preferably be tested to exclude false-positive results due to amplicon carryover. Other novel diagnostic approaches for MERS which have short turnaround times, high sensitivities and specificities include reverse transcription loop-mediated isothermal amplification and reverse transcription isothermal recombinase polymerase amplification assays which may be useful in areas without easy access to laboratories equipped with RT-PCR and/or sequencing technologies (200, 201). Further validation using more clinical

926 specimens is required to assess their field performance.

927

928 **Antibody Detection Assays**

929 A number of assays for detection of non-neutralizing and neutralizing antibodies to MERS-CoV
930 proteins have been developed but require further validation because some antibodies against
931 βCoVs are generally known to cross-react within the genus (Table 9). Indeed, cross-reacting
932 antibodies have been found not only in immunofluorescence assays, but also in virus
933 neutralization tests, which are considered to be the most specific method of antibody detection
934 (202, 203). Therefore, the European Centre for Disease Prevention and Control recommends
935 against testing for immunofluorescent antibodies unless convalescent plasma is available to look
936 for 4-fold increase in antibody titer because false positive results may arise in single tests. Cases
937 with positive serology in the absence of PCR testing or sequencing should be considered
938 probable only if they meet the other criteria of the case definition
939 (http://www.who.int/csr/disease/coronavirus_infections/MERS_Lab_recos_16_Sept_2013.pdf?u
940 a=1). Nevertheless, antibody detection assays are important for retrospective diagnosis in
941 clinically and epidemiologically suspicious cases with negative molecular test results,
942 particularly in those with only upper respiratory tract specimens being tested. It can also be used
943 for monitoring the evolution of epidemics in human and animal seroepidemiological studies, and
944 contact tracing in outbreak investigations (126). The development of high throughput, non-whole
945 virus-based assays such as enzyme-linked immunosorbent and pseudoparticle neutralization
946 assays that do not required BSL-3 containment facilities may increase their utility especially in
947 rural parts of the Middle East and other affected areas.

948

Antigen Detection Assays

The development of antigen detection assays for MERS-CoV has only been reported in histopathological confirmation in infected tissues of animals and in cell cultures with positive CPE (166, 167, 174). Possible approaches include antigen detection with monoclonal antibodies or monospecific polyclonal antibodies against the abundantly expressed N protein using either enzyme immunoassay or immunofluroescence assay. These methods were found to be highly sensitive and specific for the laboratory diagnosis of SARS from sera and nasopharyngeal samples, and have the potential advantages of being non-labor-intensive and relatively high throughput without requiring a BSL-3 containment facility (3). More information on the timing of serum neutralizing antibody kinetics and viral shedding patterns in different clinical specimens is required to optimize these antigen detection assays.

Viral Culture

In contrast to other CoVs causing human infections, which are difficult to culture in *in vitro* systems, MERS-CoV grows rapidly in a wide range of human and non-human cell lines (Table 6) (116-118). Indeed, the first identification of MERS-CoV was achieved by inoculation of the patient's sputum sample in monkey kidney cell lines, including LLC-MK2 and Vero cell lines, for detection of CPE, before specific nucleic acid amplification assays were developed (9). MERS-CoV produces focal CPE with rounded refractile cells in various susceptible cell lines on day 5 after inoculation during primary isolation, and on as early as day 1 on subsequent passage (116). These changes then spread throughout the cell monolayers, leading to rounding and detachment of cells within 24 to 48 hours. Additionally, syncytium formation caused by fusion activity of the viral spike protein at neutral pH may be observed in LLC-MK2, Calu-3, Caco-2,

and Huh-7 cell lines, and Vero cells expressing TMPRSS2 (9, 52, 58, 116). Transmission electron microscopy of MERS-CoV-infected cells shows CoV-induced membrane structures that support RNA synthesis, including convoluted membranes surrounded by double-membrane vesicles measuring 150 to 320 nm with dense inner cores, in the perinuclear region, which is typical of cellular changes of CoV infection (58). Although the clinical use of viral culture for MERS-CoV is limited by the lack of BSL-3 facilities in most satellite hospitals, the ease of growing the virus in cell culture systems has greatly facilitated study on its pathogenesis and development of antiviral agents in reference research laboratories.

CLINICAL MANAGEMENT AND ANTIVIRALS

As in the case of other human CoV infections including SARS, specific antiviral agents with proven efficacy in randomized controlled trials are lacking for MERS (204, 205). Supportive care remains the mainstay of treatment for severe MERS cases with respiratory failure and extrapulmonary complications. ECMO has been increasingly used in severe viral pneumonia including some cases of MERS (18, 71, 153, 154, 156, 206). However, procedure-related factors such as the requirements of technical expertise and specific equipment, and patient factors including the presence of multiple comorbidities and coagulopathy may limit its use especially among patients in rural parts of the Middle East and Africa. Other forms of assisted ventilation and pulmonary rescue therapy, including mechanical ventilation using a lung protective strategy with a small tidal volume, non-invasive positive pressure ventilation, and inhaled nitric oxide have been tried for SARS and influenza with ARDS (3, 153). However, data on their efficacies in MERS are lacking (88, 207). Due to the apparently high incidence of acute and acute-on-chronic renal failure in patients with severe MERS, renal replacement therapy has been frequently used,

995 and was essential for tiding the patient over the oliguric phase (64, 88, 207). Circulatory failure
996 is supported by the use of inotropes and volume expansion (207). Broad-spectrum antibacterials
997 and neuraminidase inhibitors against influenza are used empirically before the diagnosis of
998 MERS is established (207). Antimicrobials guided by interval surveillance or sepsis work-up
999 should be used to treat secondary nosocomial infections in those with prolonged hospitalization
1000 and invasive ventilation, and opportunistic infections in patients who are immunocompromised,
1001 especially those who receive corticosteroid for immunomodulation. As in SARS,
1002 immunosuppressive dose of corticosteroid therapy should not be given because of its potential
1003 side effects and immunosuppression. Only stress dose of corticosteroid should be considered in
1004 patients with refractory shock and relative adrenal insufficiency
1005 (http://www.who.int/csr/disease/coronavirus_infections/InterimGuidance_ClinicalManagement_
1006 NovelCoronavirus_11Feb13u.pdf?ua=1).

1007 The improvement in outcome of MERS with a case-fatality rate of over 30% depends on
1008 the development of effective antiviral treatment for suppression of viral load. Candidate antiviral
1009 agents are identified using three general approaches (Table 10). The first and fastest approach is
1010 to test drugs with broad-spectrum antiviral activities including those with reported activities
1011 against other CoVs associated with human infection, particularly SARS-CoV. This approach has
1012 identified numerous agents with known antiviral mechanisms. Examples include interferons,
1013 ribavirin, and cyclophilin inhibitors (58, 208, 209). Type I interferons, which are important in the
1014 innate immunity against CoV infection, exhibit anti-MERS-CoV activity in various cell lines and
1015 also rhesus macaques. MERS-CoV is 50 to 100 times more sensitive to pegylated interferon-α
1016 than SARS-CoV in cell culture (58). Moreover, the combination of interferon-α2b and ribavirin,
1017 a purine nucleoside analogue that inhibits guanosine triphosphate synthesis and viral RNA

polymerase activity that has been widely used to treat SARS, has exhibited synergistic effects against MERS-CoV in cell cultures (209, 210). In rhesus macaques infected with MERS-CoV, this combination reduces virus replication, moderates host inflammatory response, and improves clinical outcome (175). However, the regimen's efficacy in humans remains uncertain. In a small cohort of MERS cases in KSA, all five patients who received a combination of interferon-α2b, ribavirin, and corticosteroid died. The delayed commencement of the antiviral regimen of at least two weeks after symptom onset in these patients might have reduced treatment benefit, as another patient who received treatment early on the day of admission survived, though MERS-CoV RNA remained detectable in his sputum samples until day 12 of treatment (211). A more recent retrospective cohort study showed that 20 severe adult MERS patients who received oral ribavirin and pegylated interferon-α2a (Pegasys; Roche Pharmaceuticals, Basel, Switzerland) for 8 to 10 days (initiatied on a median of 3 days after diagnosis) had significantly better survival rates at 14 days but not at 28 days after diagnosis as compared to 28 historical controls who received supportive care only (207). Possible reasons for the lack of long-term survival benefit in the treatment group include the small number of patients in the study and the fact that both ribavirin and pegylated interferon have high EC_{50} against MERS-CoV relative to their peak serum concentrations achievable at clinically relevant dosages. Cyclophilin inhibitors, such as cyclosporine A, are known to have antiviral activity against numerous human and animal coroanviruses including SARS-CoV. However, the clinical relevance of cyclosporin A for treating MERS is likely limited as the drug's peak serum level achievable with clinically relevant dosages is below its EC_{50} for MERS-CoV (58).

The second approach to identify candidate antivirals for MERS involves screening of chemical libraries that comprise large numbers of existing drugs or databases that contain

information on transcriptional signatures in different cell lines. The advantages of this approach include the commercial availability, known pharmacokinetics, and well-reported safety profiles of the identified drugs. The first agent with potent *in vitro* anti-MERS-CoV activity identified by this method was mycophenolic acid, an anti-rejection drug used in organ transplantation with broad-spectrum antiviral activities that acts by inhibiting inosine-5'-monophosphate dehydrogenase and depleting the lymphocyte guanosine and deoxyguanosine nucleotide pools (210). The combination of mycophenolic acid and interferon-β1b shows synergistic activity against MERS-CoV in Vero cells. The desirable pharmacokinetics of mycophenolic acid compared to ribavirin warrants further evaluation, although the potential inhibitory effect on the immune system and therefore neutralizing antibody production should be fully assessed in animal models before use in humans. The very low EC_{50} when compared with the peak serum level achieved at routine clinical dosages suggests that even a very low dose may be effective without inducing significant immunosuppression. A fatal case of MERS was reported in a renal transplant recipient who was receiving anti-rejection therapy consisting of prednisone, mycophenolate mofetil, and cyclosporine, but the dosage, serum drug level of mycophenolate mofetil, and the resulting lymphocyte count were not reported (68, 176). Following the identification of mycophenolic acid as an inhibitor of MERS-CoV replication *in vitro*, many other drugs have been found to exhibit *in vitro* anti-MERS-CoV activity in Vero and/or Huh-7 cells using a similar drug discovery approach. These drugs belong to a number of major pharmacological categories including peptidic or small-molecule HIV entry inhibitors, antiparasitics, antibacterials, and inhibitors of clathrin-mediated endocytosis, neurotransmitters, estrogen receptor, kinase signaling, lipid or sterol metabolism, protein processing, and DNA synthesis or repair (41, 177, 212-215). However, none of them has been tested in animal models

for MERS, and many of them have doubtful clinical relevance in human infection because of unachievable peak serum levels in relation to their EC_{50} against MERS-CoV. Two notable exceptions which warrant further evaluation in clinical trials are lopinavir and chloroquine. Lopinavir, which is routinely available as a lopinavir/ritonavir combination, shows inhibitory effects on MERS-CoV infection *in vitro* in Huh-7 cells at concentrations observed in blood during clinical use and has a well established toxicity profile (212, 213, http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317139281416). Moreover, lopinavir/ritonavir has been used successfully in the treatment of SARS in a case-control study (216). Viremia resolved after two days of combinational lopinavir/ritonavir, pegylated interferon, and ribavirin therapy in a MERS patient (184). However, virus shedding in the airway was persistent despite treatment (184). Chloroquine is an anti-malarial drug that inhibits MERS-CoV *in vitro* in Huh-7 and Vero E6 cells at a concentration achievable by standard clinical oral dosage through multiple possible mechanisms including inhibition of the pH-sensitive cathepsin L cell entry pathway through elevation of endosomal pH (212, 213, 217, http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317139281416). However, previously chloroquine has not been shown to work in BALB/c mice infected by SARS-CoV, possibly due to the lack of inhibition of other cell entry pathways utilized by the virus (218).

The third approach to identify treatment for MERS requires the development of specific antiviral agents based on novel insights into the viral genome and structural biology of MERS-CoV (219, 220). Understandably, the development of such candidate drugs is more time-consuming than that of the first two approaches. However, these tailor-made antiviral agents represent the most specific and possibly most effective therapeutic options against MERS-CoV. Of particular interests are agents that target the MERS-CoV S protein, which has essential roles

in virus-host cell receptor interaction and immunogenicity. A number of potent monoclonal antibodies targeting different epitopes on the RBD in the S1 subunit of the MERS-CoV S protein have been identified by biopanning of ultra-large non-immune human antibody libraries displayed in yeast or phage baited by the RBD (37-40). These monoclonal antibodies bind to the RBD with 10- to 450-fold higher affinity than does the RBD to the human DPP4, conferring broader and higher neutralizing activity. The production of these monoclonal antibodies in high titers may help to overcome the potential cultural hurdle in collecting large amounts of convalescent plasma from patients in the Middle East and the possibility of adverse outcomes associated with immune enhancement with low antibody titer previously observed in *in vitro* and animal experiments on SARS (221, 222). Moreover, possible selection of virus mutants capable of escaping from antibody-mediated neutralization may be mitigated by using divergent combinations of two or more synergistically acting neutralizing monoclonal antibodies that target non-cross-resistant epitopes on the RBD (40). *In vitro* inhibition of S protein-mediated cell-cell fusion and virus entry into host cell can also be achieved by specially designed antiviral peptides that span the sequence of the HR2 domain of the S2 subunit of the MERS-CoV S protein. Analogus to the HIV fusion inhibitor Enfuvirtide which binds to glycoprotein 41 of HIV to block membrane fusion and virus entry, the MERS-CoV antiviral peptides block the fusion process of MERS-CoV by preventing the interaction between the HR1 and HR2 domains required for the formation of the heterologus six-helix bundle in viral fusion core formation (44, 45). Other drug candidates that target specific enzymes of MERS-CoV include inhibitors of viral proteases and helicase. The rapid determination of crystal structure for these enzymes have facilitated the development of candidate drugs to be further tested in animal studies to evaluate their pharmacokinetics and *in vivo* inhibitory effects, especially in view of the reported mutations in

1110 the papain-like protease of recently circulating MERS-CoV strains (146, 223-226). Inhibition of
1111 MERS-CoV infection can also be achieved by agents that target the functional host cell receptor
1112 DPP4. Because of the abundance of DPP4 in epithelial and endothelial cells, high titers of
1113 monoclonal antibodies against specific binding regions of DPP4, but not the commercially
1114 available reversible, competitive DPP4 antagonists such as sitagliptin, vildagliptin, and
1115 saxagliptin, efficiently inhibit virus-cell receptor interaction (46, 50). Agents that manipulate the
1116 levels of adenosine deaminase, a natural DPP4 antagonist, may also be considered (49). The
1117 clinical efficacy of anti-DPP4 monoclonal antibodies and adenosine deaminase analogues
1118 remains uncertain because expression of catalytically inactive DPP4 still allows for MERS-CoV
1119 infection *in vitro* (227). Furthermore, the risk of physiological disturbances, immunopathology,
1120 and T cell suppression should be assessed in animal studies given the wide distribution of DPP4
1121 in different human cell types and its multiple essential metabolic and immunological functions
1122 (228, 229). Alternatively, inhibitors of host cellular proteases including TMPRSS2 and
1123 cathepsins, which affect virus entry into host cells, may be considered. However, the recent
1124 finding that cathepsin activity is essential for Ebola virus infection in cell lines but not for viral
1125 spread and pathogenesis in mice highlights the necessity to confirm the roles of cellular protease
1126 inhibitors in *in vivo* spread of MERS-CoV (230, 231). Alternative host proteases that cleave the
1127 MERS-CoV S protein should also be searched to broaden the range of existing antiviral options
1128 (51).
1129

1130 **INFECTION CONTROL AND LABORATORY SAFETY**

1131 Similar to epidemics caused by other novel emerging respiratory viruses with no herd immunity
1132 in the general population and limited effective treatment and immunization options, infection

control measures to interrupt the chain of transmission remains the cornerstone to control the MERS epidemic (3, 4, 153, 232-234). Based on the available epidemiological data, the scenario is most compatible with a combination of animal-to-human and person-to-person transmission. In endemic regions, multi-source sporadic animal-to-human transmissions occur in the community, which may be amplified under special circumstances such as the breeding seasons of dromedary camels. These primary infections may be followed by limited non-sustained person-to-person transmission among unprotected household contacts (67, 70, 73). When the patients are hospitalized, the infection is introduced into the healthcare setting where lapses in infection control measures culminate in large healthcare-associated outbreaks (66, 68, 71, 75, 235). The infection can then be disseminated beyond the Middle East by air travel of infected patients seeking medical care in other non-endemic countries (150, 236, 237).

In the community setting, the primary goals of infection control are to identify and segregate all zoonotic reservoirs and infected humans from immunologically naive persons. Besides dromedary camels, bats, and hedgehogs, other livestock species prevalent in the Middle East should be further surveyed by validated serological and virological tests to exclude unrecognized MERS-CoV infection. Before these data are available, residents in and travelers to the endemic regions should generally avoid contacting sick animals and especially camels. Contact with environments contaminated with animal bodily fluids, tissues, or feces should be avoided as MERS-CoV may be transmitted via direct contact or fomite due to prolonged environmental survival lasting for at least 48 hours at 20^{o}C in 40% relative humidity, and 24 hours at 30^{o}C in 30% relative humidity (145, 238). Consumption of unpasteurized camel milk should be cautioned against, as MERS-CoV may possibly be shed and survive in the milk of camels with active nasal or fecal virus shedding (143, 144). Early recognition of human cases

can be achieved by public education and dissemination of diagnostic tests to healthcare facilities. Testing should be performed even among asymptomatic or mildly symptomatic persons with known exposures to potential animal reservoirs or laboratory-confirmed human cases. They should also undergo medical surveillance and quarantine in healthcare facilities or at home until the incubation period is over (http://www.who.int/csr/disease/coronavirus_infections/IPCnCoVguidance_06May13.pdf?ua=1). Air travel should be restricted for laboratory-confirmed cases unless it is necessary to transfer the patient to other countries for medical care. In such cases, compliance with infection control measures including hand hygiene, wearing of personal protective equipment, and standard and transmission-based precautions should be applied by the aircraft staff and accompanying medical personnel. Though there is no documented in-flight transmission of MERS-CoV so far, the risk is estimated to be one new infection in a five-hour flight in first class, and 15 infections from a "super-spreader" in a 13-hour flight in economy class (236). Temperature checks at borders and health declarations for travelers are used in some regions, but their value in controlling international spread is unproven. The Hajj, which attracts millions of pilgrims from over 180 countries to gather in Mecca every year, poses a theoretical risk of causing massive outbreaks of MERS as in the super-spreading events of SARS. Though MERS has not been reported among pilgrims attending the annual Hajj in 2012 and 2013, the small number of subjects tested and the lack of samples collected during the pilgrimage are major limitations of the few surveillance studies conducted so far (239-241). Thus, persons at risk of developing severe infection should consider postponing the Hajj until the epidemic is under control (242, 243).

In the hospital setting, triage, early diagnosis, compliance with appropriate infection control measures, prompt isolation of suspected cases, and timely contact tracing of case contacts

are the key strategies to prevent nosocomial transmission. Indeed, the disappearance of the three clades of MERS-CoV found earlier in the epidemic suggests the possible effects of enhanced surveillance and early isolation of human cases in successfully interrupting person-to-person transmission (146). In addition to standard, contact, and droplet precautions, airborne precautions should be applied for aerosol-generating procedures such as intubation, non-invasive ventilation, manual ventilation before intubation, bronchoscopy, tracheostomy, and suctioning of the airway (244, http://www.who.int/csr/disease/coronavirus_infections/IPCnCoVguidance_06May13.pdf?ua=1). Designated healthcare workers and disposable equipments for managing laboratory-confirmed cases in adequately ventilated single rooms or airborne infection isolation rooms should be considered to limit the number of exposed contacts. All healthcare workers caring for patients with suspected or confirmed MERS should undergo medical surveillance with daily temperature checks and monitoring of the development of acute respiratory symptoms. Quarantine after unprotected exposure is necessary to prevent unrecognized asymptomatic infection that may serve as the source of nosocomial and community outbreaks (70). The duration of observation should last for at least two incubation periods as applied in the medical surveillance of other respiratory tract infections such as pandemic influenza A/H1N1/2009 (245). Although it has been suggested that transmission-based precautions for MERS patients may be stopped 24 hours after the resolution of symptoms, laboratory testing to exclude persistent virus shedding should be conducted as viral RNA can be detected in the respiratory tract specimens and/or blood of critically ill patients for over three weeks after symptom onset (88, 176, 182, 184, 211). Rarely, asymptomatic cases may also have prolonged virus shedding for more than five weeks after case contact (246). The infectivity of such prolonged viral shedding should be further evaluated to

optimize infection control strategies. Patients who have no evidence of pneumonia or who have recovered from pneumonia but remain positive for MERS-CoV RNA by RT-PCR may be discharged from the hospital and isolated at home under appropriate supervision (247). Collection of potentially infectious specimens should be performed by trained staff wearing appropriate personal protective equipment. The specimens should be transported in leak-proof double containers by hand instead of pneumatic-tube systems (http://www.who.int/csr/disease/coronavirus_infections/IPCnCoVguidance_06May13.pdf?ua=1). To prevent laboratory-related outbreaks as reported in SARS, all laboratories handling live MERS-CoV should strictly comply with WHO standards for BSL-3 laboratories.

VACCINATION

Active Immunization

Active immunization to protect at-risk humans and camels is a research priority in the control of MERS because of the lack of herd immunity and effective antivirals for humans. Based on previous experience gained from vaccine design for SARS, which shows the S protein to be one of the major immunogenic components of CoVs, a number of vaccines that target the S protein of MERS-CoV are being developed and evaluated in cell culture or animal experiments (Table 11). A viral vector-based vaccine using recombinant modified vaccinia virus Ankara expressing full-length MERS-CoV S protein induced high levels of neutralizing antibodies in BALB/c mice after intramuscular immunization (248). The possibility of induction of immunopathology as in the case of a similar viral vector-based vaccine for SARS that led to enhanced hepatitis in ferrets needs to be carefully assessed in subsequent investigations (222). Alternatively, several candidate

recombinant vaccines containing either full-length MERS-CoV S protein or the RBD of the S1 subunit have been studied for their theoretical advantages of safety and ease of consistent production based on constant conditions and well-defined immunogenic fragments. A baculovirus-based expression system and a Venezuelan Equine Encephalitis Replicon Particles approach have been successfully applied for the development of full-length MERS-CoV S protein-based recombinant vaccines (174, 249). Identification and exclusion of non-neutralizing epitopes in the immunopredominant domain of the MERS-CoV S protein may help to reduce the risk of antibody-mediated disease enhancement during future optimization of these vaccines (250). RBD-based subunit vaccines have elicited neutralizing activity against MERS-CoV in cell culture-based assays, BALB/c mice, and rabbits (31, 34, 36, 42, 251). Among five different available RBD constructs, a truncated 212-aa fragment at residues 377 to 588 of RBD fused with human IgG Fc fragment (S377-588-Fc) showed the highest DPP4-binding affinity and induced the highest titers of IgG and neutralizing antibodies in BALB/c mice and rabbits respectively (36). Intranasal vaccination of this S377-588-Fc showed stronger systemic cellular and local mucosal responses as compared to subcutaneous vaccination (43). Future research directions for these promising subunit vaccine candidates include the optimization of adjuvant substances which are required to increase the immunogenicity of subunit vaccines (252), and the inclusion of chimeric S proteins containing multiple neutralizing epitopes from divergent subgroups, as there are considerable variations in the receptor-binding subdomain region of S1 within subgroups of MERS-CoV and across different CoV groups (202).

Passive Immunization

Passive immunization using convalescent plasma or hyperimmune globulin with high titers of neutralizing antibody has been used for emerging respiratory viral infections including SARS and pandemic influenza A/H1N1/2009 with relatively few side effects (253-256). The clinical use of such therapy for MERS has not yet been evaluated in randomized controlled trials. MERS-CoV-S-driven transduction in Caco-2 cells is inhibited by convalescent patient serum in a concentration-dependent manner (51). In BALB/c mice transduced by adenoviral vectors expressing human DPP4, adoptive transfer of sera containing anti-MERS-CoV-S antibodies blocked virus attachment and accelerated virus clearance (174). The increasing number of patients recovering from MERS and enhanced international collaboration for the preparation of convalescent plasma samples will accelerate the availability of passive immunization before neutralizing monoclonal antibodies become commercially available.

ANIMAL MODELS AND ANIMALS SUSCEPTIBLE TO MERS-CoV

Contrary to SARS-CoV which can cause infection in a diverse range of susceptible mammalian species, studies on MERS-CoV have been limited by the lack of animal models which are representative of MERS in humans (Table 12). The Koch's postulates for MERS-CoV as a causative agent of MERS were fulfilled with a primate model using rhesus macaques, which demonstrated mild to moderate clinical and histopathological features as compared to the infection in humans (165). However, clinical signs varied between animals, and were usually transient, lasting for only 3 days or less in most animals, which corroborated with the robust but self-limiting inflammatory response and leukocyte activation in blood and lungs of tested animals (166). Recently, common marmosets were also found to be susceptible to MERS-CoV infection and resembled moderate to severe MERS in humans with viremia and disseminated

1269 infection as evidenced by the presence of viral RNA in blood and multiple organs (168).
1270 Nevertheless, extrapulmonary manifestations that are commonly seen in human cases of MERS,
1271 such as acute renal failure and diarrhea, were absent in both the rhesus macaque and common
1272 marmoset models. Jamaican fruit bats infected with MERS-CoV do not develop clinical signs of
1273 infection despite having respiratory and intestinal tract virus shedding up to day 9 post-infection
1274 (257). Large animals including camels and goats were also found to be susceptible to MERS-
1275 CoV infection, but they developed predominantly upper respiratory tract symptoms without
1276 pneumonia (257-259). Unlike human infection in which feces and urine might be positive for
1277 viral RNA, the extrapulmonary specimens of infected camels and goats were negative. Most
1278 small animal models that worked for SARS-CoV, including BALB/c mouse, Syrian hamster, and
1279 ferret, were not susceptible to MERS-CoV infection. Infected animals had minimal clinical signs,
1280 no detectable virus in respiratory tract and extrapulmonary specimens, and did not have
1281 seroconversion. These findings suggest that MERS-CoV fails to enter these host cells because of
1282 variable DPP4 binding affinities for MERS-CoV S RBD among different species (48). A mouse
1283 model using C57BL/6 and BALB/c mice with prior transduction of respiratory epithelial cells
1284 with adenoviral vectors expressing human DPP4 inoculated with MERS-CoV intranasally
1285 showed virological, immunological, and histopathological features compatible with interstitial
1286 pneumonia, but the clinical signs were mild and evidence of infection was confined to the lungs
1287 without extrapulmonary involvement (174). Furthermore, it requires infection of the mice with
1288 the adenoviral vectors prior to every experiment, and it is unknown whether the differences in
1289 the targeted cells between the murine and human lungs may affect the immunological response
1290 and clinical progress after infection. Nonetheless, this inhaled-adenoviral vector method allows
1291 the quick use of a wide variety of pre-existing genetically modulated mice with

immunodeficiencies to dissect the elements of host responses to MERS-CoV, and can be used to test candidate drugs and vaccines *in vivo*. It also provides a rapid model for any novel emerging respiratory viruses before appropriate receptor-transgenic mouse models become available. Further refinement of small animal models that are more representative of MERS in humans is urgently needed for evaluation of the efficacy of therapeutic and immunization options with *in vitro* activity.

CONCLUSIONS

In contrast to the public health chaos in the early phase of the SARS outbreak, the global health community has demonstrated efficient and collaborative efforts to handle the MERS epidemic. The clinical experience gained in SARS and the genomic data accumulated for other human and animal CoVs discovered after SARS have facilitated the rapid development of diagnostic assays, design of candidate antiviral agents and vaccines, rationalization of infection control measures, and identification of zoonotic reservoirs for MERS (93, 104-107, 260-271). The MERS epidemic has greatly enhanced our understanding of coronavirology and provided lessons that will be useful for tackling future CoV outbreaks. Camels are now recognized as an important animal reservoir for lineage A and C βCoVs and other viruses (140, 272, 273). Continued surveillance of novel CoVs among different animal species, especially bats and mammals with frequent close contact with humans, will strengthen our preparedness to face other emerging CoVs resulting from interspecies transmissions in the future. The identification of DPP4 as a functional receptor of MERS-CoV has expanded the list of membrane ectopeptidases known to be targeted by CoVs and has increased our understanding on the pathogenesis of CoV infections. Finally, the newly identified antiviral agents in drug-repurposing programs for MERS represent additional drug

candidates that can be evaluated for novel CoVs that lack specific treatment options. Looking ahead, the successful control of the expanding MERS epidemic will depend on the development of an effective camel vaccine to stop ongoing camel-to-human transmissions, compliance with infection control measures, and timely contacting tracing to prevent secondary healthcare-associated outbreaks. The key research priorities to optimize the clinical outcomes of MERS include more in-depth understanding on the pathogenesis from post-mortem studies and serial patient samples, testing of antiviral and vaccine candidates in more representative small animal models, and evaluation of the efficacy of currently available therapeutic options in randomized controlled trials in humans. Monitoring of the molecular evolution of MERS-CoV will facilitate early recognition of further viral adaptations for efficient person-to-person transmission.

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1335 **Table 1** Comparison between MERS and SARS

Characteristics	Middle East respiratory syndrome (MERS)	Severe acute respiratory syndrome (SARS)	References
Epidemiology			
Year of first identification	2012	2003	(2, 9)
Geographical origin	Middle East with imported cases in Europe, Africa, Asia, & North America	South China with imported cases causing large outbreaks in Canada & Asia	(a, 3)
Natural reservoir[b]	?Bats (*Neoromicia* sp. in Africa)	Chinese horse-shoe bats (*Rhinolophus sinicus* & other *Rhinolophus* sp. in China)	(3, 102, 110, 111, 274, 275)
Amplification or intermediate host[b]	Dromedary camels (Middle East & Africa)	Game food mammals (civets & raccoon dogs in southern China)	(3, 12, 114, 121, 133)
Epidemic centers of outbreaks or premises of acquisition	1. ?Camel farms 2. Hospital or household with MERS patients	1. Wild life markets & restaurants 2. Hospitals & laboratories 3. Housing estate with faulty sewage system & hotels 4. Planes	(3, 12, 75, 138, 139, 276-278)
Seasonality	May be related to camel breeding season	Winter	(c, d, 3)
Main types of transmission[e]	1. Animal-to-human 2. Person-to-person	1. Person-to-person 2. Animal-to-human	(3, 73, 138)
In-flight transmission	Not yet documented	Numerous episodes, related to physical proximity to the index patient	(3, 278)
Modes of transmission	?Droplet, contact, airborne	Contact, droplet, airborne	(3, 75, 234)
Infection control measures	Standard, contact, & droplet precautions; airborne precautions for aerosol-generating procedures	Standard, contact, & droplet precautions; airborne precautions for aerosol-generating procedures	(3, 75, 234)
Incubation period (days)	2-15	2-14, occasionally up to 21 days	(3, 63, 75, 234)
Basic reproduction number (R_0)	0.3-1.3	0.3-4.1	(3, 90, 150, 151, 279-281)
Virus-host interaction			
Causative virus	MERS-CoV	SARS-CoV	(2, 9, 165, 282)
Viral phylogeny	Lineage C βCoV	Lineage B βCoV	(2, 9)
Host receptor	DPP4 (CD26)	ACE2	(46, 283)
Major host proteases that activate spike protein	1. TMPRSS2 2. Cathepsin L 3. Furin	1. Cathepsin L 2. TMPRSS2 3. HAT	(44, 51, 52, 54, 284-287)
Dominant cell entry pathway	Cell membrane fusion	Endosomal fusion	(44, 51, 284, 288)
Cytopathic effects	Prominent syncytium formation	Few if any syncytia	(2, 3, 23,

			60, 116)
Spectrum of cell line susceptibility[f]	Broad range of animal & human tissue cells	Only a few human & primate cell lines can be infected	(3, 116-118)
Viral proteins with interferon antagonist activity	PLpro, accessory proteins 4a, 4b, & 5, & membrane protein	nsp1 protein, PLpro, accessory proteins 3b & 6, & nucleocapsid & membrane proteins	(3, 24, 25, 27, 28, 172, 289-292)
Rapid evolution of virus in human	Not yet detected	Overall Ka/Ks ratio of >1 suggests rapid evolution with strong positive selection in human strains with deletion of 29bp signature sequence or 82bp in ORF8	(3, 114, 146, 293)
Clinical features			
Presenting clinical syndrome	1. Acute community- or hospital-acquired pneumonia in elderly & patients with multiple comorbidities 2. Upper respiratory tract infection, influenza-like illness or asymptomatic infection in children & immunocompetent hosts	Acute community- or hospital-acquired pneumonia in immunocompetent & immunocompromised hosts	(2, 63, 294)
Common extrapulmonary manifestation	1. Acute renal failure 2. Diarrhea	Diarrhea	(63, 160, 196)
Radiological changes	Focal to diffuse interstitial ground glass opacities and/or consolidations	Focal to diffuse ground glass opacities and/or consolidations with pneumomediastinum	(3, 63, 152)
Common changes in blood tests	Leukopenia, lymphopenia, thrombocytopenia, impaired liver function at presentation; renal function impairment, leukocytosis & neutrophilia with progressive illness	leukopenia, lymphopenia, thrombocytopenia, ↑ alanine & aspartate aminotransferase levels	(3, 63)
Severe complications	ARDS, acute renal failure	ARDS	(3, 63)
Case-fatality rate	>35%	~10%	(g, 3, 63)
Peak viral load in respiratory secretion	Unclear	~Day 10 after symptom onset	(3, 160, 196)
Onset of neutralizing antibody	≤12 days after symptom onset	~Day 5-10 after symptom onset	(3, 66, 72, 81, 183, 295)
Specimens for diagnosis with positive viral RNA (reverse transcription-polymerase chain reaction) or culture (cell culture)	1. Lower respiratory tract: sputum, endotracheal aspirate, and/or bronchoalveolar lavage 2. Upper respiratory tract: nasopharyngeal aspirate or swab, nasal and/or throat swab 3. Extra-pulmonary: urine, feces, and/or blood 4. Tissue: biopsied and/or autopsied specimens (findings not yet reported)	1. Lower respiratory tract: sputum, endotracheal aspirate, and/or bronchoalveolar lavage 2. Upper respiratory tract: nasopharyngeal aspirate or swab, nasal and/or throat swab 3. Extra-pulmonary: urine, feces, blood, and/or cerebrospinal fluid 4. Tissue: biopsied and/or autopsied specimens	(3, 195, 296)
Criteria for positive RT-PCR test	Follow WHO criteria	Follow WHO criteria	(h, 3)

Criteria for positive antibody testing	No international standard	4-fold rise in serum (taken at least 14 days apart) neutralizing anti-SARS-CoV antibody titer (often just 4-fold rise in immunofluorescence antibody against fixed whole SARS-CoV if BSL-3 facility was not available)	(h, 3)
Key treatment measures	Ventilatory support & intensive care (ECMO & hemodialysis)	Ventilatory support & intensive care	(3, 88, 204, 234)
Antivirals used in humans in non-randomized trials	Ribavirin & interferon-α2b	Interferons (infacon1, interferon-b, leukocytic interferons) Combinations of protease inhibitor with ribavirin	(3, 207, 216)
Active immunization	Vaccines containing RBD of S1 (mice)	Recombinant S protein fragment (mice)	(3, 36, 252, 297)
Passive immunization	Adoptive transfer of sera containing anti-MERS-CoV-S antibodies blocked virus attachment in mice	Convalescent plasma therapy used in humans	(3, 174, 298)
Animal models for testing antivirals & vaccines[i]	Common marmoset; no representative small animal model of severe human disease yet	Representative models using various mammalian species including small animal models	(3, 168)

Abbreviations: ACE2, angiotensin-converting enzyme 2; ARDS, acute respiratory distress syndrome; BSL, Biosafety Level; CoV, coronavirus; DPP4, dipeptidyl peptidase-4; ECMO, extracorporeal membrane oxygenation; HAT, human airway trypsin-like protease; MERS, Middle East respiratory syndrome; ORF, open reading frame; PLpro, papain-like protease; RBD, receptor-binding domain; S, spike; SARS, severe acute respiratory syndrome; TMPRSS2, transmembrane protease serine protease-2.

[a] http://www.who.int/csr/disease/coronavirus_infections/MERS-CoV_summary_update_20140611.pdf?ua=1

[b] Please refer to Table 5 for details on animal reservoirs of MERS-CoV

[c] http://www.who.int/csr/disease/coronavirus_infections/MERS_CoV_Update_09_May_2014.pdf

[d] http://www.who.int/csr/disease/coronavirus_infections/MERS-CoV_summary_update_20140611.pdf?ua=1

[e] Both animal (especially dromedary camels)-to-human and person-to-person transmission in nosocomial outbreaks are considered to be important factors for the persistent MERS outbreak. Person-to-person transmission of SARS-CoV in “super-spreading events” and

major nosocomial outbreaks is considered to be the major transmission type in the large-scale epidemic of SARS.

[f] Please refer to Table 6 for details on tissue and host tropism of MERS-CoV

[g] http://www.who.int/csr/don/17-december-2014-mers/en/

[h] http://www.who.int/csr/disease/coronavirus_infections/MERS_Lab_recos_16_Sept_2013.pdf?ua=1

[i] Please refer to Table 12 for details on other animal modes of MERS

1351 **TABLE 2** Nomenclature and putative functional characteristics of MERS-CoV gene products with analogy to SARS-CoV[a]

Gene nomenclature (no. of amino acid residues in product)	Gene product and/or putative functional domain(s)	Characteristics and/or effect on cellular response of host	References
ORF1a/b			
nsp1 (193)	Unknown	May induce template-dependent endonucleolytic cleavage of host mRNA but not viral RNA; & may interact with cyclophilins which may be blocked by cyclosporine A.	(16, 20-22, 252, 299, 300)
nsp2 (660)	Unknown	May interact with prohibitin 1 & 2, & disrupts intracellular signaling.	(16, 20-22, 252, 301)
nsp3 (1887)	Papain-like protease	Structurally similar to the papain-like protease of SARS-CoV albeit only 30% sequence identity, consisting of a right-hand-like architecture with palm, thumb, & fingers domains. Specific conserved structural features include the ubiquitin-like domain, a catalytic triad consisting of C1594-H1761-D1776, & the ubiquitin-binding domain at the zinc finger. Functions: 1. Proteolytic processing of the viral replicase polyprotein at 3 sites (nsp1-2, 2-3, & 3-4) to generate nsps that contribute to subgenomic RNA synthesis. 2. DeISGylating (ISG15-linked ISGylation) & deubiquitinating (K48- & K63-linked ubiqutination) activities 3. Interferon antagonist: reduces induction of NF-κB, blocks phosphorylation & nuclear translocation of IRF3, & blocks upregulation of cytokines CCL5, interferon-β, & CXCL10 in HEK293T cells.	(16, 20-22, 28, 172, 173, 252, 302-305)
	ADP-ribose 1”-phosphatase	Putative dephosphorylation of Appr-1”-p, a side product of cellular tRNA splicing, to ADP-ribose.	(16, 20-22, 252)
	Transmembrane domain 1	Uncertain function, but may be similar to other CoVs including SARS-CoV in anchoring the viral replication complex through recruitment of intracellular membranes to form a reticulovesicular network of CMs & DMVs interconnected via the outer membrane with the rough endoplasmic reticulum.	(16, 20-22, 252, 306)
nsp4 (507)	Transmembrane domain 2	Similar to nsp3 & may help to form part of the viral replication complex.	(16, 20-22, 252, 306)
nsp5 (306)	Main, chymotrypsin-like, or 3C-like protease	Proteolytic processing of the replicative polyprotein at specific sites & forming key functional enzymes such as replicase & helicase.	(16, 20, 22, 252)
nsp6 (292)	Transmembrane domain 3	Membrane-spanning integral component of the viral replication complex involved in DMV formation; substitutions lead to resistance to the viral RNA synthesis inhibitor K22.	(16, 20-22, 252, 306)
nsp7 (83)	Unknown	In SARS-CoV, nsp7 & -8 are part of a unique multimeric RNA polymerase complex.	(16, 20-22, 252, 307)
nsp8 (199)	Primase		(16, 20-22, 252)

nsp9 (110)	Unknown	In SARS-CoV, nsp9 is an essential protein dimer with RNA/DNA binding activity.	(16, 20-22, 253, 308)
nsp10 (140)	Unknown	In SARS-CoV, nsp10 is required for nsp16 to bind both m7GpppA-RNA substrate & S-adenosyl-L-methionine cofactor; nsp16 possesses the canonical scaffold of MTase & associates with nsp10 at 1:1 ratio.	(16, 20-22, 253, 309)
nsp11 (14)	Unknown	Unknown	(16, 20-22, 252)
nsp12 (933)	RNA-dependent RNA polymerase	Replication & transcription to produce genome- & subgenome-sized RNAs of both polarities.	(16, 20-22, 252)
nsp13 (598)	Superfamily 1 helicase	Putative dNTPase & RNA 5'-triphosphatase activities.	(16, 20-22, 252)
	Zinc-binding domain		(16, 20-22, 252)
nsp14 (524)	3'-to'5' exonuclease	Putative endoribonuclease activity in the replication of the giant RNA genome.	(16, 20-22, 252)
	N7-methyltransferase		(16, 20-22, 252)
nsp15 (343)	Nidoviral endoribonuclease specific for U	Putative RNA endonuclease that is essential in the CoV replication cycle.	(16, 20-22, 252)
nsp16 (303)	S-adenosylmethionine-dependent ribose 2'-*O*-methyltransferase	In SARS-CoV, nsp16 is critical for capping of viral mRNA & prevents recognition by host sensor molecules.	(16, 20-22, 252, 310)
ORF2 (1353)	Spike (S) protein	A type I transmembrane glycoprotein displayed on viral membrane surface critical for receptor binding & membrane fusion.	(16, 20-22, 252)
ORF3 (103)	Accessory protein 3 (single transmembrane domain)	Deletion of ORF3, -4, & -5 accessory cluster showed ~1.5 logs reduction in viral titer compared with recombinant MERS-CoV, & resulted in enhanced expression of subgenomic gRNA2 encoding the S protein associated with an increased fusion phenotype; not essential for virus replication in Vero A66 & Huh-7 cells.	(16, 20-22, 188, 252, 311)
ORF4a (109)	Accessory protein 4a (dsRNA-binding motif)	A dsRNA-binding protein of with the dsRNA-binding domain (residues 3 to 83) that potently antagonizes host interferon response via inhibition of interferon production (interferon-β promoter activity, IRF-3/7 & NF-κB activation), ISRE promoter element signaling pathways, and/or suppression of PACT-induced activation of RIG-I & MDA5 in an RNA-dependent manner; not essential for virus replication in Vero A66 & Huh-7 cells.	(16, 20-22, 24, 25, 252, 311)
ORF4b (246)	Accessory protein 4b (single transmembrane domain)	May have interferon antagonist activity; not essential for virus replication in Vero A66 & Huh-7 cells.	(16, 20-22, 24-27, 252, 311)
ORF5 (224)	Accessory protein 5 (three transmembrane domains)	Interferon antagonist with no effect on interferon-β promoter activation; not essential for virus replication in Vero A66 & Huh-7 cells.	(16, 20-22, 27, 188, 252, 311)

ORF6 (82)	Envelope (E) protein	Putative ion channel activity & is involved in viral budding & release; essential for efficient virus propagation in Vero A66 & Huh-7 cells.	(16, 20-22, 252, 311)
ORF7 (219)	Membrane (M) protein	Surface protein that incorporates viral components into virions & interacts with N protein in infected cells; interferon antagonist.	(16, 20-22, 24, 252)
ORF8a (413)	Nucleocapsid (N) protein	Interacts with C-terminal domain of M protein for binding & packaging of viral RNA in assembly of the virion.	(16, 20-22, 252)
ORF8b (112)	Unknown	Unknown	(16, 20-22, 252)

Abbreviations: CCL5, chemokine ligand 5; CM, convoluted membrane; CoV, coronavirus; CXCL10, chemokine (C-X-C motif) ligand 10; DMV, double membrane vesicle; ds, double-stranded; IRF3, interferon regulatory factor 3; ISG, Interferon-Stimulated Gene; nsp, non-structural protein.

[a] The putative functions of the accessory gene products of MERS-CoV and SARS-CoV may not directly correlate as the accessory genes of these two viruses are not homologous.

1357 **TABLE 3** Sequence of events with epidemiological importance related to MERS

Date[a]	Place or Institution	Important event	References
19 April 2012	Zarqa, Jordan	**1st healthcare-associated cluster:** an outbreak of severe respiratory disease among 13 patients & healthcare workers in an ICU. The index patient & a close contact (ICU nurse) were subsequently confirmed to be infected with MERS-CoV in November 2012.	(66)
6 to 24 June 2012	Jeddah, KSA	**1st laboratory-confirmed case:** a 60-year-old man was admitted to a regional hospital for severe acute community-acquired pneumonia complicated with acute renal failure & later died. A novel CoV was isolated in cell culture of a sputum sample obtained on admission. The virus was initially named **human coronavirus-Erasmus Medical Center (HCoV-EMC)**.	(9)
3 September 2012	London, UK	**1st imported case in UK:** a 49-year-old man in Qatar with travel history to KSA was transferred from Doha, Qatar to an ICU in London, UK on 11 September 2012 for severe acute community-acquired pneumonia. A novel CoV was detected in combined nose & throat swab, sputum, & tracheal aspirate samples. The replicase gene fragment of this strain shared 99.5% identity with the 1st HCoV-EMC strain.	(18, 84)
23 September 2012	WHO	**WHO Disease Outbreak News**: report of the first 2 laboratory-confirmed cases.	c
25 September 2012	WHO	**1st interim case definition** for HCoV-EMC infection was issued.	d
26 September 2012	EMC, Rotterdam, the Netherlands	**1st complete genome** of HCoV-EMC was available in GenBank (accession number: JX869059).	(16)
27 September 2012	ECDC	**Protocols for real-time RT-PCR (upE & ORF1b) assays** published in *Eurosurveillance*.	(312)
5 October to 14 November 2012	KSA	**1st household cluster:** three household family members of a 70-year-old man with laboratory-confirmed HCoV-EMC infection were hospitalized for severe respiratory disease.	(67)
9 October 2012	Riyadh, KSA	**1st survived case:** a 45-year-old man who was admitted for severe respiratory disease & renal failure recovered from HCoV-EMC infection.	(64)
13 October 2012	Essen, Germany	**1st imported case in Germany**	(69)
21 December 2012	WHO	**1st interim recommendations** for laboratory testing for HCoV-EMC were issued.	e
24 January to 16 February 2013	UK	**1st cluster outside of the Middle East:** a 60-year old man with recent travel history to KSA was admitted to an ICU for laboratory-confirmed HCoV-EMC. Two of his relatives who were close contacts also developed laboratory-confirmed MERS.	(73)
5 February 2013	UK	**1st mild case:** the 30-year-old female relative in the cluster only had mild, influenza-like illness symptoms & spontaneously recovered.	(73)
8 March 2013	UAE	**1st case in UAE**	(72)
8 April to May 2013	Al-Hasa, KSA	**1st large-scale cluster:** >20 laboratory-confirmed cases of HCoV-EMC were reported in household & hospital contacts in the eastern province of KSA.	(75)
22 April 2013	Valenciennes, France	**1st imported case in France**	(68, 71)
6 May 2013	Tunisia	**1st imported cases in Tunisia**	(74)
15 May 2013	Coronavirus Study Group, ICTV	**Formal naming** of the novel CoV as **Middle East respiratory syndrome coronavirus**.	(17)
25 May 2013	Italy	**1st imported case in Italy**	f

2 June 2013[b]	Italy	**1st pediatric case:** a 2-year-old girl who was a close contact of the 1st imported case in Italy (subsequently reclassified as a probable case).	(313)
9 August 2013	Oman	1st report on the detection of **anti-MERS-CoV antibodies in dromedaries** in the Middle East.	(121)
23 August 2013	CDC	1st report on the detection of a short (182-nt) fragment of the viral RdRp gene from a fecal pellet of a ***Taphozous perforatus*** **bat in KSA** which showed 100% identity to that of MERS-CoV (strain HCoV-EMC/2012).	(109)
16 September 2013	CDC	1st report on the detection of a MERS-CoV-like virus (*Neoromicia* coronavirus) with 85.6% nt identity (complete genome) in the fecal sample of a ***Neoromicia capensis*** **bat in South Africa**.	(110, 111)
26 October 2013	Oman	**1st case in Oman**	g
17 December 2013	Qatar	1st report on the detection of **MERS-CoV RNA in nose swabs from dromedaries** by RT-PCR.	(133)
13 February 2014	Kuwait	**1st case in Kuwait**	h
17 March 2014	Yemen	**1st case in Yemen**	i
9 April 2014	Malaysia	**1st imported case in Malaysia**	(78)
13 April 2014	The Philippines	**1st imported case in the Philippines**	j
17 April 2014	Greece	**1st imported case in Greece**	(76)
18 April 2014	USA	**1st imported case in USA**	(77, 81)
22 April 2014	Egypt	**1st imported case in Egypt**	k
mid-March to May 2014	KSA & UAE	**Sudden surge** of >400 cases associated with an increase in the number of primary cases amplified by several large healthcare-associated outbreaks in KSA & UAE.	l, m
22 April 2014	Lebanon	**1st case in Lebanon**	n
1 May 2014	The Netherlands	**1st imported case in the Netherlands**	(79)
11 May 2014	Iran	**1st cases in Iran**	o
23 May 2014	Algeria	**1st imported cases in Algeria**	p
4 June 2014	KSA	1st report on **camel-to-human transmission** of MERS-CoV.	
22 September 2014	Austria	**1st imported case in Austria**	q
25 September 2014	Turkey	**1st imported case in Turkey**	r
17 December 2014	WHO	A total of **938 laboratory-confirmed cases** of MERS including at least **343 deaths** were reported.	s

Abbreviations: CoV, coronavirus; CDC, Centers for Disease Control and Prevention; ECDC, European Centre for Disease Prevention and Control; ICTV, International Committee on Taxonomy of Viruses; ICU, intensive care unit; KSA, Kingdom of Saudi Arabia; UAE, United Arab Emirates; UK, United Kingdom; USA, the United States of America; WHO, World Health Organization.

[a] The date of reported cases represents the date of symptom onset unless otherwise specified.
[b] The date of reporting by WHO.
[c] http://www.who.int/csr/don/2012_09_23/en/
[d] http://www.who.int/csr/don/2012_09_25/en/
[e] http://www.who.int/csr/disease/coronavirus_infections/LaboratoryTestingNovelCoronavirus_21Dec12.pdf?ua=1

[f] http://www.who.int/csr/don/2013_06_01_ncov/en/
[g] http://www.who.int/csr/don/2013_10_31/en/
[h] http://www.who.int/csr/don/2014_03_20_mers/en/
[i] http://www.who.int/csr/don/2014_05_07_mers_yemen/en/
[j] http://www.who.int/csr/don/2014_04_17_mers/en/
[k] http://www.who.int/csr/don/2014_05_01_mers/en/
[l] http://www.who.int/csr/disease/coronavirus_infections/MERS_CoV_Update_09_May_2014.pdf
[m] http://www.who.int/csr/disease/coronavirus_infections/MERS-CoV_summary_update_20140611.pdf?ua=1
[n] http://www.who.int/csr/don/2014_05_15_mers/en/
[o] http://www.who.int/csr/don/2014_06_11_mers/en/
[p] http://www.who.int/csr/don/2014_06_14_mers/en/
[q] http://www.who.int/csr/don/02-october-2014-mers-austria/en/
[r] http://www.who.int/csr/don/24-october-2014-mers/en/
[s] http://www.who.int/csr/don/17-december-2014-mers/en/

1381 **TABLE 4** Underlying comorbidities of patients with laboratory-confirmed MERS

Underlying comorbidities	Clinical cohorts (references)							
	(87)	(66)	(63)	(75)	(80)	(88)	(314)	Others (86, 152)
Time period	April 2012 to 22 October 2013	April 2012	1 September 2012 to 15 June 2013	1 March 2013 to 19 April 2013	1 April 2013 to 3 June 2013	May 2013 to August 2013	1 October 2012 to 31 May 2014	
Setting / Data source	161 cases reported to WHO	Retrospective outbreak investigation in Jordan	All cases reported by the KSA Ministry of Health to WHO	Outbreak investigation in 4 hospitals in Al-Hasa, KSA	A 350-bed general hospital in KSA	3 intensive care units in KSA	70 cases at a single center in Riyadh, KSA	Case reports or case series
Any comorbidity	91/120 (75.8%); fatal (86.8%) > non-fatal (42.4%) cases	NA	45/47 (95.7%); 28/45 (62.2%) fatal	NA	12/12 (100%)	NA	57/70 (81.4%)	Fatal (40/55; 72.7%) > non-fatal (30/73; 41.1%) cases
Chronic pulmonary disease	NA	NA	12/47 (25.6%); 10/12 (83.3%) fatal	10/23 (43.5%)	6/15 (40.0%)	Asthma (1/12; 8.3%)	NA	NA
Chronic renal disease	16/120 (13.3%); 20.8% of fatal cases; 2^o (23.0%) > 1^o (4.3%) cases	NA	23/47 (48.9%); 17/23 (73.9%) fatal	NA	5/15 (33.3%)	5/12 (41.7%); 1/12 (8.3%) required dialysis	NA	NA
Chronic cardiac disease	9/120 (7.5%); at least 2 fatal; 1^o (7/47, 14.9%) > 2^o (2/61, 3.3%) cases	1/8 (12.5%)	13/47 (27.7%); 10/13 (76.9%) fatal	9/23 (39.1%)	8/15 (53.3%) including 3/15 (20.0%) with CHF	MI (4/12; 33.3%), cardiac surgery (3/12; 25.0%), CHF (2/12; 16.7%), valvular disease (1/12; 8.3%), & PVD (2/12; 16.7%)	NA	Chemotherapy -induced cardiomyopathy (1/7; 14.3%)

Diabetes mellitus	12/120 (10.0%); 3.8% of fatal cases; 1^{o} (11/47, 23.4%) > 2^{o} (1/61, 1.6%) cases	NA	32/47 (68.1%); 21/32 (65.6%) fatal	17/23 (73.9%)	13/15 (86.7%)	8/12 (66.7%)	NA	3/7 (42.9%)
Hypertension	NA	2/8 (25.0%)	16/47 (34.0%); 13/16 (81.3%) fatal	NA	NA	6/12 (50.0%)	NA	3/7 (42.9%)
Obesity	NA	NA	8/47 (17.0%); 5/8 (62.5%) fatal	5/21 (23.8%)	Mean BMI: 32.02±6.78 kg/m^2	Median BMI: 31.8 (21.6 to 46.1) kg/m^2; 3/12 (33.3%) were obese	7/70 (10.0%)	1/7 (14.3%)
Smoking	NA	2/8 (25.0%)	11/47 (23.4%); 7/11 (63.6%) fatal	NA	NA	4/12 (33.3%)	9/70 (12.9%)	2/7 (28.6%)
Malignancy	NA	NA	1/47 (2.1%); fatal	NA	1/15 (6.7%)	1/12 (8.3%)	NA	2/7 (28.6%)
Others	NA	Pregnancy	Immunosuppressive therapy (3/47, 6.4%; all 3 fatal)	NA	NA	Stroke, kidney & liver transplant, & neuromuscular disease	Pregnancy	Dyslipidemia (1/7; 14.3%)

1382 Abbreviations: BMI, body mass index; CHF, congestive heart failure; KSA, Kingdom of Saudi Arabia; MI, myocardial infarction;

1383 NA, not available; PVD, peripheral vascular disease; vs, versus; WHO, World Health Organization.

1384 **TABLE 5** Evidence of zoonotic sources of MERS-CoV and closely related CoVs

Animal species (virus)	Country (area) / Specimen collection date	Main findings	References
Bats			
Superfamily *Vespertilionoidea*			
Family *Vespertilionidae*			
Asia			
Tylonycteris pachypus (Ty-BatCoV HKU4)	China (Hong Kong) / April 2005 to August 2012	Detected in 29/99 (29.3%) alimentary samples; shared 90.0% (RdRp), 67.4% (S), & 72.3% (N) aa identities with MERS-CoV (HCoV-EMC/2012)	(13, 99)
Pipistrellus abramus (Pi-BatCoV HKU5)	China (Hong Kong) / April 2005 to August 2012	Detected in 55/216 (25.5%) alimentary samples; shared 92.3% (RdRp), 64.5% (S), & 70.5% (N) aa identities with MERS-CoV (HCoV-EMC/2012)	(13, 99)
Vespertilio superans (Bat CoV-BetaCoV/SC2013)	China (Southwestern part) / June 2013	Detected in 5/32 (15.6%) anal swabs; shared 75.7% (complete genome of 1 strain) nt identity; & 96.7% (816-nt RdRp fragment) & 69.0% (S) aa identities with MERS-CoV (HCoV-EMC/2012)	(315)
Africa			
Neoromicia capensis (NeoCoV)	South Africa (KwaZulu-Natal & Western Cape Provinces) / 2011	Detected in 1/62 (1.6%) fecal sample; shared 85.6% (complete genome) nt identity; & 64.6% (S), 89.0% (E), 94.5% (M), & 91.7% (N) aa identities with MERS-CoV from humans & camels; placing them in the same viral species based on taxonomic criteria.	(110, 111)
Europe			
Pipistrellus pipistrellus, *Pipistrellus nathusii,* & *Pipistrellus pygmaeus* (*Pipistrellus* bat βCoVs)	Romania (Tulcea county) & Ukraine (Kiev region) / 2009-2011	Detected in 40/272 (14.7%) fecal samples; shared 98.2% (816-nt RdRp fragment) aa identity with MERS-CoV (HCoV-EMC/2012)	(316)
Pipistrellus kuhlii, Hypsugo savii, Nyctalus noctula, & an unknown *Pipistrellus* sp. (βCoVs)	Italy (Lombardia & Emilia regions) / 2010-2012	10 βCoVs detected in fecal specimens of *Pipistrellus kuhlii* (7), *Hypsugo savii* (1), *Nyctalus noctula* (1), & an unknown *Pipistrellus* sp. (1) bats; shared 85.2% to 87% nt identity & 95.3% to 96.1% (329-nt RdRp fragment) aa identity with MERS-CoV (HCoV-EMC/2012)	(317)
Superfamily *Emballonuroidea*			
Family *Emballonuridae*			
Taphozous perforatus	KSA (Bisha) / October 2012	A βCoV detected in 1/29 (3.4%) fecal sample; shared 100% nt identity (182-nt RdRp fragment) with MERS-CoV (HCoV-	(109)

(betaCoV)		EMC/2012)	
Superfamily *Molossoidea*			
Family *Molossidae*			
Nyctinomops laticaudatus (Mex_CoV-9)	Mexico (Campeche) / 2012	Detected in 1/5 (20.0%) rectal swabs; shared 96.5% (329-nt RdRp fragment) aa identity with MERS-CoV (HCoV-EMC/2012)	(318)
Superfamily *Noctilionoidea*			
Family *Mormoopidae*			
Pteronotus davyi (BatCoV-P.davyi49/Mexico/2012)	Mexico (La Huerta) / 2007-2010	Detected in 1/4 (25.0%) intestinal sample; shared 71.0% (439-nt RdRp fragment) nt identity with MERS-CoV (HCoV-EMC/2012)	(319)
Superfamily *Rhinolophoidea*			
Family *Nycteridae*			
Nycteris gambiensis (*Nycteris* bat CoV)	Ghana (Bouyem, Forikrom, & Kwamang) / 2009-2011	Detected in 46/185 (24.9%) fecal samples; shared 92.5% aa identity (816-nt RdRp fragment) with MERS-CoV (HCoV-EMC/2012)	(316)
Hedgehogs			
Europe			
Erinaceus europaeus (*Erinaceous* CoV)	Northern Germany / unknown date	Two clades detected in 146/248 (58.9%) fecal samples; shared 89.4% (816-nt RdRp fragment), 58.2% (S), 72.0% (E), 79.4% (M), & 72.1% (N) aa identities with MERS-CoV (HCoV-EMC/2012); RNA concentration was higher in the intestine & fecal samples than other solid organs, blood, or urine, suggestive of viral replication in the lower intestine & fecal-oral transmission; 13/27 (48.2%) sera contained non-neutralizing antibodies	(113)
Camelids			
Middle East			
Camelus dromedarius	KSA (countrywide) / 1992 to 2010; & November to December 2013	Serum Ab: 150/203 (73.9%) (2013) & 72%-100% (1992 to 2010); adults (95%) > juveniles (55%) Viral RNA: nasal > rectal swabs; juveniles (36/104; 34.6%) > adults (15/98; 15.3%) Virus isolation: two nasal swabs cultured in Vero E6 cells	(123, 137)

Camelus dromedarius	KSA (Riyadh & Al Ahsa) / 2012 to 2013	Serum nAb: 280/310 (90.3% with titer ≥1:20) adults (233/245; 95.1%) > juveniles (47/65; 72.3%)	(127)
Camelus dromedarius	KSA (Jeddah) / 3 November 2013	Direct camel-to-human transmission: phylogenetical (identical full genome sequences of patient strain & an epidemiologically-linked camel strain) & serological (the virus was circulating in the epidemiologically-linked camels but not in the patient before the human infection occurred) evidence	(138)
Camelus dromedarius	KSA (Jeddah) / 14 November to 9 December 2013	Serum Ab: 4-fold rise in paired sera in 2/9 (22.2%) Viral RNA: detected in nasal swabs of both camels (upE & ORF1a)	(128)
Camelus dromedarius	KSA (Al-Hasa) / November 2013 to February 2014 (peak calving season)	Serum nAb: 280/310 (90.3%) Viral RNA: nasal > fecal specimens Viral genome: highly stable with an estimated mutation rate of 0 nt substitutions per site per day Clinical: both calves & adults could be infected; symptoms included mild respiratory symptoms (cough, sneezing, respiratory discharge), ↑ body temperature, & ↓ appetite; acute infection was not associated with prolonged viremia or viral shedding	(320)
Camelus dromedarius	UAE (Dubai) / 2003 & 2013	Serum Ab: 151/151 (100%) (2003) & 481/500 (96.2%) (2013); high titers of nAb >1:640 in 509/651 (78.2%)	(124)
Camelus dromedarius	UAE (Dubai) / February to October 2005	Serum nAb: 9/11 (81.8%)	(125)
Camelus dromedarius	Oman / March 2013 & Spain (Canary Islands) / April 2012 to May 2013	Serum Ab: 50/50 (100%) of Omani & 15/105 (14.3%) of Spanish camels; all 50/50 (100%) of Omani (titers 1/320 to 1/2560) & 9/105 (9%) of Spanish camels had nAb (titers 1/20 to 1/320)	(121)
Camelus dromedarius	Oman (countrywide) / December 2013	Viral RNA: high concentrations in nasal & conjunvtival swabs of 5/76 (6.6%) camels (≥2 gene targets)	(321)
Camelus dromedarius	Jordan (al Zarqa governorate) / June to September 2013	Serum nAb: 11/11 (100%)	(126)
Camelus dromedarius	Qatar / 17 October 2013	Serum nAb: 14/14 (100%); titers 1/160 to 1/5120 Viral RNA: 5/14 (35.7%) nasal swabs by 3 gene targets (upE, N, & ORF1a), 1/14 (7.1%) by 2 gene targets, & 5/14 (35.7%) by 1 gene target Viral genome: 3/5 samples shared 100% identity (357-nt S fragment) with sequences from 2 epidemiologically-linked patients; further sequencing of 4.2kb concatenated fragments of a camel	(133)

		strain & 2 epidemiologically-linked patient strains: only 1 nt difference in ORF1a & 1 nt difference in ORF4b	
Camelus dromedarius	Qatar (Doha) / February 2014	Viral RNA: 1/53 (1.9%) nasal swab from an 8-month-old camel (1/53, 1.9%) (upE & N) Viral genome: complete genome (MERS-CoV camel/Qatar_2_2014) shared 99.5% to 99.9% nt identities with other camel & patient strains	(131)
Camelus dromedarius	Qatar (Al Shahaniya & Dukhan) / April 2014	Serum & milk Ab: all 33 camels had IgG in serum & milk Viral RNA: detected in the nose swabs and/or feces of 7/12 camels, & the milk of 5/7 of these camels in Al Shahaniya	(144)
Camelus dromedarius	KSA (Al Hasa, As Sulayyil, Hafar Al-Batin, Medina) / 1993, Egypt / 2014, & Australia (central Australia & Queensland) / 2014	Serum nAb: 118/131 (90.1%) of KSA camels & 0/25 (0%) of Australian camels	(322)
Africa			
Camelus dromedarius	Somalia / 1983 to 1984, Sudan / June & July 1983, Egypt / June & July 1997	Serum nAb: Somalia (70/86, 81.4%), Sudan (49/60, 81.0%) & Egypt (34/43, 79.1%) by MNT	(132)
Camelus dromedarius	Kenya / 1992 to 2013	Serum Ab: 213/774 (27.5%); including 119/774 (15.4%) with nAb; seropositive camels were found in all sampling sites throughout the study period; ↑ seroprevalence was significantly correlated with ↑ camel population density	(130)
Camelus dromedarius	Nigeria / 2010 to 2011, Tunisia / 2009 & 2013, & Ethiopia / 2011 to 2013	Serum Ab: Nigeria (94.0% of adults) & Ethiopia (93.0% of juveniles & 97.0% of adults); lower rates in Tunisia (54.0% of adults & 30.0% of juveniles)	(323)
Camelus dromedarius	Egypt (Cairo & Qalyubia governorate) / June 2013	Serum nAb: 103/110 (93.6% with titer ≥1:20) by MNT & 108/110 (98% with titer ≥1:20) by spike ppNT	(122)
Camelus dromedarius	Egypt (Alexandra, Cairo, & Nile Delta region) / June to December 2013	Serum nAb: 48/52 (92.3% with titers between 1:20 to ≥1:640); 0/179 abattoir workers Viral RNA: 4/110 (3.6%) nasal swabs (upE & ORF1a)	(134)

Abbreviations: aa, amino acid; KSA, Kingdom of Saudi Arabia; N, nucleocapsid; nAb, neutralizing antibody; nt, nucleotide; ORF, open reading frame; MNT, micro-neutralization test; RT-PCR, reverse transcription polymerase chain reaction; ppNT, pseudoparticle neutralization test; RBD, receptor-binding domain; RdRp; RNA-dependent RNA polymerase; S, spike; UAE, United Arab Emirates.

1388 **TABLE 6** Tissue and host tropism of MERS-CoV demonstrated in cell culture systems

Cell culture system	Anatomic site or animal species	Main findings[a]	References
Cell lines			
Human cell types			
Lower respiratory tract			
A549	Lung adenocarcinoma	Replication with ↑ viral load (~1-2), N protein expression & CPE	(116)
Calu-3	Polarized bronchial epithelia	Replication with ↑ viral load (~4-5), N protein expression & CPE (cell rounding, detachment, & prominent syncytia formation)	(116)
		Replication in Calu-3 cells with ↑ viral load (~5-6) & CPE at 24 hpi; infection & release of virions through both the apical & basolateral routes	(185)
HFL	Embryonic lung fibroblasts	Replication with ↑ viral load (~4-5), N protein expression & CPE	(116)
Differentiated HTBE	Human tracheobronchial epithelia	Replication with ↑ viral load (~2.5-4.5) in differentiated HTBE cells; virions released exclusively from the apical but not the basolateral side	(186)
Nondifferentiated HTBE	Human tracheobronchial epithelia	Replication with ↑ viral load (<1) in nondifferentiated but much less than that observed in differentiated HTBE cells	(186)
HAE	Pseudostratified human airway epithelia	Productive infection in HAE cultures peaks at 48 hpi: host cell factors required for cell entry, RNA synthesis, & virus assembly & release are available in human	(187)
		Replication in HAE, lung fibroblasts, type II pneumocytes, & microvascular endothelial cells; most efficient in HAE & lung fibroblasts	(188)
HBEpC	Human primary bronchial epithelium	Replication with ↑ viral load (~0.5-1) (~1000-fold lower concentrations of virus progeny than in HREpC) & without CPE	(157)
Kidney			
HEK 293	Human embryonic kidney	Replication with ↑ viral load (~4-5), N protein expression & CPE	(116)
769-P	Renal cell adenocarcinoma	Replication with ↑ viral load (~3-4)	(117)
HREpC	Human primary kidney epithelium	Replication with ↑ viral load (~3-4) (~1000-fold higher concentrations of virus progeny than in HBEpC) & CPE (rounding & detachment of cells with cell death in the majority of cells after only 20 hpi)	(157)
Colon			
Caco-2	Colorectal adenocarcinoma	Replication with ↑ viral load (~4-5), N protein expression & CPE (cell rounding, detachment, & prominent syncytia formation)	(116)
LoVo	Metastatic colonic adenocarcinoma	Replication in LoVo cells with ↑ viral load (~5-6) & CPE at 4 dpi	(185)
Liver			
Huh-7	Hepatocellular carcinoma	Replication with ↑ viral load (~4-5), N protein expression & CPE (cell aggregates with marked shrinkage)	(116)
Neuromuscular cells			

NT2	Neuro-committed teratocarcinoma	Replication with ↑ viral load (~2-3), but no N protein expression & CPE	(116)
Immune cells			
THP-1	Peripheral blood monocytes from AML	Replication with ↑ viral load (<1), but no N protein expression & CPE	(116)
U937	Monocytes from histiocytic lymphoma	Replication with ↑ viral load (<0.5), but no N protein expression & CPE	(116)
H9	T lymphocytes from T-cell leukemia	Replication with ↑ viral load (<0.5), but no N protein expression & CPE.	(116)
Jurkat_CD26DPP4+	Human T lymphocytes transfected with a human DPP4-encoded plasmid	Conversion from non-susceptible state to susceptible state with productive viral infection after plasmid transfection	(185)
His-1	Malignant histiocytoma	Replication with ↑ viral load (~3-4), N protein expression & CPE	(116)
Nonhuman cell types			
Primates			
LLC-MK2	Rhesus monkey kidney	Replication with ↑ viral load (~4-5), N protein expression & CPE	(116)
Vero	African green monkey kidney	Replication with ↑ viral load (~4-5), N protein expression & CPE	(116)
Vero-TMPRSS2	African green monkey kidney cells expressing TMPRSS2	Early appearance of large syncytia at18hpi & virus particle-induced cell-cell fusion at 3hpi	(52)
Vero E6	African green monkey kidney	Replication with ↑ viral load (~4-5) & N protein expression; slower & less obvious CPE than those in Vero cells	(58, 116)
COS-7 with DPP4	African green monkey fibroblasts transfected with a human DPP4-encoded plasmid	Conversion from non-susceptible state to susceptible state with productive viral infection after plasmid transfection	(46)
Bats			
RoNi/7	Old World bat (*Rousettus aegyptiacus*) kidney	Replication with ↑ viral load (~3-4)	(117)
PipNi/1	Old World bat (*Pipistrellus pipistrellus*) kidney	Replication with ↑ viral load (~1-2)	(117)
PipNi/3	Old World bat (*Pipistrellus pipistrellus*) kidney	Replication with ↑ viral load (~1-2)	(117)
RhiLu	Old World bat (*Rhinolophus landeri*) lung	Replication with ↑ viral load (~2-3)	(117)
MyDauNi/2	Old World bat (*Myotis daubentonii*) kidney	Replication with ↑ viral load (~1-2)	(117)
CarNi/1	New World bat (*Carollia perspicillata*) kidney	Replication with ↑ viral load (<0.5)	(117)
EFF	New World bat (*Eptesicus fuscus*) embryo	Susceptible to MERS-CoV pseudovirus infection	(23)
EidNi/41.3	Old World bat (*Eidolon helvum*) adult kidney	Replication with ↑ viral load (~10^6 PFU/ml)	(324)

EpoNi/22.1	Old World bat (*Epomops buettikoferi*) adult kidney	Replication with ↑ viral load (~10^4 PFU/ml)	(324)
HypLu/45.1	Old World bat (*Hypsignathus monstrosus*) fetal lung	Replication with ↑ viral load (~10^5 PFU/ml)	(324)
HypNi/1.1	Old World bat (*Hypsignathus monstrosus*) fetal kidney	Replication with ↑ viral load (~10^5 PFU/ml)	(324)
PESU-B5L	New World bat (*Pipistrellus subflavus*) adult lung	Did not support productive MERS-CoV infection unless transfected with a plasmid expressing human DPP4	(324)
RO5T	Old World bat (*Rousettus aegyptiacus*) embryo	Did not support productive MERS-CoV infection unless transfected with a plasmid expressing human DPP4	(324)
RO6E	Old World bat (*Rousettus aegyptiacus*) embryo	Did not support productive MERS-CoV infection unless transfected with a plasmid expressing human DPP4	(324)
RoNi/7.1	Old World bat (*Rousettus aegyptiacus*) adult kidney	Replication with ↑ viral load (~10^6 PFU/ml)	(324)
RoNi/7.2	Old World bat (*Rousettus aegyptiacus*) adult kidney	Replication with ↑ viral load (~10^6 PFU/ml)	(324)
Tb1Lu	New World bat (*Tadarida brasiliensis*) adult lung	Did not support productive MERS-CoV infection unless transfected with a plasmid expressing human DPP4	(324)
Camelids			
TT-R.B	Arabian camel (*Camelus dromedarius*) umbilical cord	Replication with ↑ viral load (~1) but without production of infectious virus particles	(118)
LGK-1-R	Alpaca (*Llama pacos*) kidney	Replication with ↑ viral load (~2-3) & production of infectious virus particles	(118)
Other mammals			
ZLu-R	Goat (*Capra hircus*) lung	Replication with ↑ viral load (~1-2) & production of infectious virus particles	(118)
ZN-R	Goat (*Capra hircus*) kidney	Replication with ↑ viral load (~3-4) & production of infectious virus particles	(118)
PK-15	Pig kidney	Replication with ↑ viral load (~4-5), N protein expression & CPE	(116)
PS	Pig kidney	Replication with ↑ viral load (<1)	(117)
RK-13	Rabbit kidney	Replication with ↑ viral load (~1-2), but no N protein expression & CPE	(116)
CL-1	Civet lung fibroblasts	Replication with ↑ viral load (~1-2), N protein expression & CPE	(116)
MDCK with human DPP4	Dog kidney transfected with a human DPP4-encoded plasmid	Conversion from non-susceptible state to susceptible state with productive viral infection after plasmid transfection	(46)
LR7 with human DPP4	Mouse fibroblasts transfected with a human DPP4-encoded plasmid	Conversion from non-susceptible state to susceptible state with productive viral infection after plasmid transfection	(46)
CRFK with human DPP4	Cat kidney cortex epithelium transfected with a human DPP4-encoded plasmid	Conversion from non-susceptible state to susceptible state with productive viral infection after plasmid transfection	(46)

BHK with human DPP4	Baby hamster kidney cells expressing human DPP4	Conversion from non-susceptible state to susceptible state after transfection with a human but not hamster or ferret DPP4-encoded expression vector	(325)
Primary ferret kidney with human DPP4	Primary ferret kidney cells expressing human DPP4	Conversion from non-susceptible state to susceptible state with after transfection with a human but not hamster or ferret DPP4-encoded expression vector	(325)
Ex-vivo organ or cell cultures			
Respiratory tract			
Lower respiratory tract	Human lung	Infection & replication in most cell types of the human alveolar compartment (ciliated & non-ciliated cells in simple columnar & simple bronchial epithelium, types I & II pneumocytes, endothelial cells of large & small pulmonary vessels, but not alveolar macrophages)	(189)
	Human bronchus & lung	Productive replication in both human bronchial & lung *ex vivo* organ cultures (non-ciliated bronchial epithelium, bronchiolar epithelial cells, alveolar epithelial cells, & endothelial cells); virions were found within the cytoplasm of bronchial epithelial cells & budding virions were found in alveolar epithelial cells (type II)	(190)
	Human lung	Infection of airway epithelial cells (pneumocytes & epithelial cells of terminal bronchioles, endothelial cells, & lung macrophages)	(191)
Immune cells			
Peripheral blood mononuclear cells	Human monocyte-derived macrophages (MDMs)	Productively infection & replication in MDMs with ↑ viral load (~3-4) & aberrant induction of inflammatory cytokines & chemokines (higher expression levels of IL-12, IFN-γ, IP-10, MCP-1, MIP-1α, IL-8, CCL-5, MHC class I & costimulatory molecules > SARS-CoV-infected MDMs)	(191)
	Human monocyte-derived dendritic cells (MoDCs)	Productive infection of MoDCs with ↑ viral load (~2-3) & significantly higher expression levels inflammatory cytokines & chemokines (IL-12, IFN-γ, IP-10, CCL-5, MHC class II & the costimulatory molecule CD86) than SARS-CoV-infected MoDCs	(193)

Abbreviations: AML, acute monocytic leukemia; CCL, chemokine C-C motif ligand; CPE, cytopathic effects; dpi, days post infection; hpi, hours post infection; IFN, interferon; IL, interleukin; IP, interferon-γ-induced protein; MCP, monocyte chemotactic protein; MHC, major histocompatibility complex; MIP, macrophage inflammatory protein; N, nucleocapsid; PFU, plaque-forming unit; TMPRSS2, transmembrane protease serine protease-2.

[a]Values of viral loads are presented in $\log_{10}$ virus RNA genome copies equivalents per mL of cell culture supernatant unless otherwise specified.

1395 **TABLE 7** Clinical, laboratory, and radiological features of MERS

Clinical, laboratory, and radiological features	Clinical cohorts (references)						
	(66)	(63)	(75)	(80)	(88)	(314)	Others (9, 18, 64, 67, 69, 71-73, 86, 152, 157, 326)
Time period	April 2012	1 September 2012 to 15 June 2013	1 March 2013 to 19 April 2013	1 April 2013 to 3 June 2013	May 2013 to August 2013	1 October 2012 to 31 May 2014	
Setting / Data source	Retrospective outbreak investigation in Jordan	All cases reported by the KSA Ministry of Health to WHO	Outbreak investigation in 4 hospitals in Al-Hasa, KSA	A 350-bed general hospital in KSA	3 intensive care units in KSA	70 cases at a single center in Riyadh, KSA	Case reports or case series
Clinical features							
Systemic							
Fever >38°C	8/9 (88.9%)	46/47 (97.9%)	20/23 (87.0%)	6/15 (40.0%)	8/12 (66.7%)	43/70 (61.4%)	6/7 (85.7%)
Chills and/or rigors	1/9 (11.1%)	41/47 (87.2%)	NA	1/15 (6.7%)	NA	NA	NA
Respiratory							
Rhinorrhea	1/9 (11.1%)	2/47 (4.3%)	NA	NA	1/12 (8.3%)	NA	NA
Sore throat	NA	10/47 (21.3%)	20/23 (87.0%)	1/15 (6.7%)	1/12 (8.3%)	NA	NA
Cough	8/9 (88.9%)	39/47 (83.0%)	NA	NA	10/12 (83.3%)	38/70 (54.3%)	7/7 (100%)
Sputum	NA	17/47 (36.2%)	NA	NA	2/12 (16.7%)	23/70 (23.9%)	3/7 (42.9%)
Hemoptysis	NA	8/47 (17.0%)	NA	1/15 (6.7%)	1/12 (8.3%)	NA	NA
Wheezing	NA	NA	NA	2/15 (13.3%)	2/12 (16.7%)	6/70 (8.6%)	NA
Chest pain	4/9 (44.4%)	7/47 (14.9%)	NA	1/15 (6.7%)	NA	NA	NA
Dyspnea	5/9 (55.6%)	34/47 (72.3%)	11/23 (47.8%)	10/15 (66.7%)	11/12 (91.7%)	42/70 (60.0%)	4/7 (57.1%)
Renal							
Acute renal failure	NA	NA	NA	NA	7/12 (58.3%)	30/70 (42.9%)	7/7 (100%) in one cohort; & 9/12 (75.0%) in another with at least 6/9 (75.0%) fatal; median time = 11±2 days from symptom onset

Gastrointestinal							
Nausea	NA	10/47 (21.3%)	NA	NA	1/12 (8.3%)	NA	NA
Vomiting	NA	10/47 (21.3%)	4/23 (17.4%)	1/15 (6.7%)	NA	21/70 (30.0%)	NA
Diarrhea	NA	12/47 (25.5%)	5/23 (21.7%)	1/15 (6.7%)	2/12 (16.7%)	21/70 (30.0%)	NA
Abdominal pain	NA	8/47 (17.0%)	NA	NA	Acute abdomen (3/12, 25.0%): ischemic bowel requiring hemi-colectomy (1) & negative laparotomies (2)	17/70 (24.3%)	1/7 (14.3%)
Other symptoms							
Myalgia	NA	15/47 (31.9%)	NA	1/15 (6.7%)	NA	14/70 (20.0%)	1/7 (14.3%)
Headache	NA	6/47 (12.8%)	NA	1/15 (6.7%)	2/12 (16.7%)	9/70 (12.9%)	
Malaise	3/9 (33.3%)	NA	NA	NA	2/12 (16.7%)	29/70 (41.4%)	1/7 (14.3%)
Complications							
Co-infections							
Bacterial & fungal	NA	0/47 (0%)	NA	NA	*Staphylococcus aureus* (1/12, 8.3%) & *Streptococcus pneumoniae* (1/12, 8.3%)	*Clostridium difficile*, multidrug-resistant bacteria (22/70, 31.4%) including CRAB, VRE, MRSA, & candidemia	*Klebsiella pneumoniae*, *S. aureus*, *S. epidermidis*, *Acinetobacter* sp., *Pseudomonas aeruginosa*; *Aspergillus fumigatus*, & *candidemia* (*Candida albicans & C. glabrata*)
Viral	NA	0/47 (0%)	NA	NA	Influenza B (1/12, 8.3%)	0/70 (0%)	Influenza A(H1N1)pdm09 (1) & type 2 parainfluenza (2)
ICU admission[a]	4/8 (50.0%)	42/47 (89.4%)	18/23 (78.3%); time from symptom onset	8/15 (53.3%)	12/12 (100%); time from symptom onset	49/70 (70.0%)	60/133 (45.1%)

			= 5 days (1 to 10 days)		to ICU admission = 2 days; duration = 30 days (7 to 104 days)		
Mechanical ventilation[a]	2/8 (25.0%)	34/47 (72.3%); time from presentation = 7 days (3 to 11 days)	18/23 (78.3%); time from symptom onset = 7 days (3 to 11 days)	NA	12/12 (100%); time from symptom onset to mechanical ventilation = 4.5 days; duration = 16 days (4 to 30 days)	49/70 (70.0%)	NA
Others	Pericarditis, pleural & pericardial effusions, arrhythmias (SVT & VT), & delirium	NA	NA	NA	Vasopressors: (8/12, 66.7%)	Delirium: (18/70, 25.7%), seizure (6/70; 8.6%), arrhythmias (11/70, 15.7%), pneumothorax (5/70, 7.1%), rhabdomyolysis (10/70, 14.3%)	2nd trimester stillbirth at 5 months of gestation
Death[a]	2/8 (25.0%); time from symptom onset = 16.5 day	28/47 (59.6%); time from presentation = 14 days (5 to 36 days); CFR ↑ with ↑ age	At least 15/23 (65.2%); time from symptom onset = 11 days (5 to 27 days)	13/17 (76.5%)	7/12 (58.3%) at day 90 of symptom onset	42/70 (60.0%)	291/837 (34.8%) (April 2012 to 23 July 2014) (86)
Laboratory features							
Hematological abnormalities							
Leukocytosis	NA	NA	3/23 (13.0%)	2/17 (11.8%)	NA	Yes	NA
Leukopenia	2/7 (28.6%)	7/47 (14.9%)	2/23 (8.7%)	1/17 (5.9%)	NA	NA	3/7 (42.9%)
Normal neutrophil count	NA	43/47 (91.5%)	NA	NA	NA	Yes	NA
Lymphocytosis	NA	5/47 (10.6%)	NA	NA	NA	NA	NA
Lymphopenia	6/7 (85.7%)	16/47 (34.0%)	NA	6/17 (35.3%)	9/12 (75.0%) on ICU admission & 11/12	Yes (median lymphocyte count,	7/7 (100%)

					(91.7%) during ICU stay	0.85x10^9/l	
Thrombocytosis	NA	NA	1/23 (4.3%)	NA	NA	NA	NA
Thrombocytopenia	NA	17/47 (36.2%)	4/23 (17.4%)	NA	2/12 (16.7%) on ICU admission & 7/12 (58.3%) during ICU stay	NA	3/7 (42.9%)
Others	DIC	NA	NA	NA	NA	DIC (10, 14.3%), anemia (median 10.7 g/dl), neutropenia	Anemia, ↑PT, ↑APTT, ↑INR, & DIC
Biochemical abnormalities							
Elevated serum ALT	NA	5 (10.6%)	NA	3/17 (17.6%)	2/12 (16.7%) on ICU admission & 5/12 (41.7%) during ICU stay	22/70 (31.4%)	NA
Elevated serum AST	NA	7/47 (14.9%)	3/13 (23.1%)	9/17 (52.9%)	2/12 (16.7%) on ICU admission & 8/12 (66.7%) during ICU stay	22/70 (31.4%); median 59 IU/l	NA
Elevated serum LDH	NA	23/47 (48.9%)	NA	8/17 (47.1%)	NA	NA	NA
Others	NA	NA	NA	NA	NA	Hypoalbuminemia	Hyponatremia, hyperkalemia, hypoalbuminemia, & ↑ serum urea, creatine kinase, troponin, C-reactive protein, & procalcitonin levels
Radiological findings	7/7 (100%) had CXR lesions in ≤3 days of presentation (uni- / bilateral ↑ bronchovascular	47/47 (100%) had CXR lesions (mild to extensive uni- / bilateral ↑ bronchovascular markings, air-	20/23 (87.0%) had CXR lesions at presentation (↑ bronchovascular markings, uni- / bilateral	Single (6/15; 40.0%) & multiple (9/15; 60.0%) CXR infiltrates; interstitial infiltrates	12/12 (100%) had CXR lesions (unilobar to bilateral diffuse air-space infiltrates)	Bi- (53/66; 80.3%) & unilateral (10/66; 15.2%) had CXR lesions	Bi- (6/7; 85.7%) & unilateral (1/7; 14.3%) had CT lesions; ground-glass opacities & consolidations

	markings, consolidation, elevated diaphragm, & cardiomegaly with pericardial effusion)	space opacities, patchy infiltrates, interstitial changes, patchy to confluent air-space consolidation, nodular opacities, reticular opacities, reticulonodular shadows, pleural effusion, & total opacification of lung segments & lobes)	infiltrates, & diffuse reticulonodular shadows)	(10/15; 66.7%) & cardiomegaly (8/15; 53.3%)	(5/7; 71.4%), isolated ground-glass opacities (1/7; 14.3%); isolated consolidation (1/7; 14.3%); smooth septal thickening (3/7; 42.9%); lower lung-predominant (5/7; 71.4%); none had tree-in-bud pattern, cavitation, or intrathoracic lymphadeopathy

Abbreviations: ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; CFR, case-fatality rate; CRAB, carbapenem-resistant *Acinetobacter baumannii*, CT, computerized tomography scan; CXR, chest radiograph; DIC, disseminated intravascular coagulation; ICU, intensive care unit; INR, international normalized ratio; KSA, the Kingdom of Saudi Arabia; LDH, lactate dehydrogenase; MRSA, methicillin-resistant *Staphylococcus aureus*, NA, not available; SVT, supraventricular tachycardia; UK, the United Kingdom; VRE, vancomycin-resistant enterococci, VT, ventricular tachycardia; WHO, World Health Organization.

[a] Values represent median time intervals

1403 **TABLE 8** Characteristics of nucleic acid amplification tests for laboratory diagnosis of MERS

Diagnostic method and target gene	Clinical specimen(s)	Recommended use	Technical LOD	Remarks	References
Nucleic acid amplification					
upE assay (upstream of E gene)	Respiratory swab, sputum, & endotracheal aspirate	Screening	1.6 to 3.4 RNA copies/reaction	Most widely used test globally	(312)
ORF1a assay (ORF1a gene)	BAL, NPA	Confirmatory for upE-positive samples	4.1 RNA copies/reaction	As sensitive as upE assay	(62, 69)
RealStar® MERS-CoV RT-PCR kit 1.0	Aspiration tube flushed with PBS, BAL, mouth exudates, nose exudates, stool, urine, CVC flushed with PBS	Screening	upE assay: 5.3 copies/reaction ORF1a assay: 9.3 copies/reaction	As sensitive as the in-house upE & 1A assays; rapid & less labor-intensive than the in-house assays	(327)
ORF1b assay (ORF1b gene)	Respiratory swab, sputum, & endotracheal aspirate	Confirmatory for upE positive samples	64 RNA copies/reaction	Less sensitive than upE & 1A assays; no overlap with those of known pan-CoV assays	(312)
RdRpSeq assay (RdRp gene & sequencing)	BAL, NPA	Screening (pan-CoV RT-PCR) & confirmatory (sequencing)	0.3 to 3.0 PFU/ml	May cross-react with other βCoVs as the gene target is highly conserved	(62, 69)
NSeq assay (N gene & sequencing)	BAL, NPA	Screening (RT-PCR) & confirmatory (sequencing)	0.03 to 0.3 PFU/ml	Highly sensitive & specific for MERS-CoV; may have deletion or mutation in the amplified fragment	(62, 69)
N2 assay (N gene)	URT, LRT, serum, stool	Screening with upE to enhance sensitivity & specificity	5 to 10 RNA copies/reaction	As sensitive as upE assay	(328)
N3 assay (N gene)	URT, LRT, serum, stool	Confirmatory of upE- or N2-positive samples	5 to 10 RNA copies/reaction	As sensitive as upE assay	(328)
RT-RPA assay (N gene)	No clinical specimen: culture supernatant	Field use (point-of-care test)	10 RNA copies/reaction	As sensitive as RT-PCR, faster TAT (≤30 minutes), & mobile	(200)
RT-LAMP	Medium containing pharyngeal swabs (healthy adults) mixed with MERS-CoV	Field use	3.4 RNA copies/reaction	As sensitive as upE & ORF1a assays, faster TAT(≤30 minutes)	(201)

Abbreviations: BAL, bronchoalveolar lavage; CoV, coronavirus; CVC, central venous catheter; Ig, immunoglobulin; LOD, lower limit of detection; LRT, lower respiratory tract; PCR, polymerase chain reaction; N, nucleocapsid; NPA, nasopharyngeal aspirate; ORF, open reading frame; RdRp, RNA-dependent RNA polyemerase; RT-LAMP, reverse transcription loop-mediated isothermal amplification; RT-PCR, reverse transcription polymerase chain reaction; RT-PRA, reverse transcription isothermal Recombinase Polymerase Amplification; TAT, turnaround time; URT, upper respiratory tract.

1409 **TABLE 9** Characteristics of antibody detection assays for laboratory diagnosis of MERS and related seroepidemiological data in

1410 human

Diagnostic method and detection target	Antigen used	Source of tested sera	Cross-reactivity	Main findings	References
IFA					
Indirect IFA (anti-MERS-CoV Ab)	Whole virus	2 laboratory-confirmed cases & blood donors	1/85 (1.2%) cross-reactive IgM in blood donors; detected in cells overexpressing recombinant S or N proteins	Better cell morphology; used as a screening test in a 2-stage protocol	(62, 69, 183)
		130 blood donors & 226 slaughterhouse workers (Jeddah & Makkah, KSA)	8/226 slaughterhouse workers had cross-reactive Ab in IFA	No evidence of widespread circulation of MERS-CoV in Jeddah & Makkah, KSA	(98)
Indirect IFA (anti-MERS-CoV Ab)	Whole virus	Animal handlers, SARS patients, & healthy blood donors in southern China	2/94 (2.1%) of animal handlers, 17/28 (60.7%) SARS patients, & 0/152 (0%) of healthy blood donors had cross-reactive anti-MERS-CoV Ab	An epitope around HR2 domain of S2 subunit may induce cross-reactivity in IFA against βCoVs.	(203)
IFA on Vero B4 cells (anti-MERS-CoV Ab)	Recombinant S & N proteins	2 serum samples from 1 patient (weeks 3 & 8)	None in samples from a few German blood donors; detected in cells overexpressing recombinant S or N proteins	Does not require optimization of infection dose & duration, & BSL-3 containment	(62, 69)
		1laboratory-confirmed case & 85 contacts	None	Helps to confirm the positive tests in conventional IFA	(183)
ELISA					
ELISA (anti-S & anti-N Ab)	S & N proteins expressed in VRP	Mouse sera	Cross-reactive anti-N Ab against MERS-CoV & other lineage 2c βCoVs; little cross-reactive anti-S Ab; no cross-reactive anti-N or anti-S Ab between MERS-CoV & SARS-	Strain specific anti-S responses with very low level of cross-reactivity within or across CoV subgroups; cross-reactive anti-N Ab within but not across CoV subgroups	(202)

			CoV or αCoVs		
Western blot					
Western blot (anti-S & anti-N Ab)	Recombinant S & N proteins	2 serum samples from 1 patient (weeks 3 & 8)	Not tested	Confirms the presence of anti-S & anti-N Ab detected in IFA	(62)
Western blot (anti-S & anti-N Ab)	S & N proteins expressed in VRP	Mouse sera	Cross-reactive anti-N Ab against MERS-CoV & other lineage C βCoVs, little cross-reactive anti-S Ab; no cross-reactive anti-N or anti-S Ab between MERS-CoV & SARS-CoV or αCoVs	Strain specific anti-S responses with very low level of cross-reactivity within or across CoV subgroups; cross-reactive anti-N Ab within but not across CoV subgroups	(202)
Protein microarray	Soluble S1 subunit of S protein	Patients with MERS, SARS, and/or other human CoV infections; & sera from cynomolgus macaques & rabbit infected with MERS-CoV	None	Allows 1-stage, high-throughput, testing with minimal sample requirement & can use dried blood spots for testing to facilitate sample transfer	(329)
Neutralization test					(195)
PRNT (anti-MERS-CoV Ab)	Whole virus	1laboratory-confirmed case & 85 contacts	None	Used as a confirmatory test in a 2-stage protocol	(183)
		130 blood donors & 226 slaughterhouse workers (Jeddah & Makkah, KSA)	8/226 slaughterhouse workers had cross-reactive Ab in IFA but not PRNT	PRNT is more specific than IFA	(98)
PRNT (anti-MERS-CoV Ab)	Whole virus	Patients with MERS, SARS, and/or other human CoV infections; & sera from camels &other animals	None in human samples	Used as a confirmatory test in a 2-stage protocol	(121)
PRNT (anti-S & anti-N Ab)	S & N proteins expressed from VRP	Mouse sera & 1patient with MERS	Very low levels of cross-neutralization of MERS-CoV by mouse antisera to SARS-CoV using high concentrations of serum	S but not N protein is the major determinant of neutralizing Ab response to MERS-CoV; N proteins of CoVs cross-react within but not between subgroups; S proteins of CoVs have little cross-neutralization or cross-reactivity within subgroup 2c or any other subgroup	(202)

Neutralization of MERS-CoV-S-driven transduction (anti-S Ab	S proteins expressed by lentiviral vectors	Sear from hospitalized children & male blood donors in KSA	None	Estimated MERS-CoV seroprevalence in the study area was <2.3% in children during 2010 to 2011, & <3.3% in male adults in 2012	(51, 97)
Microneutralization assay (neutralizing anti-MERS-CoV Ab)	Whole virus	Animal handlers, SARS patients, & healthy blood donors in southern China	0/94 (0%), 7/28 (25.0%) of SARS patients, & 0/152 (0%) of healthy blood donors had low-titer cross-reactive neutralizing anti-MERS-CoV Ab	An epitope around HR2 domain of S2 subunit may induce cross-reactive neutralizing Ab against βCoVs	(203)
Microneutralization assay (neutralizing anti-MERS-CoV Ab)	Whole virus	Human sera from general populations in Egypt & Hong Kong; MERS & SARS patients; & animal sera from Egypt	None in human samples	10 times less sensitive than the ppNT assay	(122)
ppNT assay (neutralizing anti-S Ab)	S pseudoparticle expressed by a replication-incompetent HIV virus containing a luciferase reporter gene	Human sera from general populations in Egypt & Hong Kong; MERS & SARS patients; & animal sera from Egypt	None in human samples	10 times more sensitive than the conventional microneutralization assay, does not require BSL-3 containment	(122)

Abbreviations: Ab, antibody; BAL, bronchoalveolar lavage; BSL, Biosafety Level; CPE, cytopathic effects; CVC, central venous catheter; ELISA, enzyme-linked immunosorbent assay; HIV, human immunodeficiency virus; HR2, heptad repeat 2; Ig, immunoglobulin; IFA, immunofluorescence assay; KSA, Kingdom of Saudi Arabia; LRT, lower respiratory tract; MNT, microneutralization test; N, nucleocapsid protein; NPA, nasopharyngeal aspirate; PCR, polymerase chain reaction; ppNT, pseudoparticle neutralization; PNRT, plaque reduction neutralization test; RT-PRA, reverse transcription isothermal Recombinase Polymerase Amplification; S, Spike; TAT, turnaround time; $TCID_{50}$, 50% tissue culture infective dose; URT, upper respiratory tract; VRP, Venezuelan equine encephalitis virus replicons.

1418 **TABLE 10** Antiviral agents and immunomodulators against MERS-CoV

Antiviral agents and/or immunomodulator(s)	Drug target and/or proposed mechanism	Study setting and methods (virus strain)	Main findings	References
***In vitro* studies**				
Interferons				
IFN-universal type 1	Exogenous IFN	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 113.8 U/ml	(330)
Pegylated IFN-α	Exogenous IFN	Vero (HCoV-EMC/2012)	↓ CPE at ≥1ng/ml	(58)
IFN-α2a	Exogenous IFN	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 160.8 U/ml	(330)
IFN-α2b	Exogenous IFN	Vero (HCoV-EMC/2012)	IC_{50} = 58.08 µg/ml	(209)
		LLC-MK2 (HCoV-EMC/2012)	IC_{50} = 13.26 µg/ml	(209)
		Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 21.4 U/ml	(330)
IFN-α2b (Intron A)	Exogenous IFN	Vero (HCoV-EMC/2012)	IC_{50} = 6709.79 IU/ml	(210)
IFN-β1a (Avonex)	Exogenous IFN	Vero (HCoV-EMC/2012)	IC_{50} = 5073.33 IU/ml	(210)
IFN-β1a (Rebif)	Exogenous IFN	Vero (HCoV-EMC/2012)	IC_{50} = 480.54 IU/ml	(210)
IFN-β1b (Betaferon)	Exogenous IFN	Vero (HCoV-EMC/2012)	IC_{50} = 17.64 IU/ml	(210)
		Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 1.37 U/ml	(330)
IFN-γ	Exogenous IFN	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 56.5 U/ml	(330)
Cyclophilin inhibitors				
Cyclosporin A	Inhibitor of cyclophilins & their interactions with Nsp1	Vero (HCoV-EMC/2012)	Complete inhibition of infection at 9 µM of cyclosporin A	(58)
		Huh-7 (HCoV-EMC/2012)	Partial & complete inhibition of infection at 7.5 µM & 15 µM of cyclosporin A respectively	(58)
Viral protease inhibitors				
Lopinavir	3C-like protease inhibitor	Huh-7 (HCoV-EMC/2012)	IC_{50} = 8.0 µM, SI = 3.1; 2 other MERS-CoV strains (MERS-HCoV/KSA/UK/Eng-2/2012 & MERS-HCoV/Qatar/UK/Eng-1/2012) tested were less sensitive; inhibition of a post-entry step	(213)
N3	3C-like protease inhibitor	Not available	IC_{50} = 0.28 µmol/l	(223)
CE-5	3C-like protease inhibitor	HEK293T (HCoV-EMC/2012)	IC_{50} = 12.5 µM	(224)

GRL-001	3C-like protease inhibitor	Vero (Hu/England-N1/2012)	Completely blocked viral replication at early time points (<24 hpi), ↓ viral replication by ~100-fold at 24 hpi, & ↓virus-induced cytopathology in infected cells	(225)
Helicase inhibitors				
SSYA10-001	Helicase inhibitor	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 25 μM, SI ≥ 20	(226)
Cellular protease inhibitors				
Camostat mesylate	TMPRSS2 inhibitor	Vero-TMPRSS2 (HCoV-EMC/2012)	↓ cell entry by ~15-fold (10 μM) & inhibited syncytia formation in a dose-dependent manner (1 to 100 μM)	(52)
		Calu-3 (HCoV-EMC/2012)	↓ cell entry by ~10-fold (10 μM), inhibited the multistep growth of the virus by ~90-fold (10 μM) to ~270-fold (100 μM), & delayed virus-induced cell death by 2 (10 μM) to 5 days (100 μM)	(52)
Leupeptin	Protease inhibitor	Calu-3 (HCoV-EMC/2012)	↓ virus entry into cells (10 & 100 μM)	(52)
E-64-D	Broad-spectrum cathepsin inhibitor	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 1.275 μM	(212)
EST	Cathepsin inhibitor	Vero-TMPRSS2 (HCoV-EMC/2012)	↓ virus entry into cells by ~3-fold (10 μM)	(52)
Cathepsin L inhibitor III	Cathepsin L-specific inhibitor	Vero E6 & LLC-MK2 (HCoV-EMC/2012)	↓ entry of MERS-CoV pseudovirus by 97%	(23)
MDL-28170	Cathepsins B & L inhibitor	MRC5 (HCoV-EMC/2012)	MERS-CoV-S mediated transduction was blocked	(51)
Nucleic acid and/or protein synthesis inhibitors				
Anisomycin	Protein & DNA synthesis inhibitor by inhibiting peptidyl transferase or 80S ribosome system	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 0.003 μM	(212)
Cycloheximide	Protein synthesis inhibitor	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 0.189 μM	(212)
Dasatinib	Tyrosine kinase inhibitor (ABL1 pathway)	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 5.468 μM	(212)
Emetine dihydrochloride	Protein synthesis inhibitor by binding	Vero E6 (Hu/Jordan-	IC_{50} = 0.014 μM	(212)

hydrate	to 40S ribosomal subunit	N3/2012)		
Gemcitabine hydrochloride	Nucleoside analog & DNA synthesis inhibitor	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 1.216 μM	(212)
Homoharringtonine (omacetaxine mepesuccinate)	Protein synthesis inhibitor	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 0.0718 μM	(212)
Imatinib mesylate	Tyrosine kinase inhibitor (ABL1 pathway)	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 17.689 μM	(212)
K22	Specifically targets membrane-bound viral RNA synthesis	HAE (HCoV-EMC/2012)	↓ viral replication by >4-log & substantial reduction of dsRNA (50 μM)	(306)
Mycophenolic acid	Inhibitor of IMPDH & depletion of guanosine & deoxyguanosine nucleotide pools	Vero (HCoV-EMC/2012)	IC_{50} = 0.17 μg/ml	(210)
		Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 2.87 μM	(330)
Ribavirin	Nucleoside polymerase inhibitor	Vero (HCoV-EMC/2012)	IC_{50} = 41.45 μg/ml	(209)
		Vero (HCoV-EMC/2012)	IC_{50} = 9.99 μg/ml	(210)
		LLC-MK2 (EMC/2012)	IC_{50} = 16.33 μg/ml	(209)
		Vero E6 (Hu/Jordan-N3/2012)	IC_{50} ≥250 μM	(330)
mAb against Spike protein				
Mersmab1	mAb against RBD of S1 subunit of S protein	Huh-7 (HCoV-EMC/2012)	Blocked entry of MERS-CoV-S-mediated pseudovirus into cells with ND_{50} <0.16 μg/ml	(37)
		Vero E6 (HCoV-EMC/2012)	Neutralizing inhibitory activity with ND_{50} <2 μg/ml	(37)
		Calu-3 (HCoV-EMC/2012)	Neutralizing activity with CPE inhibition	(37)
MERS-4 mAb	mAb against RBD of S1 subunit of S protein	Huh-7 (IC_{50}) & COS7 (syncytia formation) (HCoV-EMC/2012)	Inhibited syncytia formation & neutralizing inhibitory activity with IC_{50} = 0.37 nM (pseudovirus) & 3.33nM (live)	(39)
MERS-27 mAb	mAb against RBD of S1 subunit of S protein	Huh-7 (IC_{50}) & COS7 (syncytia formation) (HCoV-EMC/2012)	Neutralizing inhibitory activity with IC_{50} = 63.96 nM (pseudovirus) & 13.33nM (live)	(39)
m336 mAb	mAb against RBD of S1 subunit of S protein	Vero (live virus) & DPP4-expressing Huh-7 (pseudovirus) (HCoV-EMC/2012)	Neutralizing inhibitory activity with IC_{50} <0.01 μg/ml (live) & 0.07 μg/ml (pseudovirus); inhibited RBD-DPP4 binding (IC_{50} = 0.034 μg/ml)	(38)

m337 mAb	mAb against RBD of S1 subunit of S protein	Vero (live virus) & DPP4-expressing Huh-7 (pseudovirus) (HCoV-EMC/2012)	Neutralizing inhibitory activity with IC_{50} <0.01 µg/ml (pseudovirus) & <10 µg/ml (live); inhibited RBD-DPP4 binding (IC_{50} = 0.044 µg/ml)	(38)
m337 mAb	mAb against RBD of S1 subunit of S protein	Vero (live virus) & DPP4-expressing Huh-7 (pseudovirus) (HCoV-EMC/2012)	Neutralizing inhibitory activity with IC_{50} <0.1 µg/ml (pseudovirus) & <1 µg/ml (live); inhibited RBD-DPP4 binding (IC_{50} = 0.041 µg/ml)	(38)
1E9 scFvFc	Single-chain variable domain fragment against RBD of S1 subunit of S protein fused with hFc	Vero (live virus) & hDPP4-expressing 293T (pseudovirus) cells (HCoV-EMC/2012)	Neutralizing inhibitory activity (IC_{50} = 3.21 µg/ml)	(40)
1F8 scFvFc	Single-chain variable domain fragment against RBD of S1 subunit of Sprotein fused with hFc	Vero (live virus) & hDPP4-expressing 293T (pseudovirus) cells (HCoV-EMC/2012)	Neutralizing inhibitory activity (IC_{50} = 6.27 µg/ml)	(40)
3A1 scFvFc	Single-chain variable domain fragment against RBD of S1 subunit of S protein fused with hFc	Vero (live virus) & hDPP4-expressing 293T (pseudovirus) cells (HCoV-EMC/2012)	Neutralizing inhibitory activity (IC_{50} = 1.46 µg/ml)	(40)
3B12 scFvFc	Single-chain variable domain fragment against RBD of S1 subunit of S protein fused with hFc	Vero (live virus) & hDPP4-expressing 293T (pseudovirus) cells (HCoV-EMC/2012)	Neutralizing inhibitory activity (IC_{50} = 1.25 µg/ml)	(40)
3C12 scFvFc	Single-chain variable domain fragment against RBD of S1 subunit of S protein fused with hFc	Vero (live virus) & hDPP4-expressing 293T (pseudovirus) cells (HCoV-EMC/2012)	Neutralizing inhibitory activity (IC_{50} = 2.00 µg/ml)	(40)
3B11 scFvFc	Single-chain variable domain fragment against RBD of S1 subunit of S protein fused with hFc	Vero (live virus) & hDPP4-expressing 293T (pseudovirus) cells (HCoV-EMC/2012)	Neutralizing inhibitory activity (IC_{50} = 1.83 µg/ml)	(40)
M14D3 scFvFc	Single-chain variable domain fragment against RBD of S1 subunit of S protein fused with hFc	Vero (live virus) & hDPP4-expressing 293T (pseudovirus) cells (HCoV-EMC/2012)	Neutralizing inhibitory activity (IC_{50} = 4.30 µg/ml)	(40)
mAb against DPP4				
Clone 2F9 mAb	mAb against DPP4	Huh-7 (?strain)	Near complete inhibition of NSP4 expression in infected cells	(50)
Clone YS110 mAb	mAb against DPP4	Huh-7 (?strain)	Partial inhibition of NSP4	(50)

			expression in infected cells	
Inhibitors of clathrin-mediated endocytosis				
Astemizole	Antihistamine & anticholinergic; inhibitor of clarthrin-mediated endocytosis	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 4.884 μM	(212, 214)
Clomipramine hydrochloride	Tricyclic antidepressant; inhibitor of clarthrin-mediated endocytosis	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 9.332 μM	(212, 214)
Chlorpromazine	Antipsychotic (phenothiazine); inhibitor of clathrin-mediated endocytosis	Huh-7 (HCoV-EMC/2012)	IC_{50} = 4.9 μM, SI = 4.3. Inhibition of an early step with or without another post-entry step in the replicative cycle.	(213, 214)
		Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 9.514 μM.	(212, 214)
Fluphenazine hydrochloride	Antipsychotic (piperazine); inhibitor of clarthrin-mediated endocytosis	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 5.868 μM	(212, 214)
Promethazine hydrochloride	Antihistamine & antipsychotic (phenothiazine); inhibitor of clarthrin-mediated endocytosis	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 11.802 μM	(212, 214)
Tamoxifen citrate	Estrogen receptor inhibitor; inhibitor of clarthrin-mediated endocytosis	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 10.117 μM	(212, 214)
Thiothixene	Antipsychotic (thioxanthene); inhibitor of clarthrin-mediated endocytosis	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 9.297 μM	(212, 214)
Trifluprомazine hydrochloride	Antipsychotic (phenothiazine); inhibitor of clarthrin-mediated endocytosis	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 5.758 μM	(212, 214)
Other cell entry inhibitors				
HR2P peptide	HR2-based fusion inhibitor; inhibitor of clarthrin-mediated endocytosis	Vero (HCoV-EMC/2012)	IC_{50} = 0.6 μM	(44, 214)
		Calu-3 (HCoV-EMC/2012)	IC_{50} = 0.6 μM	(44)
		HFL (HCoV-EMC/2012)	IC_{50} = 13.9 μM	(44)
P1 peptide	HR2-based fusion inhibitor	Huh-7 (HCoV-EMC/2012)	Inhibited MERS-CoV pseudovirus with IC_{50} = 3.013μM.	(45)
dec-RVKR-CMK	Furin inhibitor	Huh-7, MRC-5, WI-38, Vero, & NHBE cells (HCoV-EMC/2012)	Dose-dependent & significant ↓ virus infection in various cell types.	(54)
S377-588-Fc protein	Recombinant truncated RBD of S protein fused with human IgG Fc fragment	Calu-3 (HCoV-EMC/2012)	Complete CPE inhibition (25 μg/ml)	(42)

HP-HSA	3-hydroxyphthalic anhydride-modified human serum albumin targeting HIV-1 gp120 and/or CD4 receptor	Huh-7 & NBL-7 (MERS-CoV pseudovirus expressing full-length S protein of HCoV-EMC/2012)	Around 90% of pseudovirus entry inhibition (20 µM); minimal cytotoxicity in Huh-7 cells at up to 100 µM	(41)
ADS-J1	Small molecule entry inhibitor targeting HIV gp41	Huh-7 & NBL-7 (MERS-CoV pseudovirus expressing full-length S protein of HCoV-EMC/2012)	CC50 = 26.9 µM, IC50 = 0.6 µM, & SI = 45	(41)
C34	Peptidic HIV entry inhibitor	Huh-7 & NBL-7 (MERS-CoV pseudovirus expressing full-length S protein of HCoV-EMC/2012)	Around 50% of pseudovirus inhibition at 20 µM in NBL cells but no activity in Huh-7 cells.	(41)
T20	Peptidic HIV entry inhibitor	Huh-7 & NBL-7 (MERS-CoV pseudovirus expressing full-length S protein of HCoV-EMC/2012)	Around 50% of pseudovirus inhibition at 20 µM in NBL cells but no activity in Huh-7 cells.	(41)
Adenosine deaminase	Natural DPP4 ligand	Huh-7 (HCoV-EMC/2012)	Dose-dependent inhibition of MERS-CoV infection	(49)
		Human DPP4 plasmid-transfected MDCK (HCoV-EMC/2012)	Blocks S1 binding & MERS-CoV infection despite expression of DPP4	(49)
Miscellaneous				
Amodiaquine dihydrochloride dihydrate	Histamine N-methyltransferase inhibitor	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 6.212 µM	(212)
Benztropine mesylate	Anticholinergic	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 16.627 µM	(212)
Chloroquine	Anti-parasitic	Huh-7 (HCoV-EMC/2012)	IC_{50} = 3.0 µM, SI = 19.4. Inhibition of an early step in the replicative cycle.	(213)
		Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 6.275 µM.	(212)
Chlorphenoxamine hydrochloride	Antihistamine & anticholinergic	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 12.646 µM	(212)
Dabrafenib	Raf inhibitor	Huh-7 (HCoV-EMC/2012)	45% inhibition (10 µM)	(215)
ESI-09	Epac-specific inhibitor	Calu-3 (HCoV-EMC/2012)	Dose-dependent CPE inhibition (1 to 10 µM) & viral yield reduction (2.5 to 40 µM); treatment before infection unnecessary; extended therapeutic window (≥20 hours); inhibitory effects starts at 6 hpi;	(331)

			CC_{50} >50 µM; changed DPP4 expression pattern on the membrane of Calu-3 cells	
		Vero E6 (HCoV-EMC/2012)	Dose-dependent CPE inhibition & viral yield reduction	(331)
Everolimus	mTOR inhibitor	Huh-7 (HCoV-EMC/2012)	56% to 59% inhibition (10 µM)	(215)
Fluspirilene	Antipsychotic (diphenylbutylpiperidine)	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 7.477 µM	(212)
Hydroxychloroquine sulfate	Anti-parasitic	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 8.279 µM.	(212)
Loperamide	µ-opioid receptor agonist	Huh-7 (HCoV-EMC/2012)	IC_{50} = 4.8 µM; SI = 3.2; inhibition of an early step in the replication cycle	(213)
Mefloquine	Inhibition of heme polyermase; serotonin agonist	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 7.416 µM	(212)
Miltefosine	AKT inhibitor	Huh-7 (HCoV-EMC/2012)	28% inhibition (10 µM)	(215)
SB203580	Kinase inhibitor	Vero E6 (HCoV-EMC/2012)	Pretreatment of infected cells with SB203580 decreased 15% & 7% of the log10 viral titer at 24 hpi & 48 hpi respectively	(177)
Selumetinib	ERK/MAPK signaling inhibitor	Huh-7 (HCoV-EMC/2012)	>95% inhibition (10 µM)	(215)
Sorafenib	Raf inhibitor	Huh-7 (HCoV-EMC/2012)	93% inhibition (10 µM)	(215)
Terconazole	Sterol metabolism inhibitor	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 12.203 µM	(212)
Thiethylperazine maleate	Antiemetic (phenothiazine)	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 7.865 µM	(212)
Toremifene citrate	Estrogen receptor inhibitor	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 12.915 µM	(212)
Trametinib	ERK/MAPK signaling inhibitor	Huh-7 (HCoV-EMC/2012)	>95% inhibition (0.1 µM)	(215)
Triparanol	Sterol metabolism inhibitor	Vero E6 (Hu/Jordan-N3/2012)	IC_{50} = 5.283 µM	(212)
Combinational treatment				
Ribavirin / IFN-α2b (1:5)	Nucleoside polymerase inhibitor / exogenous IFN	Vero (HCoV-EMC/2012)	Additional ↓ viral titer by 0.40 to 2.16-logs with ribavirin	(209)
Mycophenolic acid / IFN-β1b	IMPDH inhibitor / exogenous IFN	Vero (HCoV-EMC/2012)	IC_{50} of mycopheonlic acid = 1.7-2.8 times lower with 6.25-12.5 IU/ml of IFN-β1b; IC_{50} of IFN-β1b 1.1-1.8 times lower with 0.016-0.063 µg/ml of mycophenolic acid	(210)
MERS-4 & MERS-27	mAbs against RBD of S1 subunit of S	Huh-7 (HCoV-EMC/2012)	Synergistic neutralizing effect	(39)

mAbs	protein		against pseudovirus	
Animal experiments				
Ribavirin / IFN-α2b	Nucleoside polymerase inhibitor / exogenous IFN	Rhesus macaques (HCoV-EMC/2012) Regimen: loading dose of 30mg/kg of ribavirin i.v. & 5 MIU/kg of IFN-α2b s.c.; followed by 10mg/kg q8h of ribavirin i.m. & 5MIU/kg of IFN-α2b s.c. q16h until 72 hpi	Compared to untreated, infected macaques, treated macaques had no breathing abnormalities, minimal radiological evidence of pneumonia, lower levels of serum & pulmonary proinflammatory markers, few viral genome copies, lower expression of inflammatory genes, & less severe histopathological changes in lungs	(175)
Poly I:C	TLR3 agonist	Ad5-hDPP4-transduced mice (HCoV-EMC/2012)	Accelerated virus clearance from lungs of infected mice	(174)
Human trials				
Ribavirin / IFN-α2b / corticosteroid	Nucleoside polymerase inhibitor / exogenous interferon / corticosteroid	5 critically ill MERS patients Regimen: oral ribavirin, s.c. IFN-α2b, & i.v. and/or oral corticosteroid (methylprednisolone and/or prednisolone)	Mean age = 57.6 (24-81) years; 3 males & 2 females; admitted 4 (2-10) days after symptom onset; all had co-morbidities; time between admission & antiviral treatment = 16.8 (11-21) days & corticosteroid 15.8 (6-22) days; side effects = hemolytic anemia, thrombocytopenia, pancreatitis, ↑ lipase, & deranged liver & renal function tests; all died after a mean of 39.6 (32-52) days after admission	(332)
Ribavirin / IFN-α2b ± corticosteroid	Nucleoside polymerase inhibitor / exogenous IFN ± corticosteroid	2 epidemiologically-linked MERS patients Regimen: oral ribavirin & s.c. IFN-α2b for 2 weeks (& i.v. methylprednisolone 500mg q24h for 3 days for index case)	Both the index case (treatment) & contact (prophylaxis) had clinical & radiological improvement after receiving ribavirin & IFN-α2b	(211)
Ribavirin / IFN-α2a	Nucleoside polymerase inhibitor / exogenous IFN ± corticosteroid	20 severe MERS patients Regimen: oral ribavirin for 8-10 days & pegylated IFN-	Compared to the comparator group (28 severe MERS patients who received supportive care only), the treatment group had significantly	(207)

		α2a 180 μg/week for 2 weeks; 11/19 (58%) patients received corticosteroid	improved survival at 14 days but not 28 days after the diagnosis of MERS; significantly greater reduction in hemoglobin level was noted in the treatment group	
Ribavirin / lopinavir / IFN-α2a	Nucleoside polymerase inhibitor / protease inhibitor / exogenous IFN	1 severe MERS patient Regimen: oral ribavirin 1200mg q8h & lopinavir/ritonavir (400/100mg) q12h for 8 days, & pegylated IFN-α2a 180 μg/week for 2 weeks	Viremia resolved 2 days after initiation of antiviral treatment (started on day 13 of illness); persistent virus shedding in respiratory tract secretions until 4th week of illness	(184)

Abbreviations: ABL1, Abelson murine leukemia viral oncogene homolog 1; Ad5-hDPP4, adenovirus expressiong human host-cell receptor dipeptidyl peptidase 4; AKT, protein kinase B; CC_{50}, 50% inhibition of cell survival; DPP4, dipeptidyl peptidase 4; Epac, exchange proteins directly activated by cAMP; ERK/MAPK, extracellular signal-regulated kinases/mitogen-activated protein kinases; HAE, primary human airway epithelia; hFc, constant region fragment of human IgG; hpi, hours post infection; HR, heptad repeat; IC_{50}, 50% maximal inhibitory concentration; IFN, interferon; IMPDH, inosine-5'-monophosphate dehydrogenase; i.v., intravenous; mAb, monoclonal antibody; MIU, mega international units; mTOR, mammalian target of rapamycin; ND_{50}, 50% neutralization dose; Nsp1, non-structural protein 1; RBD, receptor-binding domain; S, spike; s.c., subcutaneous; SI, selectivity index; TLR3, Toll-like receptor 3; TMPRSS2, type II transmembrane serine protease.

1427 **TABLE 11** Active and passive immunization against MERS

Vaccine	Components (virus strain)	Animal model (administration)	Main findings (animal model)	References
Active immunization				
MVA-MERS-S	Recombinant modified vaccinia virus Ankara expressing full-length MERS-CoV S protein (HCoV-EMC/2012)	BALB/c mice (2 i.m. immunizations at days 0 & 21)	High levels of nAb were induced	(248)
VRP-S	Venezuelan Equine Encephalitis Replicon Particles containing S protein of MERS-CoV (HCoV-EMC/2012)	Ad5-hDPP4-transduced BALB/c mice (2 immunizations in the footpads at days 0 & 28)	Reduction of viral titers to nearly undetectable levels by 1 dpi	(174)
Spike protein nanoparticles	Purified S protein nanoparticles produced in Sf9 cells infected with specific recombinant baculovirus cloned with MERS-CoV S protein gene sequence (Al-Hasa_1_2013)	BALB/c mice, 6 to 8 weeks old (2 i.m. immunizations on days 0 & 21)	Inducted nAb in mice receiving MERS-CoV S inoculation with adjuvants Matrix M1 or Alum, but not in those receiving MERS-CoV S inoculation alone (Matrix M1 > Alum > no adjuvant); nAb levels were not significantly different between regimens consisting of 1 µg & 3 µg, & between sera obtained on days 21 &45	(249)
S-RBD-Fc	Recombinant protein containing RBD (residues 377 to 662) of S1 (HCoV-EMC/2012)	Mice (2 s.c. immunizations on days 0 & 14)	Sera of vaccinated mice showed neutralizing activity (>96%) against MERS-CoV pseudo- (Huh-7 cells) & live (Vero E6 cells) virus infection	(41)
358-to-588 S1-Fc	RBD (residues 358 to 588) of S1 fused with human IgG Fc fragment (HCoV-EMC/2012)	Vero cells (inoculation of sera containing polyclonal Ab raised in immunized rabbits)	Polyclonal antibodies against 358-to-588 S1-Fc variant efficiently neutralized virus infectivity	(34)
S377-588-Fc	Truncated 212-aa fragment of RBD (residues 377 to 588) of S1 fused with human IgG Fc fragment (HCoV-EMC/2012)	BALB/c mice, 6 to 8 weeks old (3 s.c. immunizations)	↑ neutralizing IgG1 (Th2) & IgG2a (Th1) Ab responses specific for the RBD in the S1 subunit were induced after each immunization with Montanide ISA 51 adjuvant	(31, 42)
		BALB/c mice, 4 to 6 weeks old (5 s.c. or i.n. immunizations at days 0, 21, 42, 3 months & 6 months)	i.n. vaccination with Poly(I:C) adjuvant induced similar degree of systemic humoral immune responses, including nAb, & more robust systemic cellular & local (lung) mucosal immune responses as comparable to those induced by s.c. vaccination with Montanide ISA 51 adjuvant	(43)

		BALB/c mice, 6 to 8 weeks old (3 s.c. immunizations); & rabbits (3 immunizations)	Among 5 versions of RBD fragments, the S377-588-Fc showed the highest DPP4-binding affinity, & induced the highest-titer IgG Ab in mice & neutralizing Ab in rabbits	(36)
rRBD (combined with different adjuvants)	Recombinant RBD protein containing a 240-aa fragment of RBD (residues 367-606) of S1(HCoV-EMC/2012) combined with different adjuvants [Alum alone, Alum plus CpG-ODNs, Alum plus Poly(I:C), or CpG-ODNs plus IFA]	BALB/c mice, 6 to 8 weeks old (3 i.m. or s.c. immunizations at days 0, 21 & 42)	The combination of rRBD and Alum plus CpG-ODNs given by the i.m. route provided the most robust RBD-specific humoral and cellular immunity.	(251)
Passive immunization				
Adoptive transfer of sera	Sera containing anti-MERS-CoV-S Ab (HCoV-EMC/2012)	Ad5-hDPP4-transduced BALB/c mice (sera obtained 2-4 weeks after immunization with VRP-S, & transferred into mice i.p. 1 day before infection)	Adoptive transfer of sera containing anti-MERS-CoV-S Ab blocked virus attachment & accelerated virus clearance to nearly undetectable levels by 5 dpi	(174)

Abbreviations: aa, amino acid; Ab, antibody; Ad5-hDPP4, adenoviral vectors expressing human dipeptidyl peptidase 4; Alum, aluminium hydroxide; CpG-ODNs, cysteine-phosphate-guanine oligodeoxynucleotides; dpi, days post infection; IFA, incomplete Freund's adjuvant; i.m., intramuscular; i.n., intranasal; i.p., intraperitoneal; nAb, neutralizing antibody; Poly(I:C), polyriboinosinic acid; RBD, receptor-binding domain; S, Spike; s.c., subcutaneous.

1433 **TABLE 12** Animals tested for susceptibility to MERS-CoV in experimental and natural infection

Animal species & age	Dose and route of inoculation (virus strain)	Point of evaluation (days)	Clinical, virological, & immunological findings	Histopathological & IHC results	References
Susceptible					
Rhesus macaques (*Macaca mulatta*); 6-10 years	7×10^6 $TCID_{50}$ i.t., i.n., oral & ocular (HCoV-EMC/2012)	Up to 6	**Clinical:** mild to moderate symptoms including nasal swelling, piloerection, ↓ bowel opening, ↑ or ↓ respiratory rate, ↓ food intake, & hunched posture on 1-6 dpi; leukocytosis with neutrophilia & lymphopenia on 1 dpi **Virological:** viral RNA detected in upper & lower respiratory tract specimens, conjunctiva, & lymphoid tissues (mediastinal & tonsils) from 1 dpi, & in 1 macaque's urogenital swab on 1 dpi **Immunological:** significant up-regulation of genes associated with proinflammatory process (IL-6, CXCL1, MMP9); rapid resolution of controlled interferon-mediated innate immune response	**Macroscopic:** multifocal to coalescent, mild to marked interstitial pneumonia **Microscopic:** thickening of alveolar septae by edema fluid & fibrin with predominantly macrophages; BOOP-like changes with multinucleate syncytia formed by alveolar macrophages, fibrin aggregates, & occluded small airways by sloughed pulmonary epithelium, & perivascular infiltrates of inflammatory cells; type II pneumocyte hyperplasia; hyaline membrane formation **IHC:** viral Ag detected in types I & II pneumocytes, & macrophages/monocytes or dendritic cells	(165, 166)
Rhesus macaques (*Macaca mulatta*); 2-3 years	6.5×10^7 $TCID_{50}$ i.t. (HCoV-EMC/2012)	Up to 28	**Clinical:** fever & reduced water intake on 1-2 dpi; CXR showed varying degrees of localized infiltration & interstitial markings on 3-5 dpi **Virological:** viral RNA detected in lungs on 3 dpi **Immunological:** neutralizing Ab detected at 7 dpi, & peaked at 14 dpi	**Macroscopic:** congestion & palpable nodules scattered in distribution **Microscopic:** multifocal mild-to-moderate interstitial pneumonia & exudative changes in lungs **IHC:** viral Ag detected in types I & II pneumocytes, & alveolar macrophages	(167)
Common marmosets (*Callithrix jacchus*); 2-6 years	5.2×10^6 $TCID_{50}$ i.t., i.n., oral & ocular (HCoV-EMC/2012)	Up to 55	**Clinical:** moderate to severe symptoms including ↑ respiratory rate, open mouth and/or labored breathing, frothy hemorrhagic discharge from mouth, ↓ food intake, & ↓ activity level since 1-3 dpi & peaked o 4-6 dpi. Clinical scores retuned to baseline by 13 dpi; 2/9 animals	**Macroscopic:** multifocal, extensive, severe lesions especially in lower lobes; lungs were firm, failed to collapse, & fluid filled **Microscopic:** multifocal to coalescing, moderate to marked acute bronchointerstitial pneumonia centered on terminal bronchioles, with influx of	(168)

			were euthanized because of severe disease; CXR showed varying degrees of interstitial infiltration on 3-4 dpi **Virological:** viral RNA detected in upper (since 1 dpi) & lower respiratory tract specimens, blood, & multiple organs (conjunctiva, lymph nodes, tonsils, kidneys, heart, adrenal glands, liver, spleen, pancreas, colon, ileum, frontal lobe, cerebellum, brain stem, urinary bladder, & testes) since 3 dpi **Immunological:** tissue differentiation with development of pulmonary fibrosis as evidenced by activation of pathways associated with chemotaxis & ell migration, cell cycle progression, cell proliferation, fibrogenesis, inflammation, vascularization, endothelial activation, smooth muscle cell proliferation, & tissue repair; upregulation of innate & adaptive immune genes; induction of type I IFNs, IL-2, IL-4, & IL-6; inhibition of type II IFNs, IL-1 & TNFα	neutrophils & macrophages; thickening of alveolar septa; edema, hemorrhage & fibrin filled the alveolar spaces (3-4 dpi); type II pneumocyte hyperplasia & formation of hyaline membrane (6 dpi) **IHC:** viral Ag detected in affected areas, especially in type I pneumocytes & alveolar macrophages	
C57BL/6 & BALB/c mice with Ad5-hDPP4 transduction; 6-12 weeks (young) & 18-22 months (aged)	1×10^5 PFU i.n. (HCoV-EMC/2012)	Up to 14	**Clinical:** young BALB/C mice failed to gain weight, aged C57BL/6 & BALC/c mice lost weight **Virological:** clearance of virus by 6-8 dpi in young mice & 10-14 in aged mice **Immunological:** requirement of type I IFN induction & signaling, CD8 T cells & Ab for virus clearance; low level of cross-reactivity between MERS-CoV & SARS-CoV	**Macroscopic:** vascular congestion & inflammation **Microscopic:** perivascular & peribronchial lymphoid infiltration initially, with progression to an interstitial pneumonia **IHC:** viral Ag detected in lungs	(175)
Dromdary camels (*Camelus dromedarius*); 2-5 years (adults)	10^7 $TCID_{50}$ i.t., i.n. & ocular (HCoV-EMC/2012)		**Clinical:** mild upper respiratory tract symptoms including rhinoorhea & mild ↑ temperature **Virological:** infectious virus detected in nasal (up to 7 dpi & 10^8 PFU/ml) & oral (up to 5 dpi & 10^2 PFU/ml) swabs; viral RNA detected in nasal (up to 35 dpi &	**Macroscopic:** lesions found in the upper respiratory tract, trachea, bronchi & bronchioles, but not in the alveoli (up to 28 dpi) **Microscopic:** mild to moderate acute intraepithelial & submucosal inflammation with multifocal necrosis, loss of	(259)

			10^6 $TCID_{50}$ equivalent/ml) & oral (up to 35 dpi & 10^4 $TCID_{50}$ equivalent/ml) swabs **Immunological:** neutralizing Ab detected at 14 dpi, & peaked at 35 dpi	pseudostratified epithelial cells & infiltration of small numbers of neutroprophils & macrophages (up to 28 dpi) **IHC:** viral Ag detected in affected areas (up to 28 dpi)	
Goats	N/A	N/A	**Clinical:** asymptomatic to mildly symptomatic **Immunological:** seroconversion in all 14 goats by 14 dpi	N/A	(258)
Jamaican fruit bats	N/A	N/A	**Clinical:** no clinical signs or elevation in temperature **Virological:** virus shedding from respiratory & intestinal tract for up to 9 dpi	N/A	(257)
Non-susceptible					
Syrian hamster (*Mesocricetus auratus*)	4×10^2 $TCID_{50}$ aerosols, 10^3 $TCID_{50}$ i.t., or 10^6 $TCID_{50}$ i.t. (HCoV-EMC/2012)	Up to 21	**Clinical:** no significant weight loss or fever **Virological:** no viral RNA detected in nasal, oropharyngeal, urogenital & rectal swabs from 1-11 dpi; & lungs, spleen & mandibular lymph nodes on 2, 4, & 8 dpi **Immunological:** no seroconversion	**Macroscopic:** no gross lesions **Microscopic:** no lesions in trachea, heart, lung, spleen, liver, kidney, ileum, colon, urinary bladder, nasal turbinates, & brain tissues	(333)
BALB/c, 129/SvEv, & 129/SvEv STAT1 knockout mice; 8 weeks	120 or 1200 $TCID_{50}$ i.n. (HCoV-EMC/2012)	Up to 9	**Clinical:** no significant weight loss **Virological:** no detectable virus in lungs	**Microscopic:** no sign of viral infection (apoptotic cells & syncytia formation); 129S6/SvEv & 129/SvEv STAT1 knockout mice had only minor signs of pathological lesions or inflammatory response, with a few lesions of focal interstitial pneumonitis composed of neutrophils & macrophages; BALB/c mice had perivascular cuffing with scattered neutrophils & foci of pneumonia around proximal airways	(334)
Ferret (*Mustela putorius furo*)	1×10^6 $TCID_{50}$ i.n. & i.t. (HCoV-EMC/2012)	Up to 14	**Virological:** no infectious virus was detected in nose & throat swabs **Immunological:** no seroconversion	***In vitro*:** ferret primary kidney cells did not bind recombinant S protein S1 & could not be infected with MERS-CoV, despite DPP4 surface expression	(49)

1434 Abbreviations: Ab, antibody; Ad5-hDPP4, adenoviral vectors expressing human dipeptidyl peptidase 4; Ag, antigen; BOOP,

bronchiolitis obliterans organizing pneumonia; CXCL1, chemokine C-X-C ligand 1; dpi, days post inoculation; IFN, interferon; IHC, immunohistochemistry; IL, interleukin; i.n., intranasal; i.t., intratracheal; MMP9, matrix metalloproteinase 9; N/A, not available; PFU, plaque-forming unit; S, spike; TCID50, 50% tissue culture infectious dose.

FIGURE LEGENDS

FIG. 1A. Taxonomy of *Coronaviridae* according to the International Committee on Taxonomy of Viruses.

FIG. 1B. Phylogenetic tree of 50 coronaviruses with partial nucleotide sequences of RNA-dependent RNA polymerase. The tree was constructed by the neighbor-joining method using MEGA 5.0. The scale bar indicates the estimated number of substitutions per 20 nucleotides. Abbreviations (accession number): AntelopeCoV, sable antelope coronavirus (EF424621); BCoV, bovine coronavirus (NC_003045); BdCoV HKU22, bottlenose dolphin coronavirus HKU22 (KF793826); BuCoV HKU11, bulbul coronavirus HKU11 (FJ376619); BWCoV-SW1, beluga whale coronavirus SW1 (NC_010646); CMCoV HKU21, common moorhen coronavirus HKU21 (NC_016996); DcCoV HKU23, dromedary camel coronavirus HKU23 (KF906251); ECoV, equine coronavirus (NC_010327); ErinaceousCoV, Betacoronavirus *Erinaceus*/VMC/DEU/2012 (NC_022643); FIPV, feline infectious peritonitis virus (AY994055); HCoV-229E, human coronavirus 229E (NC_002645); HCoV-HKU1, human coronavirus HKU1 (NC_006577); HCoV-NL63, human coronavirus NL63 (NC_005831); HCoV-OC43, human coronavirus OC43 (NC_005147); Hi-BatCoV HKU10, *Hipposideros* bat coronavirus HKU10 (JQ989269); IBV-partridge, partridge coronavirus (AY646283); IBV-peafowl, peafowl coronavirus (AY641576); MERS-CoV, Middle East respiratory syndrome coronavirus (NC_019843.3); MERS-CoV KSA-CAMEL-363, Middle East respiratory syndrome coronavirus isolate KSA-CAMEL-363 (KJ713298); MHV, murine hepatitis virus (NC_001846); Mi-BatCoV 1A, *Miniopterus* bat coronavirus 1A (NC_010437); Mi-BatCoV 1B, *Miniopterus* bat coronavirus

1461 1B (NC_010436); Mi-BatCoV HKU7, *Miniopterus* bat coronavirus HKU7 (DQ249226); Mi-
1462 BatCoV HKU8, *Miniopterus* bat coronavirus HKU8 (NC_010438); MRCoV HKU18, magpie
1463 robin coronavirus HKU18(NC_016993); MunCoV HKU13, munia coronavirus HKU13
1464 (FJ376622); My-BatCoV HKU6, *Myotis* bat coronavirus HKU6 (DQ249224); NeoCoV,
1465 coronavirus *Neoromicia*/PML-PHE1/RSA/2011 (KC869678); NHCoV HKU19, night heron
1466 coronavirus HKU19 (NC_016994); PEDV, porcine epidemic diarrhoea virus (NC_003436);
1467 PHEV, porcine haemagglutinating encephalomyelitis virus (NC_007732); Pi-BatCoV-HKU5,
1468 *Pipistrellus* bat coronavirus HKU5 (NC_009020); PorCoV HKU15, porcine coronavirus HKU15
1469 (NC_016990); PRCV, porcine respiratory coronavirus (DQ811787); RbCoV HKU14, rabbit
1470 coronavirus HKU14 (NC_017083); RCoV parker, rat coronavirus parker (NC_012936); Rh-
1471 BatCoV HKU2, *Rhinolophus* bat coronavirus HKU2 (EF203064); Ro-BatCoV-HKU9, *Rousettus*
1472 bat coronavirusHKU9 (NC_009021); Ro-BatCoV HKU10, *Rousettus* bat coronavirus HKU10
1473 (JQ989270); SARS-CoV, SARS coronavirus (NC_004718); SARSr-CiCoV, SARS-related palm
1474 civet coronavirus (AY304488); SARSr-Rh-BatCoV HKU3, SARS-related *Rhinolophus* bat
1475 coronavirus HKU3 (DQ022305); Sc-BatCoV 512, *Scotophilus* bat coronavirus 512
1476 (NC_009657); SpCoV HKU17, sparrow coronavirus HKU17 (NC_016992); TCoV, turkey
1477 coronavirus (NC_010800); TGEV, transmissible gastroenteritis virus (NC_002306); ThCoV
1478 HKU12, thrush coronavirus HKU12 (FJ376621); Ty-BatCoV-HKU4, *Tylonycteris* bat
1479 coronavirus HKU4 (NC_009019); WECoV HKU16, white-eye coronavirus HKU16
1480 (NC_016991); WiCoV HKU20, wigeon coronavirus HKU20 (NC_016995).
1481
1482 **FIG. 2.** Genome arrangement of MERS-CoV with emphasis on the clinical applications of the
1483 key non-structural and structural genes. * denotes furin cleavage sites. Abbreviations: 3CLpro,

3C-like protease; AP, accessory protein; CP, cytoplasmic domain; E, envelope; FP, fusion peptide; Hel, helicase; HR, heptad repeat; IFN, interferon; M, membrane; mAb, monoclonal antibody; N, nucleocapsid; nsp, non-structural protein; ORF, open reading frame; pp, polyprotein; PLpro, papain-like protease; RBD, receptor binding domain; RdRp, polymerase; RT-RPA; reverse transcription isothermal Recombinase Polymerase Amplification; S, spike; SP, signal peptide; TM, transmembrane domain.

FIG. 3. Candidate antiviral agents for MERS-CoV in relation to the viral replication cycle. (+) and (-) denotes positive- and negative-strand RNA respectively. Abbreviations: AKT, protein kinase B; Cyps, cyclophilins; DPP4, dipeptidyl peptidase-4; E, envelope; ER, endoplasmic reticulum; ERGIC, endoplasmic reticulum Golgi intermediate compartment; ERK, extracellular signal-regulated kinases; HR2P, heptad repeat 2 peptide; IFN, interferon; M, membrane; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinases; MPA, mycophenolic acid; mRNA, messenger RNA; mTOR, mammalian target of rapamycin; N, nucleocpasid; NFAT, nuclear factor of activated T-cells; nsp, non-structural protein; ORF, open reading frame; PI3K, phosphatidylinositide 3-kinases; S, spike; TMPRSS2, transmembrane protease serine protease-2.

FIG. 4. Phylogenetic tree of representative human and camel strains of MERS-CoV rooted by NeoCoV (KC869678.4) according to reference (111).

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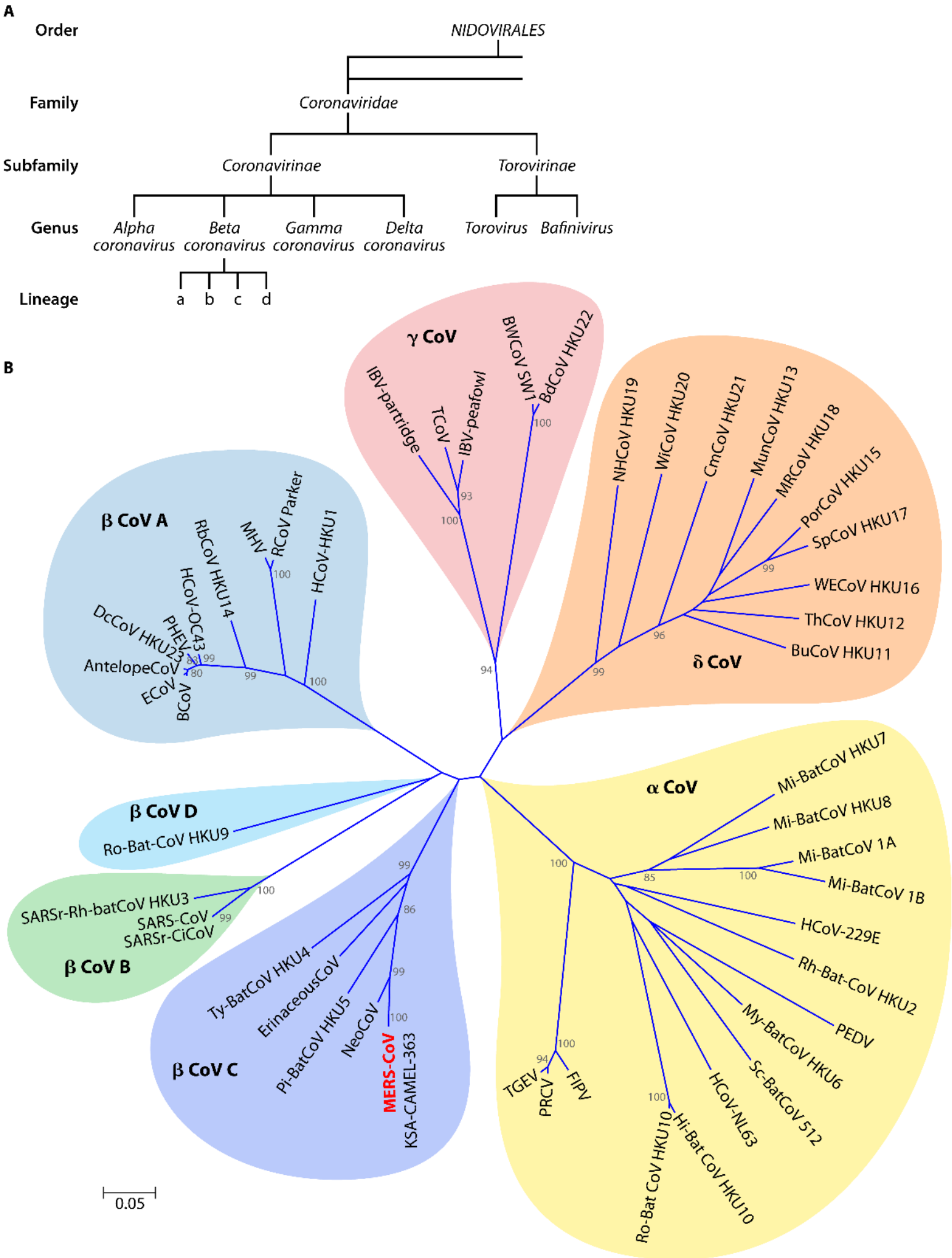
A
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Family
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Subfamily
Coronavirinae
Torovirinae
Genus
Alpha coronavirus
Beta coronavirus
Gamma coronavirus
Delta coronavirus
Torovirus
Bafinivirus
Lineage
a
b
c
d
B
γ CoV
IBV-partridge
TCoV
IBV-peafowl
BWCoV SW1
BdCoV HKU22
δ CoV
NHCoV HKU19
WiCoV HKU20
CmCoV HKU21
MunCoV HKU13
MRCoV HKU18
PorCoV HKU15
SpCoV HKU17
WECoV HKU16
ThCoV HKU12
BuCoV HKU11
β CoV A
RbCoV HKU14
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RCoV Parker
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HCoV-OC43
PHEV
DcCoV HKU23
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ECoV
BCoV
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Ro-Bat-CoV HKU9
β CoV B
SARSr-Rh-batCoV HKU3
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Mi-BatCoV HKU8
Mi-BatCoV 1A
Mi-BatCoV 1B
HCoV-229E
Rh-Bat-CoV HKU2
PEDV
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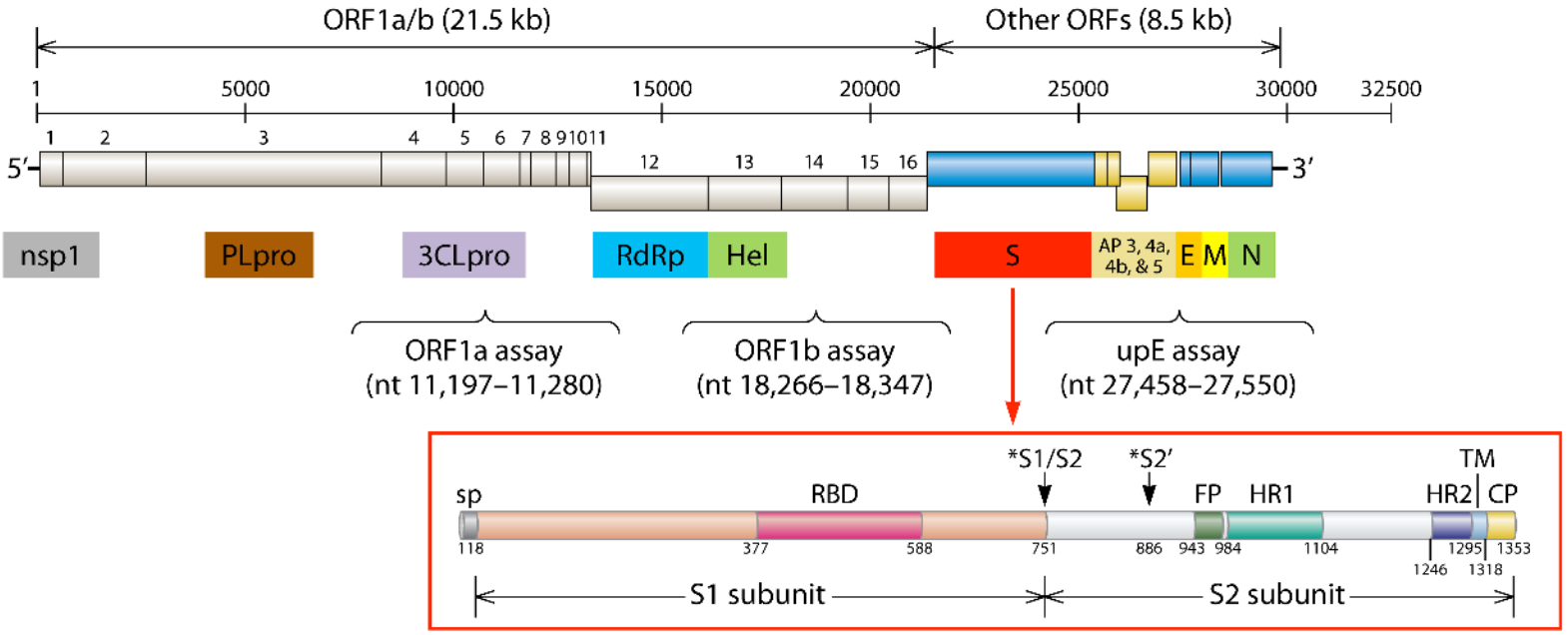


nsp1: possibly interacts with cyclophilins
Use: antiviral target (cyclophilin inhibitors)

PLpro: proteolysis, IFN antagonist, deubiquitination and deISGylation
Use: antiviral target (PLpro inhibitors)

3CLpro: proteolysis
Use: antiviral target (3CLpro inhibitors)

RdRp: viral replication and transcription
Use: diagnostic (RdRpSeq assay) and antiviral targets (polymerase inhibitors)

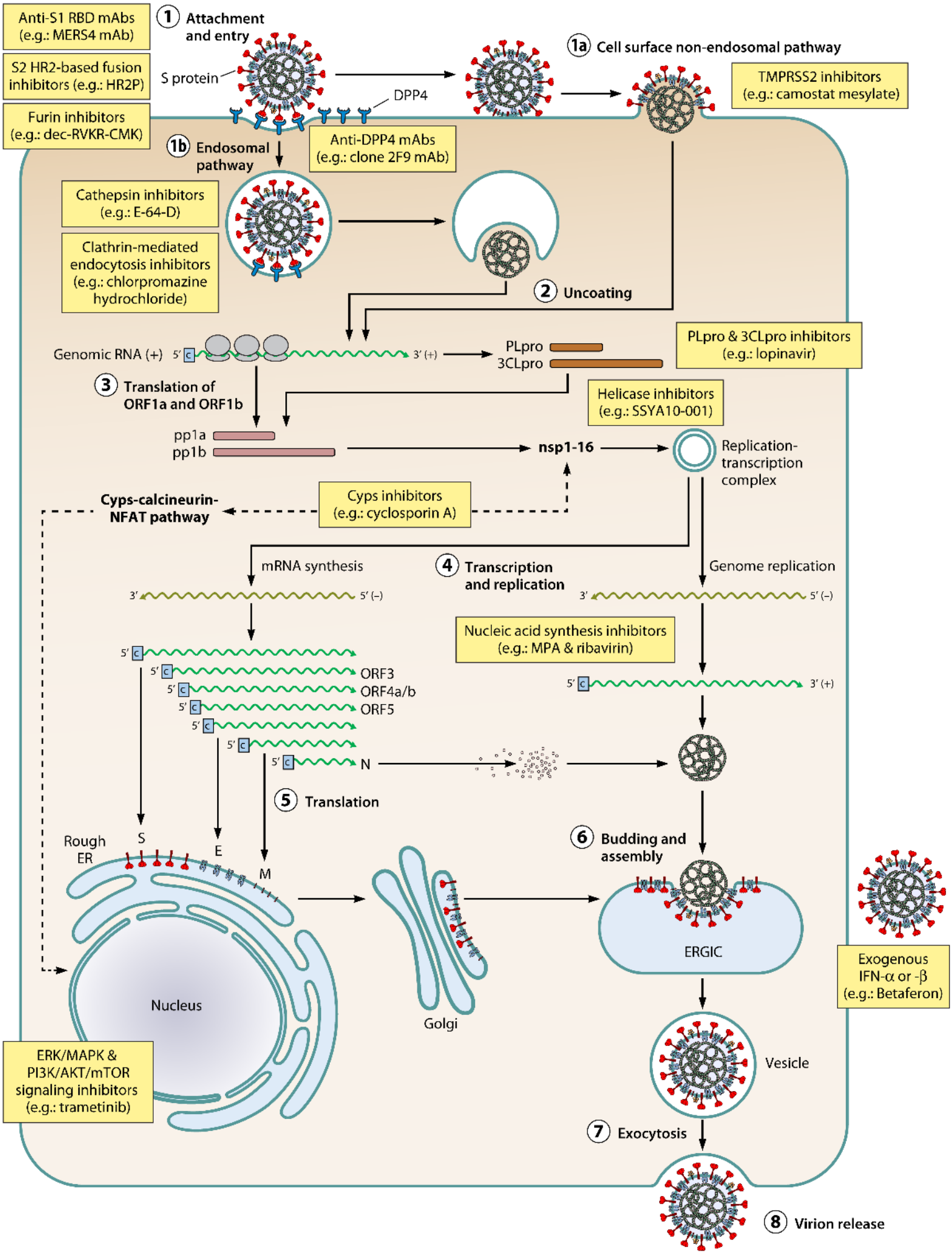
Hel: viral replication
Use: antiviral target (Hel inhibitors)

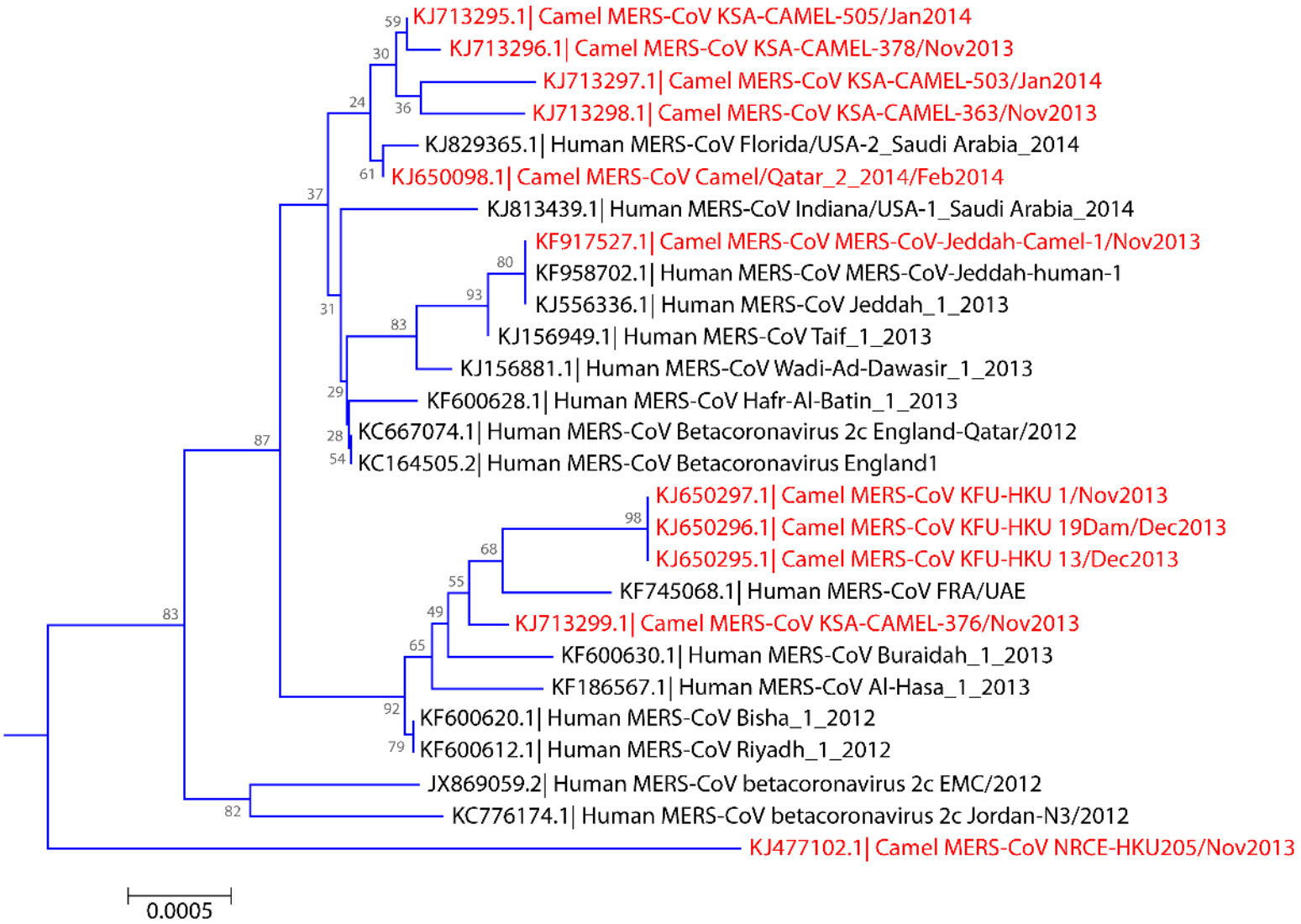
S: virus-cell receptor binding
Use: diagnostic (serology), antiviral (mAbs and antiviral peptides), and vaccination targets

E: virion assembly

M: virion assembly; IFN antagonist
Use: antiviral target (exogenous IFN)

N: virion assembly
Use: diagnostic target (Nseq, N2, N3, and RT-RPA assays; serology)


Anti-S1 RBD mAbs (e.g.: MERS4 mAb)
S2 HR2-based fusion inhibitors (e.g.: HR2P)
Furin inhibitors (e.g.: dec-RVKR-CMK)
1 Attachment and entry
S protein
DPP4
Anti-DPP4 mAbs (e.g.: clone 2F9 mAb)
1a Cell surface non-endosomal pathway
TMPRSS2 inhibitors (e.g.: camostat mesylate)
1b Endosomal pathway
Cathepsin inhibitors (e.g.: E-64-D)
Clathrin-mediated endocytosis inhibitors (e.g.: chlorpromazine hydrochloride)
2 Uncoating
Genomic RNA (+)
5′ c
3′ (+)
PLpro
3CLpro
PLpro & 3CLpro inhibitors (e.g.: lopinavir)
3 Translation of ORF1a and ORF1b
Helicase inhibitors (e.g.: SSYA10-001)
pp1a
pp1b
nsp1-16
Replication-transcription complex
Cyps-calcineurin-NFAT pathway
Cyps inhibitors (e.g.: cyclosporin A)
mRNA synthesis
4 Transcription and replication
Genome replication
3′
5′ (–)
Nucleic acid synthesis inhibitors (e.g.: MPA & ribavirin)
ORF3
ORF4a/b
ORF5
N
5 Translation
Rough ER
S
E
M
6 Budding and assembly
ERGIC
Nucleus
Golgi
Exogenous IFN-α or -β (e.g.: Betaferon)
ERK/MAPK & PI3K/AKT/mTOR signaling inhibitors (e.g.: trametinib)
Vesicle
7 Exocytosis
8 Virion release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KJ829365.1| Human MERS-CoV Florida/USA-2_Saudi Arabia_2014
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KJ813439.1| Human MERS-CoV Indiana/USA-1_Saudi Arabia_2014
KF917527.1| Camel MERS-CoV MERS-CoV-Jeddah-Camel-1/Nov2013
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KC667074.1| Human MERS-CoV Betacoronavirus 2c England-Qatar/2012
KC164505.2| Human MERS-CoV Betacoronavirus England1
KJ650297.1| Camel MERS-CoV KFU-HKU 1/Nov2013
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KJ650295.1| Camel MERS-CoV KFU-HKU 13/Dec2013
KF745068.1| Human MERS-CoV FRA/UAE
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KF186567.1| Human MERS-CoV Al-Hasa_1_2013
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KC776174.1| Human MERS-CoV betacoronavirus 2c Jordan-N3/2012
KJ477102.1| Camel MERS-CoV NRCE-HKU205/Nov2013
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