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REVIEW

Middle East respiratory syndrome coronavirus: a comprehensive review

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Abstract **The Middle East respiratory syndrome coronavirus was first identified in 2012 and has since then remained uncontrolled. Cases have been mostly reported in the Middle East, however travel-associated cases and outbreaks have also occurred. Nosocomial and zoonotic transmission of the virus appear to be the most important routes. The infection is severe and highly fatal thus necessitating rapid and efficacious interventions. Here, we performed a comprehensive review of published literature and summarized the epidemiology of the virus. In addition, we summarized the virological aspects of the infection and reviewed the animal models used as well as vaccination and antiviral tested against it.**

Keywords MERS; coronavirus; review

Introduction

Coronaviruses (CoV) became known to cause human disease in the twentieth century. HCoV-229E and HCoV-OC43 were discovered in the 1960s and shown to cause respiratory infections in humans [1,2]. With the emergence of SARS-CoV in 2003 [3], two other human coronaviruses were discovered, HCoV NL63, HCoV HKU1 [4]. In 2012, a new type of coronavirus was detected as the cause of severe respiratory illness in humans. The first case was a 60-year-old male from Saudi Arabia admitted to hospital with acute respiratory illness leading to pneumonia and acute renal failure. The virus initially named as human corona virus-EMC [5], is currently known as the Middle East respiratory syndrome coronavirus (MERS-CoV) [6].

Virology of MERS-CoV

Classification and nomenclature of MERS-CoV

Phylogenetically, MERS-CoV is a lineage C β coronavirus (β-CoV) and is closely related to bat coronaviruses HKU4 and HKU5. The rooted phylogenetic analysis showed that MERS-CoV had an amino acid sequence identity less than 90% to all other known CoVs [7]. The virus initially named by many different working groups as novel coronavirus, human coronavirus EMC, human β coronavirus 2c EMC, human β coronavirus 2c England-Qatar, human β coronavirus 2C Jordan-N3, and β coronavirus 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, and England) was later named as MERS-CoV by the coronaviruses study groups of ICTV [5,6,8].

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General virology of MERS-CoV

MERS-CoV is an enveloped virus with a positive sense RNA genome. Coronavirus genomes range between 25 to 32 kb in size. The complete sequence of HCoV-EMC-2012 resulted in 30 119 nucleotides sequence [7]. Coronavirus genomes are polycistronic with large replicase open reading frames ORF1a and ORF1b which are subsequently cleaved into 15 or 16 nonstructural proteins (NSPs). The region downstream of ORF1b encode smaller genes including the spike (S), envelope (E), membrane (M), and nucleocapsid (N) structural protein [9–11]. The functional receptor for MERS-CoV is the Dipeptidyl peptidase 4 (DPP4) which is present on human non-ciliated bronchial epithelial cells surfaces [12]. The DPP4 protein displays high amino acid sequence conservation

across different species, including the sequence that was obtained from bat cells. Cell lines susceptibility studies showed that MERS-CoV infected several human cell lines, including histiocytes as well as respiratory, kidney, intestinal, and liver cells [13]. The range of tissue tropism *in vitro* was broader than that for any other known human coronavirus [14]. MERS-CoV can also infect nonhuman primate, porcine, bat, civet, rabbit, and horse cell lines all possessing the DPP4 receptor [15].

MERS-CoV replication cycle

The replication cycle of MERS-CoV consists of numerous steps as illustrated by Lu *et al.* [30].

Viral receptor attachment

The MERS-CoV S protein is a class I fusion protein composed of two subunits: the amino N-terminal receptor binding S1 and carboxyl C-terminal membrane fusion S2 subunits. The S1/S2 junction is a protease cleavage site which is responsible for membrane fusion activation, virus entry, and syncytium formation. The S1 C domain contains the receptor binding domain (RBD), and an N domain [13]. Neutralizing monoclonal antibodies against the RBD may inhibit virus entry into cells and receptor-dependent syncytium formation in cell culture, hence vaccines containing the RBD induced high levels of neutralizing antibodies in mice and rabbits [16–18].

DPP4 is the cell key functional receptor for the MERS-CoV S protein [19]. MERS-CoV is the first CoV that has been identified to use DPP4 as a receptor [19,20]. DPP4 has important roles in glucose metabolism, T cell activation, chemotaxis modulation, cell adhesion, and apoptosis [19,21].

Membrane fusion

The S2 subunit contains five domains: a fusion peptide, the heptad repeat 1 (HR1) and HR2 domains, a transmembrane domain, and a cytoplasmic domain, which form the stalk region of S protein that facilitates fusion of the viral and cell membranes [22,23]. 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. MERS-CoV utilizes many pathways for membrane fusion depending on available host proteases, such as transmembrane protease serine protease 2 (TMPRSS2), trypsin, chymotrypsin, elastase, thermolysin, endoproteinase Lys-C, and human airway trypsin-like protease. Proteases cleave the S protein into the S1 and S2 subunits to activate the MERS-CoV S protein for endosome-independent host cell entry at the plasma membrane [24–26]. In addition to the pervious fusion proteases furin has been identified recently to play an essential role in the MERS-CoV S protein cleavage activation into their biologically active forms [27,28].

Disassembly, genome replication and expression

After cell entry, the virion particle disassembles to release the nucleocapsid and viral RNA into the cytoplasm for expression of viral polyproteins pp1a and pp1ab. Double-membrane vesicles and convoluted membranes are formed by the attachment of the hydrophobic domains of the MERS-CoV replication machinery to the limiting membrane of auto-phagosomes [29]. The viral polyproteins pp1a and pp1ab are cleaved by papain-like protease and 3C-like protease into nsp1 to nsp16 [7,30,31]. These non-structural proteins form the replication-transcription complex, which regulates transcription and viral protein expression [29].

Assembly and release of the new viruses

After the production of abundant viral RNA and structural and accessory proteins, the N protein binds to the genomic RNA in the cytoplasm to form the helical nucleocapsid (viral core). The viral core is enveloped by budding through intracellular membranes between the endoplasmic reticulum and Golgi apparatus [32]. The S, E, and M proteins are transported to the budding virion, where the nucleocapsid probably interacts with M protein to generate the basic structure and complexes with the S and E proteins to induce viral budding and release from the Golgi apparatus [33]. MERS-CoV replication cycle is completed by releasing the progeny virions through the cell membrane via exocytosis pathway.

Animal models for MERS-CoV infection

Mice

MERS-CoV strain HCoV-EMC/2012 was inoculated to three different mouse strains (immunocompetent BALB/c mice, 129S6/SvEv and innate immune-deficient 129/STAT1$^{-/-}$ mice) intranasally. No significant weight loss was observed and infectious virus could not be detected in the lungs. Only moderate pathological lesions were observed in the lungs. Hence no viral replication was observed in these strains of mice [34].

Zhao *et al.* developed a mouse model transduced with a recombinant adenovirus vector expressing hDPP4 (Ad5-hDPP4) in lung tissue. Inoculation of MERS-CoV in these mice resulted in MERS-CoV replication but without mortality. Young mice cleared from MERS-CoV in 6−8 days and old mice in 10−14 days. Perivascular and

peribronchial lymphoid infiltration was observed, with progression to an interstitial pneumonia postinfection [35].

In another study, transgenic mice expressing hDPP4 were susceptible to MERS-CoV infection. Infectious virus was isolated from lung and brain tissue and weight loss was observed [36]. Pascal *et al.* developed humanized transgenic mouse. No mortality or clinical signs was observed but interstitial pneumonia and significant lung disease were observed histopathologically, suggesting that humanized DPP4 mouse is a model for MERS-CoV infection in which pathological changes resembles MERS-CoV infection in humans [37,38].

Non-human primate models

The rhesus macaque was the first animal model used for MERS-CoV infection as it possessed DPP4 receptor [38,39]. In infected animals, an increase in respiratory rates, body temperature, cough and reduced appetite was observed with mild to moderate severity. Infectious virus isolated only from the lower respiratory tract. Viral RNA was detected in the conjunctiva, nasal mucosa, tonsils, pharynx, trachea, bronchus and lungs. Mild to marked interstitial pneumonia with dark red lesions appeared in lungs. Seroconversion of neutralizing antibodies began at 7 dpi and increased in titer with time. The development of a transient pneumonia, rapid replication, and tropism of MERS-CoV for the lower respiratory tract resembled the severity of the disease observed in humans [38,40,41].

Similarly, the common marmoset was shown to possess the DPP4 receptor [42]. Radiographic imaging showed mild to severe bilateral interstitial infiltration and extensive bronchointertitial pneumonia in infected animals. Infectious virus was detected in lower and upper respiratory tract tissue and viral RNA was detected in nasal mucosa, oropharyngeal swabs, blood, conjunctiva, lymph nodes, gastrointestinal tract, kidney, heart, adrenal gland, liver, spleen, brain and lungs [42].

Other models

Inoculation of Syrian hamsters and ferrets with MERS-CoV did not result in infection [12,43].

Rabbits may be used as a model to study pathogenesis, transmission, and disease control strategies of MERS-CoV *in vivo* as they seroconvert and shed virus after inoculation [44].

Epidemiology of MERS-CoV

In September 2012, a novel coronavirus infection was noted in ProMed Mail [45]. The virus was isolated from the sputum of a 60-year-old Saudi male, who was admitted to a hospital with pneumonia and acute kidney injury in June 2012. A few days later, another report appeared describing an almost identical virus detected in a patient in Qatar with acute respiratory syndrome and acute kidney injury. The patient had a recent travel history to Saudi Arabia and then traveled to UK for further medical care [5,46,47]. Two cases from Jordan (April 2012) were retrospectively diagnosed as MERS patients. Since that time, more than 1542 cases of MERS-CoV infection have been reported including 544 deaths [48]. The actual number of cases could be higher than those reported [49]. An outbreak of more than 180 confirmed cases including 36 deaths occurred in South Korea in May and June 2015. The median age of Korean cases were 55 years (range: 16 to 87 years), 60% were men, and 14% were health care professionals. The index case was a 68-year-old male who had recently traveled to several countries in the Arabian Peninsula [50].

Case definitions, clinical manifestation, and diagnosis

Case definitions

MERS-CoV infection cases were classified by the World Health Organization (WHO) [51], the US Centers for Disease Control and Prevention (CDC), and the Ministry of Health of Saudi Arabia (MOHSA) as asymptomatic, mild, severely symptomatic, or mortal. Cases may be classified into suspected, probable, and confirmed [52,53].

Confirmed case

Any person with laboratory confirmation of infection with MERS-CoV irrespective of clinical signs and symptoms is considered as a confirmed case. WHO criteria for laboratory confirmation require detection of viral RNA or acute and convalescent serology. The presence of nucleic acid can be confirmed by positive results from at least two sequence-specific rRT-PCRs or a single sequence-specific rRT-PCR test and direct sequencing from a separate genomic target [54]. A case confirmation by serological methods requires demonstration of seroconversion in two samples collected at least 14 days apart using at least one screening assay (enzyme-linked immunoassay, immunofluorescence assay) and a neutralization assay.

Probable case

A probable case is defined by the following criteria, a febrile acute respiratory illness as pneumonia or acute respiratory distress syndrome, direct contact with a confirmed MERS-CoV case and unavailability of MERS-CoV testing or results being inconclusive for a single inadequate specimen.

Suspected case

Any person who developed a fever and pneumonia or acute respiratory distress syndrome with a history of travel to countries in or near the Arabian Peninsula within 14 days before symptom onset or was in contact with a traveler from this region who developed a febrile respiratory illness is considered as a MERS-CoV suspected case.

Diagnosis

The WHO, CDC, and MOHSA recommended laboratory diagnostics for MERS-CoV infection [6,9,51,55,56]. MERS-CoV cases must be confirmed by at least two positive qRT-PCR tests on two different specific genomic regions or single positive qRT-PCR with a sequence of another positive genome fragment [57]. The WHO algorithm for testing MERS-CoV relies on qRT-PCR and sequencing [58]. Available real-time tests include an assay targeting the RNA upstream of the E gene (*upE*) as a highly sensitive screening assay and three confirmatory assays targeting open reading frames (ORF 1a and 1b) and/or N gene. The ORF 1a assay is of equal sensitivity to the upE assay. The ORF 1b assay is less sensitive but is useful for confirmation. These assays are specific for MERS-CoV and have not shown cross-reactivity with other respiratory human coronaviruses. For sequencing, two target genes, the RNA-dependent RNA polymerase (RdRp, present in ORF 1b) and N genes are enough to confirm the existence of MERS-CoV RNA in the samples of a patient [57].

Several serologic assays including immunofluorescence assays, protein microarray assay, enzyme-linked immunosorbent assay (ELISA) have been developed for the detection of MERS-CoV antibodies [57,59–61]. Any positive test by one of these assays should be confirmed with a neutralization assay. Single serological result may be valuable for definition of probable case and should be followed by further testing for confirmation of MERS-CoV infection [62–64].

Clinical manifestation of MERS-CoV infection

Incubation period of MERS-CoV infections was studied by Assiri *et al.* in 2013. The median incubation period was 5.2 days (95% CI 1.9−14.7 days) [65]. In another report from France of a secondary case, a patient who shared a room with an infected patient, the incubation period was estimated at 9 to 12 days [66]. In the recent outbreak in South Korea during May/June 2015, the median incubation period was 6.3 days [67]. WHO and CDC recommended that individuals that returned from the Arabian Peninsula and other affected countries must be evaluated for MERS-CoV infections up to at least 14 days [68].

Clinical features of MERS-CoV infections range from asymptomatic cases to mildly ill, severe pneumonia, acute respiratory distress syndrome, septic shock and mortal with multi-organ failure (Table 1) [64,65]. Many other clinical features such as gastrointestinal symptoms (anorexia, nausea, vomiting, abdominal pain, diarrhea), pericarditis, and disseminated intravascular coagulation were reported [65,69,70].

Specific clinical conditions (comorbidities) were apparently proportionate with high severity of MERS-CoV infections. A study by Assiri *et al.* in Saudi Arabia showed that of a total of 47 patients with MERS-CoV infection in 2013, 45 (96%) had underlying clinical conditions, including diabetes mellitus (68%), hypertension (34%), chronic cardiac disease (28%), and chronic kidney disease (49%) [65]. This high rate of comorbidities must be interpreted with some caution, since diabetes mellitus is common in Saudi Arabia, and because approximately half of those 47 were part of an outbreak in a hemodialysis unit, where rates of comorbidities might be high due to chronic kidney disease [65,71]. In another study, being on dialysis, diabetes mellitus, and age $>$ 50 years was associated with mortality [72]. In this study, testing positive for MERS-CoV in a plasma sample was a predictor of severe outcome [72].

Younger adults and children appeared to be less susceptible to MERS-CoV infection. Only one study described MERS-CoV infection in children [73]. All of those children were discovered during contact investigations of older patients. Only 2 of 11 children developed symptoms of MERS-CoV infection. These two children had underlying conditions (cystic fibrosis and Down syndrome). The other 9 children were asymptomatic. There are few reports of MERS-CoV infections in pregnant women. A five-month pregnant female developed vaginal bleeding and abdominal pain after one week, then delivered a stillborn infant [74]. Another case in the United Arab Emirates was near term phase, she gave birth to an apparently healthy baby, and died after delivery [52].

Mild and asymptomatic MERS-CoV infections have been reported, a majority of whom were identified among the contacts of patients [62,75,76]. In a report from MOHSA, more than 3000 contacts of patients were screened using qRT-PCR and seven healthcare workers with MERS-CoV infection were identified, two of whom were asymptomatic and five of whom had mild upper respiratory tract symptoms [75].

Human-to-human transmission

Epidemiological and virological studies were conducted in attempts to determine person to person transmission of MERS-CoV. They studied case clustering in household and hospital outbreaks in the UK, Tunisia, Italy, and in healthcare facilities in Saudi Arabia, France, Iran, and lately in South Korea. Those studies provided strong evidence that human-to-human transmission occurs

Table 1 Clinical features for MERS-CoV patients

First cases reported	April, 2012 (Zarqa, Jordan) June, 2012 (Jeddah, Saudi Arabia)
Incubation period	
Mean (d)	5.2 (1.9–14.7)
Range (d)	2–13
Patient characteristics	
Adults	98%
Children	2%
Age range (year)	1–94
Average age (year)	Median 50
Sex ratio (male/female)	64.5%/35.5%
Mortality	
Overall CFR	35.35%
CFR in patients with comorbidities	60%
Disease progression	
Time from onset to ventilatory support	Median 7 days
Time from onset to death	Median 11.5 days
Presenting symptoms	
Fever (>38°C)	98%
Chills or rigors	87%
Cough	83%
Dry	56%
Productive	44%
Hemoptysis	17%
Headache	11%
Myalgia	32%
Malaise	38%
Shortness of breath	72%
Nausea	21%
Vomiting	21%
Diarrhea	26%
Sore throat	14%
Rhinorrhea	6%
Comorbidities	76%
Dead subjects with underlying conditions	86%
Asymptomatic or recovered subjects with underlying conditions	42%
Laboratory results	
Chest radiography abnormalities	90%–100%
Leucopenia ($<4.0\times10^9$ cells per L)	14%
Lymphopenia ($<1.5\times10^9$ cells per L)	32%
Thrombocytopenia ($<140\times10^9$ platelets per L)	36%
High lactate dehydrogenase	48%
High alanine aminotransferase	11%
High aspartate aminotransferase	14%

CFR, case-fatality rate; MERS,Middle East respiratory syndrome.

[70,77–80]. The number of contacts infected by individuals with confirmed infections, however, appears to be limited [62], except the outbreak of South Korea in May/June 2015, where most cases were secondary and some cases were tertiary infections [67,81]. Secondary cases often were milder or symptomless [62]. Possible modes of transmission include droplet and close contact transmission, air borne transmission, and fomite transmission [82].

The majority of all laboratory-confirmed secondary cases have been associated with healthcare settings [82]. The majority of cases of Jeddah, Saudi Arabia hospital outbreak during the spring of 2014 were acquired through human-to-human transmission due to systematic weaknesses in infection control [76]. Secondary transmission rates were assessed within households and the transmission rate was around 4%, suggesting that the actual number of infection is greater than reported [62].

During the outbreak in South Korea during May/June 2015, 25 secondary infections were associated with the index case, who was hospitalized from May 15 to May 17 and 11 were tertiary [83]. The median incubation period was six days for secondary cases and six days for tertiary cases. This outbreak also clearly demonstrated roles of "superspreaders," who may be responsible for a high proportion of cases [83]. For instance, a single patient infected more than 70 other people while being treated in the emergency room of a hospital in South Korea for three days, 27–29 May 2015.

Transmissibility and epidemic potential studies of MERS-CoV revealed that the reproduction number (R_0) of patients infected with MERS-CoV ranged between 0.6 to 0.69 [84,85]. The finding of an $R_0 < 1$ suggests that MERS-CoV does not yet have pandemic potential. Other study suggested that R_0 values might reach to 0.8 to 1.3 in the absence of infection control [49]. Shedding periods of MERS-CoV in humans was reported to be long as viruses were detected in lower respiratory samples of symptomatic patients for more than two weeks [86]. At instances, prolonged shedding for 6 weeks was detected in an asymptomatic healthcare worker. These findings raise concerns that asymptomatic persons could transmit infection to others in a silent manner [87].

Geographic distribution of MERS-CoV cases

The majority of cases have occurred in Saudi Arabia and United Arab Emirates [88–90]. Many cases have also been reported outside the Arabian Peninsula in North Africa, Europe, Asia, and North America as shown in Table 2. Almost all cases reported outside the Arabian Peninsula had a travel history to it.

Notable outbreaks and clusters

The first cluster was in October/November 2012 in four men of the same family in Riyadh, Saudi Arabia, two of whom died [75]. The second cluster was reported in Jordan in April 2012 involving 10 healthcare workers exposed to fatal patients. In addition, seven surviving hospital contacts seroconverted suggesting that they had MERS-CoV infection [91]. The third cluster was reported in UK during January/ February 2013. An English resident had a travel history to Saudi Arabia and Pakistan in January, developed a severe respiratory illness, and tested positive for both MERS-CoV and H1N1 influenza A, and died in March 2013 after infecting several contacts [92].

A cluster of 43 cases of MERS-CoV was reported in Al-Hasa in Saudi Arabia during April 2013. All those cases were directly linked to human to human contact in the same hospital. There were only two confirmed cases of healthcare workers, and three family members were detected by a survey of over 200 household contacts that visited this hospital [77]. In France, May 2013, an infection of MERS-CoV was reported in a patient who recently traveled to the United Arab Emirates. A second case who shared the hospital room with the first case tested positive. The first patient died and the second patient was critically ill. A survey of 100 healthcare workers found no other infections with MERS-CoV, despite the lack of use of personal protective equipment [70].

A surge in MERS-CoV cases was reported in Saudi Arabia and the United Arab Emirates during March and April 2014 [55,76]. The majority of cases were associated with hospital-based outbreaks Jeddah, Riyadh, Tabuk, and Madinah in Saudi Arabia as well as in Al Ain, and Abu Dhabi in United Arab Emirates. Cases included several healthcare workers, visitors, patients, and ambulance staff. Person to person transmission was confirmed in $> 75\%$ of cases. The majority of infected health care workers developed mild symptomatic or asymptomatic infection, but about 15% had severe illness or died [93].

The recent outbreak of South Korea occurred in May 2015. The index case was a man who had recently traveled to Bahrain, the United Arab Emirates, Saudi Arabia, and Qatar [55]. As of late July 2015, > 180 secondary cases were reported including 36 death and many cases had been reported among household and hospital contacts [55,67]. In China, one case occurred in a man who traveled to China from Korea following exposure to two relatives with MERS-CoV infection [55].

Disease seasonality

In spite of reporting of MERS-CoV infections throughout the year, some evidence on disease seasonality occurred. The first identified cases of MERS-CoV infection were reported in April and June 2012 [5,46,47]. A high increase in cases was reported in April and May 2013 followed by a surge in case reporting in April and May 2014. Increase in case reporting in March to May 2013 were attributed to infection from infected young camels [94,95], but the increase in 2014 in Saudi Arabia and in South Korea in 2015 were due to gaps in infection control in hospitals. Small peaks in case reporting occurred in September and November of 2013 and 2014. The epicurve of infection is shown in Fig. 1.

Table 2 Number of MERS-CoV by country as of July 7, 2015 as per WHO data

Country	2012	2013	2014	2015	Total
Algeria	0	0	2	0	2
Austria	0	0	1	0	1
China	0	0	0	1	1
Egypt	0	0	1	0	1
France	0	2	0	0	2
Germany	1	1	0	1	3
Greece	0	0	1	0	1
Iran	0	0	5	1	6
Italy	0	1	0	0	1
Jordan	2	0	10	0	12
Kuwait	0	2	1	0	3
Lebanon	0	0	1	0	1
Malaysia	0	0	1	0	1
Netherlands	0	0	2	0	2
Oman	0	1	1	4	6
Philippines	0	0	0	2	2
Qatar	0	7	2	4	13
South Korea	0	0	0	185	185
Saudi Arabia	5	136	679	210	1037
Thailand	0	0	0	1	1
Tunisia	0	3	0	0	3
Turkey	0	0	1	0	1
United Arab Emirates	0	12	57	7	76
UK	1	3	0	0	4
USA	0	0	2	0	2
Yemen	0	0	1	0	1
Total	9	168	768	413	1368

Zoonotic origin of MERS-CoV

Role of bats

Sources and modes of transmission of MERS-CoV are still unclear. Initially, a bat origin of MERS-CoV was suggested based on the relation of genome sequences between MERS-CoV and bat coronaviruses [96]. Cell tropism studies showed that both bat coronavirus HKU4 and MERS-CoV shared the same cell type receptors, DPP4 [4,7,75]. MERS-CoV grows readily in several bat-derived cell lines [14]. There is no evidence for direct or indirect transmission of MERS-CoV from bats to humans. Virological studies performed in Europe, Africa, and Asia, including the Middle East, have shown that coronavirus RNA sequences are found frequently in bat feces. Some of the sequences were closely related to MERS-CoV sequences [97–99]. In a survey from Saudi Arabia, 823 fecal and rectal samples were tested by PCR for MERS-CoV, many coronaviruses sequences were detected [97]. Most of the detected sequences were unrelated to MERS-CoV, but one sequence of 190 nucleotide in the RNA-dependent RNA polymerase (RdRp) gene had a 100% identity with a MERS-CoV. This sequence was detected from feces of a *Taphozous perforatus* bat captured near the home of the index Saudi patient. Uncommon contact of humans with bats indicates that bats are not the intermediate host of MERS-CoV transmission but may be the reservoir of the virus [100].

Role of camels

Dromedary camels (*Camelus dromedarus*) appear to be the source of MERS-CoV. Other animals like sheep, goats, and cows tested negative to anti-MERS-CoV antibodies. Camel sera from Oman, Canary Islands, and Egypt were positive for anti-MERS-CoV antibodies in about 100%, 14%, and $> 90\%$ of the samples respectively [63,101, 102]. Retrospective studies on archived human sera showed no evidence of infection with MERS-CoV before 2012 [103], but anti-MERS-CoV antibodies were detected

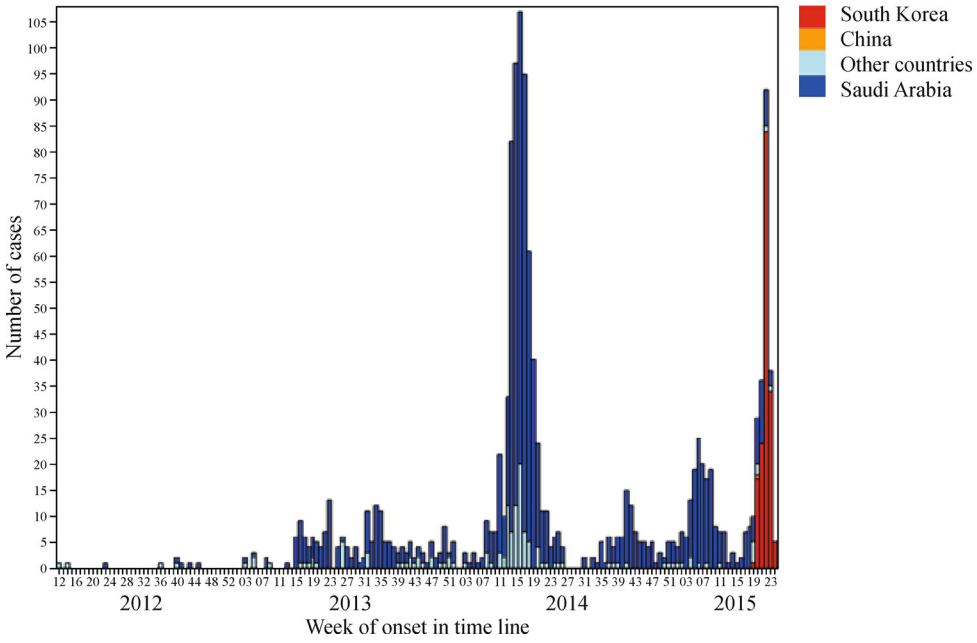

Fig. 1 Epidemiological curve of MERS-CoV up to week 23, 2015 as per WHO data.

in archived camel sera in Saudi Arabia in 1993 [95], and United Arab Emirates in 2003 [104], indicating circulation of MERS-CoV in camels for many years. Bactrian camels in Mongolia tested negative for MERS-CoV antibodies [105].

Serologic studies from around the Middle East suggested that camels are one of the sources of MERS-CoV as > 90% of adult camels tested positive and had high titers of antibodies. Seropositivity was different in juvenile camels and was usually lower than in adults. These results suggested that MERS-CoV infections in camels occurred in young ages followed by frequent boosting [94,95,102,104]. Camels in other parts of the world, far from the Middle East like in Europe, Australia, and the Americas do not have MERS-CoV antibodies and have no evidence of infection [106]. Table 3 summarizes camel serologic studies.

In a study aimed to evaluate virus infectivity and shedding in camels, three adult dromedary camels were inoculated with MERS-CoV intratracheally, intranasally, and conjunctivally. Those camels shed large quantities of virus from the upper respiratory tract and infectious virus was detected in nasal secretions for 7 days post-inoculation and viral RNA for up to 35 days post-inoculation [113].

Human infections with MERS-CoV were linked to camels. The first evidence was a study in Saudi Arabia in which the MERS-CoV full genome sequences of isolates from a man with fatal infection and from one of his camels were identical. This patient had a direct contact with his deceased camels some days before the onset of symptoms. These results suggested that MERS-CoV can infect dromedary camels and can be transmitted from them to humans by direct close contact [86]. In other studies, phylogenetic analyses of camel and human isolates of the MERS-CoV genome demonstrated that the viruses were highly identical or in some cases were similar to each other [107,108,114].

Seroepidemiological studies shown low prevalence of MERS-CoV antibodies in humans in Saudi Arabia [103,115]. A survey of 10 009 individuals representative of the general population of Saudi Arabia resulted in 15 seropositive subjects (0.15%), however, seropositivity increased 15−23 folds in camel-exposed individuals [78]. In a separate report, 7 of 87 camel shepherds and 140 slaughterhouse workers (3.1%) tested positive for MERS-CoV antibodies [103]. An overview of MERS-CoV transmission routes is illustrated in Fig.2.

Vaccines and antivirals

Vaccines

The development of an effective vaccine is critical for prevention of a MERS-CoV pandemic. Some investigators have indicated that the RBD protein of MERS-CoV S protein is a good candidate antigen as a subunit vaccine. Various RBD fragments showed the highest DPP4 binding affinity and induced the highest-titer of IgG Ab and neutralizing Ab in mice and rabbits [17,18,116–120]. A

Table 3 Serological studies in camels

Study location	Sampling time	Sample size	Test name	Animal species	Seropositive percentage	Camel age	Reference
Oman	2013	50	Protein microarray, neutralization test	Dromedary	100	Adult	[102]
Canary Islands	2013	195	Protein microarray, neutralization test	Dromedary	14	Adults and one juvenile	[102]
Egypt	2013	110	Pseudoparticle, neutralization assay	Dromedary	94	Adult	[63]
Egypt	2013	52	Pseudoparticle, neutralization assay	Dromedary	92.3	Adult	[107]
Egypt, Sudan, Somalia	1983–1997	43	ELISA, neutralization test	Dromedary	81.4	Not available	[106]
Qatar	2013	14	Neutralization assay	Dromedary	100	Not mentioned	[108]
Saudi Arabia	2010–2013	310	Pseudoparticle neutralization assay	Dromedary	90	27 juveniles, 283 adults	[109]
Saudi Arabia	2014	9	Immunofluorescence assay	Dromedary	22.2	6 adults, 3 juveniles	[73]
UAE	2005	151, 500	Spike protein microarray	Dromedary	81.8	Adult	[104]
USA, Canada	2000–2001	6	Neutralization assay	Dromedary	0	Adult	[110]
Ethiopia	2010–2011	188	Protein microarray	Dromedary	96	1–13 years	[111]
Nigeria	2010–2011	358	Protein microarray	Dromedary	94	Adult	[111]
Jordan	2013	11	Protein microarray, neutralization test	Dromedary	100	Juvenile	[101]
Tunisia	2010–2011	204	Protein microarray	Dromedary	48.5	1–16 years	[111]
Sudan	1983	60	ELISA, neutralization test	Dromedary	86.7	Adult	[106]
Somalia	1983, 1984	25, 61	ELISA, neutralization test	Dromedary	80, 85.2	Adult	[106]
Kenya	1993–2013	774	ELISA	Dromedary	27.5	Not mentioned	[112]
Australia	2014	25	Pseudoparticle, neutralization assay	Dromedary	0	Adults and juvenile	[95]
Mongolia	2015	200	Pseudoparticle neutralization test	Bactrian	0	Adults and juvenile	[105]

robust neutralizing antibody response was elicited in BALB/c mice against MERS-CoV after immunization with purified full S protein nanoparticles produced in Sf9 cells infected with specific recombinant baculovirus containing the S gene [121] or a recombinant human adenoviral vectors (rAd5 or rAd41) containing the S or S1 genes [122,123]. Vaccinia Ankara was encoded with full S protein and inoculated to BALB/c mice that developed high levels of neutralizing antibodies and had induction of humoral and cell-mediated immunity [124,125]. Another study using Ad5-hDPP4-transduced BALB/c mice immunized with Venezuelan equine encephalitis virus replicon particles containing S protein elucidated a reduction of viral titers to nearly undetectable levels and increased neutralizing antibodies [35].

Recently, Wang *et al.* developed two candidate vaccines, a subunit (full S and S1 protein fraction) and a DNA vaccine (full S and S1 gene in a mammalian VRC8400 vector). The vaccine containing the full S DNA and S1 protein was the most efficacious in mice and rhesus macaques [126].

Passive immunization

Using antibodies to deter MERS-CoV infection appears to have some promise. Transfer of sera containing anti-MERS-CoV-S protein to or seropositive camel sera to Ad5-hDPP4-transduced mice accelerated virus clearance, inhibited virus attachment, and reduced weight loss [35,37,127]. Recently, Corti *et al.* successfully isolated monoclonal antibodies from serum obtained from a MERS-CoV survivor after 200 days of infection [128]. Transduced Ad5-hDPP4 BALB/c mice were immunized with 15 mg/kg of the mAb and showed decreased lung

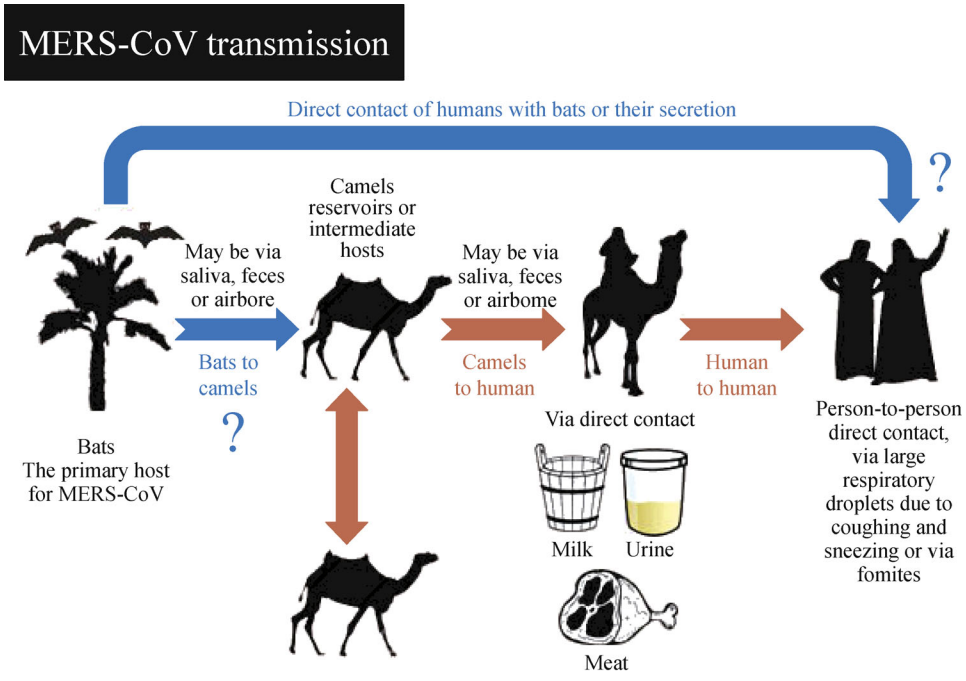

Fig. 2 Zoonotic transmission of newly emerged MERS-CoV.

viral titers, no weight loss, and decreased peribronchial lymphoid infiltration [128].

Antivirals

No approved antivirals for use against MERS-CoV infection are yet available. The first approach performed when a new unknown virus like MERS-CoV emerges is testing drugs used as antiviral for similar viruses [29,129,130]. Type I interferons and ribavirine combination exhibited acceptable results in cell culture and rhesus macaques by decreasing the host inflammatory response, replication of virus, and improved clinical outcome [129,131,132]. A human cohort study in Saudi Arabia showed that treatment with combination of ribavirin and interferon-α2b to 5 did not improve clinical outcomes but this may have been due to late treatment or due to the immunocompromised state of the patients [133]. In a retrospective study of 20 MERS-CoV infected patients treated with ribavirin and interferon α-2a, results showed 14-day and 28-day survival was improved by 70% and 28% in the treated group as compared to an untreated group [134].

The second approach is screening of approved drugs with known safety profiles and transcriptional signatures in different cell lines. Several drugs, including antiparasitics, neurotransmitters, antibacterials, inhibitors of clathrin-mediated endocytosis estrogen receptor, lipid or sterol metabolism, protein processing, and DNA synthesis or repair were tested on culture cells [119,135–139]. Lopinavir-ritonavir combined with pegylated interferon and ribavirin therapy showed improved outcomes in infected marmosets [140].

The third approach involves *in vitro* inhibition of S protein to block virus entry into host cells using designed antiviral peptides targeting the HR2 domain of the S2 subunit of the MERS-CoV and preventing the interaction between the HR1 and HR2 domains required for the formation of the heterologous six-helix bundle in viral fusion core formation [22,23]. Other drugs that act as inhibitors for viral proteases and helicase to suppress MERS-CoV infection were tested [141–145]. Other investigators studied inhibition of MERS-CoV infection by competitive inhibition of DPP4 cell receptor using compounds such as sitagliptin, vildagliptin, and saxagliptin [19,146].

Conclusions

More than three years have passed since the first detection of MERS-CoV human infection and the virus, uncontrolled, continues to cause major outbreaks in the Middle East. The recent outbreak in Korea demonstrated that a single index case can lead to 185 more infections in a short period of time, hence raising questions about the accuracy of the number of cases being reported in the Middle East. Furthermore, the Korean outbreak confirmed the high fatality rate of MERS-CoV infection as being true rather

than overestimated in case only the more severe cases are detected. In all, public health, veterinary health, and research efforts need to be consolidated in order to answer the following high priority questions:

- What is the true extent of human infection with MERS-CoV?
- What antivirals and vaccines are to be used in humans?
- What infection control measures are needed in healthcare settings to prevent nosocomial outbreaks?
- What measures need to be in place in order to prevent zoonotic infections from camels?
- Is it possible to control the virus in the camel population and if so, how?
- Are there other animal species involved in the MERS-CoV transmission cycle?

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Compliance with ethics guidelines

Mahmoud M. Shehata, Mokhtar R. Gomaa, Mohamed A. Ali, and Ghazi Kayali declare that they have no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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