Review

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Middle East respiratory syndrome coronavirus: transmission and phylogenetic evolution

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The Middle East respiratory syndrome coronavirus (MERS-CoV) was first described in 2012 and, subsequently, many cases were reported with a lower case fatality rate than initial cases. Humans can become infected within their communities and transmission can then be amplified in the healthcare setting. Contact investigation among cases shows a variable amount of spread among family members and healthcare workers. So far, circulating virus strains remain similar under continuous monitoring, with no genetic changes. Here, we discuss the transmission pattern, phylogenetic evolution, and pathogenesis of MERS-CoV infection.

Global spread of Middle East respiratory syndrome coronavirus

The initial case of MERS-CoV was reported from a patient from Bisha who was subsequently admitted to a private hospital in Jeddah, the Kingdom of Saudi Arabia (KSA) in 2012 [1]. Given that the etiology of this severe community-acquired pneumonia became known as MERS-CoV, formerly novel coronavirus, a retrospective investigation of an outbreak of 13 cases of severe acute respiratory infection clustered in a hospital in Zarqa, Jordan was conducted. The investigation revealed two confirmed and 11 probable cases in March 2012 [2]. Since then, cases have been reported from KSA [1,3–11], Jordan [2,12], France [13,14], United Arab Emirates (UAE) [13–15], Qatar [3,16], Tunisia [3] (http://www.promedmail.org/direct.php?id=1725864), UK [17], Italy [18], Oman (http://www.who.int/csr/don/2014_05_15_mers/en/), Kuwait, Yemen, Germany, The Netherlands, Greece, Malaysia, Philippines, Iran, and Egypt (http://www.cdc.gov/coronavirus/mers/index.html). The total global cases as of 16 June 2014 were 701 laboratory-confirmed cases officially reported to the World Health Organization (WHO), including 249 (35.5%) deaths (http://www.who.int/csr/don/2014_06_16_mers/en/). The number of cases per reporting country is shown in Table 1. In this review, we highlight the transmission, evolution, and pathogenesis of MERS-CoV infection.

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Interhuman transmission of MERS-CoV and genetic analysis

It is thought that most MERS-CoV cases are acquired through human-to-human transmission. An analysis of 144 confirmed and 17 probable cases showed that 95 cases (59%) were secondary cases with an epidemiologic link to other confirmed cases [3]. The acquisition of infection in these 95 cases occurred more frequently in healthcare settings (63.2%) and among family contacts (13.7%) [3]. In a recent update by the WHO, it was noted that the number of laboratory-confirmed MERS-CoV cases increased sharply at the beginning of mid-March 2014, in KSA and UAE, in relation to the occurrence of healthcare-associated outbreaks (http://www.who.int/csr/don/2014_05_28_mers/en/). The occurrences of healthcare-associated clusters were reported from KSA [3], Jordan [2,12], France [13], UAE, and Qatar [3]. Household transmission and clusters were also reported from KSA [5,6] and Tunisia [3], and transmission occurring in both healthcare and household settings was reported from UK [17] and Italy [18].

Another large healthcare-associated cluster reported from Al-Hasa, KSA involved 23 hemodialysis patients, family members, and healthcare workers [4]. It was evident epidemiologically that person-to-person transmission occurred in 21 (91.3%) of the cases [4]. Subsequent phylogenetic analysis of the virus isolates from the Al-Hasa outbreak revealed that there was more than one possibility of the MERS-CoV virus being introduced from the community [19]. The Al-Hasa MERS-CoV variants were not detected in any other part of KSA, and the Al-Hasa region has remained free of other virus variants [20]. The same analysis of 32 MERS-CoV isolates from May 2013 to September 2013 revealed four different MERS-CoV clades, namely: Hafr-Al_Batin_1 clade; Buraidah_1 clade; Riyadh_3 clade; and Al-Hasa clade [20]. The second three clades (i.e., not the Hafr-Al_Batin_1 clade) were no longer circulating or contributing to cases in KSA [20]. In addition, the Al-Hasa clade was confined to an area in the eastern region of KSA. The other three clades were detected in cases from many locations, suggesting a mobile human or nonhuman source. The duration of the existence of each clade was 98 days (range: 62–147 days) [20].

Table 1. Number of reported MES-CoV cases and number of deaths per country[a]

Country	Cases	Deaths
Saudi Arabia	689	283
United Arab Emirates	70	9
Qatar	7	4
Jordan	18	5
Oman	2	2
Kuwait	3	1
Egypt	1	0
Yemen	1	1
Lebanon	1	0
Iran	2	1
UK	4	3
Germany	2	1
France	2	1
Italy	1	0
Greece	1	0
The Netherlands	2	0
Malaysia	1	1
Tunisia	3	1
Algeria	2	0
Philippines	1	0
USA	2	0
Total	815	313

[a]As of 4 June 2014, according to European Center for Disease Prevention and Control (http://www.ecdc.europa.eu/en/press/news/_layouts/forms/News_DispForm.aspx?List=8db7286c-fe2d-476c-9133-18ff4cb1b568&ID=1016).

Recently, analysis of reported genetic data from a community cluster in the city of Hafr Al-Batin showed that at least two of the infected contacts could not have been directly infected from the index patient and, thus, an alternate source was a possibility [11]. Therefore, the reported cases seemed to originate in the community with nonsustained human-to-human transmission and subsequent amplification of cases in the healthcare setting. This is more evident with the current increase in the number of cases in the Jeddah region, where there was also amplification in the healthcare setting for these cases [21] (http://www.moh.gov.sa/en/CoronaNew/PressReleases/Pages/default.aspx). Thus, immediate isolation of these MERS-CoV cases in the healthcare setting would prevent subsequent transmission of the virus.

It is estimated that the rate of molecular evolution of MERS-CoV is 1.12×10^{-3} substitutions/site per year [20]. The spike protein is important in potentiating membrane fusion and coronavirus entry and a variation in this protein was documented in the main Saudi Arabian lineage and the Munich/Abu Dhabi/2013 cluster [19]. The genetic analysis of MERS-CoV, especially in the Al-Hasa outbreak and in the community from Hafr Al-Batin, showed multiple introduction of MERS-CoV, possibly from an animal source as well as limited human-to-human transmission, with different clades in circulation in different regions.

Transmission among family and healthcare worker contacts

Screening of healthcare workers and family contacts of presumed index cases have been extensively studied [2,7,14,16,17,22,23]. A summary of the contact investigation results is shown in Table 2 [2,7,12,14,16,17,22,23]. In the UK, contacts of two index cases and an additional two (2.17%) secondary cases out of 92 contacts were identified, but none of the 43 contacts of the secondary cases were positive [17,22]. In France, one (0.8%) of 124 contacts of the index case and none of the 39 contacts of the secondary case were positive for MERS-CoV [14]. Using serology, investigation of 85 contacts of an index case failed to demonstrate any positive cases [16], and the use of respiratory PCR and serology among 83 contacts of another case did not reveal any positive cases [23]. The largest screening of contacts is from KSA [7]. Of the 1695 healthcare contacts and 462 family contacts of confirmed MERS cases, a positive result was obtained from 19 (1.12%) healthcare workers and 14 (3.6%) cases of family contacts [7]. Thus, the rate of secondary transmission among family contact is higher than other contacts. Thus, close and prolonged contact with the index case seems to be a requirement for the spread of the disease. Another possible explanation is exposure of other family members to the same source of infection, as was evident from the Hafr Al-Batin community outbreak [11].

Zoonotic transmission of MERS-CoV

The origin of the MERS-CoV and the intermediate host is currently not well known. CoVs are grouped into: *Alphacoronavirus, Betacoronavirus* (classified as clades 2a–2d), *Gammacoronavirus*, and *Deltacoronavirus*, and all known 2c bat CoVs originate from bats [24]. It was also thought that MERS-CoV originates from bats because MERS is classified in the C betacoronaviruses [25]. A fragment of CoV sequence (182 nucleotides) was recovered from *Taphozous perforatus* (Egyptian tomb bats) [25]. The bat was found living near the home and work location of a case of MERS-CoV infection in the city of Bisha in western KSA

Table 2. Results of contact investigation

Method	Case	Number of contacts	Number (%) positive	Country	Refs
Respiratory PCR	Outbreak	13	2 (15.4)	Jordan	[2]
Serology	Outbreak	124	9 (7.5)	Jordan	[12]
Respiratory PCR	Index	92	2 (2.2)	UK	[17,22]
Respiratory PCR	Secondary	43	0 (0)	UK	[2,17]
Respiratory PCR	Index	124	1 (0.8)	France	[14]
Respiratory PCR	Secondary	39	0 (0)	France	[14]
Serology	Index	85	0 (0)	Germany	[16]
Serology	Secondary	83	0 (0)	Germany	[23]
Respiratory PCR	Index secondary	1695 (HCW)[a]; 462 (family)	19 (1.12); 14 (3.6)	KSA	[7]

[a]Abbreviation: HCW, healthcare workers.

Table 3. Summary of different studies of MERS-CoV in camels

Country of study	Date of sampling	Test	Number tested	Number positive	% positive	Refs
Oman	2013	Serology	50	50	100	[27]
Spain (Canary Islands)	2012–2013	Serology	195	15	14	[27]
Qatar	17 October 2013	Neutralization assay	14	14	100	[28]
		PCR	14	3	21	[28]
Egypt	June 2013	Serology	110	103	94	[29]
Saudi Arabia (Al-Hasa & Riyadh)	2010–2013	Serology	310	280	90	[30]
UAE	2003	Serology	151	151	100	[31]
	2013	Serology	500	481	96	[31]
KSA (Jeddah)	2014	PCR	9	2	22.2	[32]
Egypt	June–December 2013	Pseudoparticle neutralization assay	52	48	92.3	[33]
		PCR	110	4	3.6	[33]
Oman	December 2013	PCR	76	5	6.6	[34]
UAE	February–October 2005	Neutralization test	11	9	81.8	[35]
USA, Canada	2000–2001	Neutralization test	6	0	0	[35]
Ethiopia	2010–11	Serology	188	181	96	[36]
Nigeria	2010–2011	Serology	358	336	94	[36]
Tunisia	2010–2011	Serology	204	99	48.5	[36]
Jordan	June and September 2013	Serology	11	11	100	[37]
Kenya	1993–2013	Serology	774	213	27.5	[88]
Egypt	1997	Serology	43	35	81.4	[89]
Sudan	1983	Serology	60	52	86.7	[89]
Somalia	1983	Serology	25	20	80	[89]
Somalia	1984	Serology	61	52	85.2	[89]

[26]. It was also found that DPP4 receptor is also the receptor for bats HKU4 virus [87]. Analysis of many human MERS-CoV genomes showed diversity within the isolates and suggested the possibility of multiple independent zoonotic events [15,19]. More recent studies point toward camels as possible reservoirs or intermediate hosts.

The first study to point to camels as a possible source and reservoir was based on positive serological results from 50 serum samples from dromedary camels (*Camelus dromedaries*) from Oman that showed a high titer of neutralizing antibodies against MERS-CoV [27]. Subsequently, multiple studies showed positive antibodies in camels from many countries (Table 3) [27–37], including Spain (Canary Islands), Egypt, Qatar, KSA, UAE, and Jordan. The detection of MERS-CoV by PCR was also obtained from two out of nine (22.2%) camels from the farm of a patient in Jeddah, KSA [32]. Similarly, in an investigation of two human cases of MERS-CoV in Qatar, of the 14 tested dromedary camels, three tested positive by PCR for MERS-CoV [28]. In dromedary camels, there is more than one genomic variant compared with humans, who have one variant, and this suggests interspecies transmission of MERS-CoV of specific genotypes [38]. There was one amino acid change in the spike protein within the receptor-binding domain and other changes were outside the receptor-binding domain [38]. Despite the evidence linking camels to MERS-CoV, camel contact of patients was reported in only 4.3% of the total 161 cases and in 18.3% of the 49 cases with documented animal exposure [17]. More recently, MERS-CoV was isolated from the milk of five out of 12 camels, suggesting another mode of transmission [39]. However, it is unusual for milk consumption to cause respiratory illness because the pathogenesis of the initial events of MERS-CoV is thought to be upper respiratory tract infection with subsequent viremia rather than viremia causing respiratory infection. Interestingly, a recent study showed that camels may have acquired MERS-CoV from bats in sub-Saharan Africa, with subsequent importation of camels to the Arabian peninsula [40]. Identification of the major route of animal-to-human transmission is needed to understand the overall picture of persistence and evolution in the different hosts [41].

Transmission mode of MERS-CoV

Possible routes of transmission were elucidated from the largest nosocomial outbreak of MERS-CoV in the Al-Hasa region in May–June 2012 [4,42–44]. Respiratory droplet transmission was the most likely mode of transmission, with the possibility of direct or indirect contact and airborne transmission during aerosol-generating procedures as other possible routes of transmission [4,45]. Healthcare workers are advised to follow contact and droplet isolation precautions when dealing with patients with MERS-CoV with the addition of airborne infection isolation precautions during aerosol-generating procedures [45,46]. The only study describing infection among healthcare workers showed that many healthcare workers were involved in aerosol-generating procedures, such as intubation (71.4%), airway suctioning (57%), and sputum induction (28.6%) [47]. Further recommendations for the use of airborne infection isolation precautions were recently discussed in two recent publications [48] (http://www.ecdc.europa.eu/en/press/news/_layouts/forms/News_DispForm.aspx?List=8db7286c-fe2d-476c-9133-18ff4cb1b568&ID=1002). Airborne spread of severe acute respiratory syndrome (SARS)-CoV and MERS-CoV is thought to be predominantly in the form of large

respiratory droplets expelled during coughing and sneezing and via contact with fomites [48]. A recent study showed that identical MERS-CoV RNA fragments were detected in an air sample collected from the barn of a camel that shared similar MERS-CoV with an infected person [49]. However, the evidence of aerosol dissemination was lacking.

Coronaviruses are categorized based on the levels of oral–fecal and respiratory transmission, as indicated by protein intrinsic disorder predictions [50]. Based on these predictions, MERS-CoV has hard inner and outer shells that enable its persistence in the environment [50]. Thus, MERS-CoV is predicted to have the highest oral–fecal transmission rates and relatively low respiratory transmission rates [50]. In a patient with MERS-CoV, the virus was detected in stool samples obtained on days 12 and 16, with a viral load of up to 1031 RNA copies/g [15]. Patients with SARS commonly show high virus concentrations and prolonged virus excretion in stools, leading to the use of stool samples for routine virological diagnosis [51–53]. Diarrhea was reported to occur in patients with MERS-CoV [6,8,13]. An oral–fecal route of transmission of MERS-CoV was not documented in the clinical setting. MERS-CoV seems to be more stable at low temperatures and low humidity. MERS-CoV was recovered after 48 h at 20°C and 40% relative humidity (RH), and the virus was viable for 8 h at 30°C and 80% RH and for 24 h at 30°C and 30% RH [54].

In addition to the required respiratory or airborne infection isolation precautions, it is recommended to use contact isolation precautions [42,43,45]. In the SARS-CoV era, the amplification of infection in the healthcare setting is thought to be related to multiple factors, including overcrowding and reduced space between patient beds of less than 1 m; poor ventilation; use of nebulization; oxygen or non-invasive ventilation therapy; and resuscitation of patients [55,56]. The US and European Centers for Disease Control and Prevention (CDC) have called for the use of airborne infection isolation precautions when dealing with patients with MERS-CoV while they await further evidence to support their superiority over contact and droplet precautions (http://www.ecdc.europa.eu/en/press/news/_layouts/forms/News_DispForm.aspx?List=8db7286c-fe2d-476c-9133-18ff4cb1b568&ID=1002).

MERS and SARS pathogenesis comparison

Most of the data relating to the pathogenesis of SARS-CoV come from *in vitro* studies. The virus is thought to enter the cells through dipeptidyl peptidase 4 (DPP4), which is also known as CD26 [57]. DPP4 is found on the surfaces of nonciliated bronchial epithelial cells in humans [57]. The MERS-CoV spike protein interacts with cellular DPP4 and mediates viral attachment to the host cells [58]. In studies of the pathogenesis of MERS-CoV, the use of anti-DPP4 antibodies resulted in the inhibition of MERS-CoV infection of primary human bronchial epithelial cells [57]. Adenosine deaminase is a DPP4-binding protein that competes for virus binding and acts as a natural antagonist for MERS-CoV infection [59]. Thus, the presence of DPP4 dictates which species are infected with MERS-CoV and explains the difference in hosts between MERS-CoV and SARS-CoV. SARS-CoV-like viruses have been found in Himalayan palm civets (*Paguma larvata*), raccoon dogs (*Nyctereutes procyonoides*), and the Chinese ferret badger (*Melogale moschata*) [60]. The MERS-CoV was found to bind to DPP4 from various animals, such as camel, goat, cow, and sheep [61]. However, there was no serological evidence of MERS-CoV in these animals [37] and only camels were found to have MERS-CoV antibodies [27–37].

Similar to SARS-CoV, MERS-CoV papain-like proteases (PLpro) block the regulation of the cytokines CCL5, interferon β (IFN-β), and CXCL10 in stimulated cells [62]. Human, camel, and horse cells were more likely to be infected with MERS-CoV than were goat and bat cells, and the presence of a cell surface lung protease increased susceptibility to MERS-CoV [63]. MERS-CoV can infect lower respiratory, kidney, intestinal, and liver cells, as well as histiocytes [64], and may infect nonhuman primate, porcine, bat, civet, and rabbit cell lines [64,65]. When human monocyte-derived macrophages (MDMs) were infected with either MERS-CoV or SARS-CoV, neither virus significantly stimulated the expression of antiviral cytokines (IFN-α and IFN-β) but both induced similar levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). However, MERS-CoV induced significantly higher levels of IL-12, IFN-γ and chemokines (IP-10/CXCL-10, MCP-1/CCL-2, MIP-1α/CCL-3, RANTES/CCL-5, and IL-8) [66]. The presence and activation of immune cell recruiting chemokines and immunostimulating cytokines were higher and more prolonged in cases of MERS-CoV, compared with SARS-CoV and this may explain the presence of more severe disease and a higher fatality rate in patients with MERS-CoV [66]. MERS-CoV uses a viral double-stranded RNA-binding protein (4a protein) to evade the innate host response [67]. The MERS-CoV 4a protein is an immunosuppressive factor that antagonizes type I interferon production [67]. Thus, MERS-CoV might cause severe disease in humans by counteracting host immunity and the sensing of virus invasion [67]. Understanding how MERS-CoV weakens virus sensing would be informative for the development of new strategies for therapy and vaccine.

Recently, a recombinant protein containing the receptor-binding domain (RBD) of MERS-CoV spike (S) glycoprotein infused with Fc of human IgG (RBD-Fc) resulted in neutralizing antibodies in mice [68,69]. The use of the intranasal MERS-CoV RBD-Fc vaccination resulted in a similar systemic humoral immune responses to subcutaneous vaccination, and higher systemic cellular immune responses and local mucosal immune responses [70]. DPP4 in mice did not support MERS-CoV infection, but using mouse DPP4 as a scaffold showed that two critical amino acids (A288L and T330R) are important for the development of a mouse-adapted MERS-CoV strain for rapid assessment of therapy and vaccines [71]. Understanding the contribution of different antigenic components of the MERS-CoV and utilizing spike glycoprotein may result in the development of an effective vaccine.

Variation in the MERS-CoV infection response

There is wide variation in the host response and clinical outcome of MERS-CoV infection from asymptomatic, to mildly symptomatic to a fatal disease [7,8]. MERS-CoV infection was also found to have a male predominance and

more specifically among those with underlying comorbid conditions. These differences in the susceptibility may be related to underlying genetic variation, environmental exposure, or demography [72]. A patient with a poor outcome did not promote type 1 IFN and IFN-α compared with a patient who survived [73]. IFN-α promotes antigen presentation in response to viruses, impairs the development of a robust antiviral response and, thus, decreases viral clearance [73]. Two human RBD-specific neutralizing monoclonal antibodies (MERS-4 and MERS-27) inhibited infection of live MERS-CoV with IC_{50} (half-maximal inhibitory concentration) at nanomolar concentrations [74]. Given that MERS-CoV was initially limited to patients with chronic diseases [4], causing severe disease in those with comorbid conditions and tends to affect certain individuals among family contacts [7]. Understanding the factors involved in the pathogenesis of the disease would facilitate the development of therapeutic options.

Prospects for control of MERS-CoV

The emergence of SARS in 2003 and the subsequent control of the virus shed light on important measures for the control of emerging viral respiratory infections. These measures include: quick case detection, isolation of suspected cases, effective contact tracing, and the practice of basic infection control measures [75] (http://www.who.int/csr/sars/en/WHOconsensus.pdf). In a mathematical model, a combination of household-based quarantine, isolation of cases outside the household, and targeted prophylactic use was shown to be effective in the prevention of viral transmission [76]. Delays of more than 3 days in contact tracing, case identification, and quarantine adversely affected the effectiveness of isolation [77]. One study estimated the effectiveness of the isolation of symptomatic patients and the tracing and quarantine of contacts [78]. The authors concluded that SARS could be controlled by effective isolation of symptomatic patients [78]. This finding is in contrast to the case of pandemic influenza because of the high level of presymptomatic transmission that does not occur with SARS [78]. In a review of the effectiveness of community quarantine, it was found that this is an effective preventive measure, but quantification of the effect was lacking [79,80]. The delay in the isolation of patients in the healthcare setting also contributed to the spread of SARS [79]. The Al-Hasa MERS-CoV outbreak and the recent increase of MERS-CoV cases in April–May 2014 also point to inadequate infection control measures [4] (http://www.who.int/csr/disease/coronavirus_infections/MERS_CoV_RA_20140424.pdf?ua=1). Prompt identification of patients with SARS and the implementation of infection control measures resulted in the prevention of secondary transmission of SARS [81]. Different strategies may be used for the control of an outbreak in a healthcare setting. A hospital closure is needed to control nosocomial transmission if the outbreak is detected late, or closure early in an outbreak is sufficient to remove exposed persons to a designated location or keeping them in one place [82]. In a study of family members and other visiting patients with SARS, it was shown that the rate of SARS among visitors was lower when they used masks [83]. Thus, prompt identification of cases with subsequent proper isolation in the healthcare setting or community quarantine is a key measure to prevent the spread of emerging respiratory pathogens.

Box 1. Outstanding questions

- How is MERS-CoV transmitted to humans?
- What promotes interspecies transmission?
- Are camels the only animal host?
- What is the prevalence of immunity among the general population in the affected countries?
- Why is there enhanced disease severity in people with comorbidities or the elderly?
- What is the exact period of infectiousness?

Concluding remarks

In conclusion, the number of MERS-CoV cases has recently increased due to multiple introductions into humans from the animal reservoir, with no long-term sustained human-to-human transmission. There is no human reservoir of cases with few or no symptoms [84]. Infection in the healthcare setting is an important risk factor for human-to-human transmission. It is not easy to identify patients with MERS-CoV during the early stages of the disease based on clinical presentation alone because patients may have mild or atypical symptoms [84]. In a univariate analysis, the presence of a normal white blood cell count (WBC) and interstitial infiltrate may be used as clues to the presence of MERS-CoV infection [10]. Thus, it is important to have a high index of suspicion and to isolate those patients early to avoid healthcare transmission. It is also important to have early diagnostic tests based on respiratory specimens, preferably lower respiratory tract specimen for rapid diagnosis [85]. The implications of MERS-CoV for travelers with comparison with SARS-CoV were recently reviewed [21,86]. The origin of SARS-COV and MERS-CoV is an animal source with subsequent human transmission [21,86]. Although SARS resulted in a widespread infection, MERS-CoV has caused limited travel-associated human cases with no major events related to the Hajj [86]. In regard to their clinical presentation, both SARS-CoV and MERS-CoV have significant overlap of signs and symptoms [21]. Further studies are needed to identify the presence of other animal reservoirs for MERS-CoV and to pinpoint the risk for the development of severe disease (Box 1).

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