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Review Article

Middle East Respiratory Syndrome Coronavirus (MERS-CoV): A Review Article

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Abstract

The recently emerged Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in the Middle East region in 2012. The virus is phylogenetically related to bat CoV, but other animal species like camels and goats may potentially act as an intermediate host by spreading the virus to humans. This virus is thought to cause a severe disease in patients with underlying comorbidities. Laboratory response capacity during the early stages of MERS-CoV outbreak focuses on development of virological and immunological methods for diagnosis, for contact tracing and for epidemiological studies into sources, modes of transmission, identification of risk groups and animal reservoirs. Current international recommendations do not support any specific therapies; however there are a number of agents which were used during the SARS epidemic of 2003. It is possible that these might be active against the related coronavirus; in the other hand, development of affective vaccine is crucial for preventing further pandemic of MERS-CoV. In this article we reviewed available data from MERS-CoV case reports.

Keywords: MERS-CoV, Respiratory Coronavirus, Middle East Respiratory Syndrome

History and Epidemiology

Coronaviruses are enveloped RNA viruses that are broadly distributed among humans, other mammals and birds causing acute and persistent infections. Members of this family were isolated as early as 1930s as the causative agents of infectious bronchiolitis in chickens, transmissible gastroenteritis in pigs and severe hepatitis and neurologic disease in mice. In 1960s these viruses were recognized to share characteristics that merited their being grouped together. The most notable common feature demonstrated by electron microscopy was a fringe of widely spaced, cube-shape spikes that projected from the virion surface. The halo of spikes was described as giving the viral particle the appearance of solar corona which prompted the name that was adopted for this new virus group [1].

The emergence of a highly pathogenic human coronavirus in the Middle East has sparked new interest in human coronaviruses around the world. Middle east respiratory syndrome coronavirus (MERS-CoV) was identified first time in 2012, almost 10 years after the highly fatal human severe acute respiratory syndrome coronavirus (SARS-CoV) which emerged from china in 2003 [2]. There have been two known incidences of emergence of highly pathogenic coronavirus, first in 2003 SARS-CoV emerges in Guangdong province, China and spread to 37 different countries causing 8.273 confirmed cases of infection of which 775 cases (9%) were fatal [3]. Since the SARS pandemic, two

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additional human coronaviruses, HKU-1 and NL-63, have been identified, both of which caused mild respiratory infection and distributed worldwide [4, 5]. Then in 2012 MERS-CoV emerged in the kingdom of Saudi Arabia (KSA) as of March 26, 2015 MERS-CoV is confirmed to have infected 1090 people worldwide, killing 412 of them (38%) [6]. Table 1 demonstrated number of confirmed cases of MERS-CoV and number of death in the Middle East up to the intervention period according to WHO summery and update as of 5 February 2015 [7].

Cases of MERS-CoV infection have been geographically restricted to the Arabian Peninsula with the majority of cases occurring in KSA, Qatar, Jordan, Oman, UAE and Egypt. Outside of this region there have been a smaller number of cases of infection in people who have traveled to the Arabian Peninsula or had been in contact with people who had [2]. To date, the affected countries in the Middle East include Egypt, Iran, Jordan, Kuwait, Lebanon, Oman, Qatar, Saudi Arabia (SAU), United Arab Emirates (UAE) and Yemen; in Africa: Algeria, and Tunisia; in Europe: Austria, France, Germany, Greece, Italy, the Netherlands, and the United Kingdom; in Asia: Malaysia and Philippines; and in North America: the United States of America (USA). The majority of cases (>85%) have been reported from SAU. Since June 2014, two new countries (Austria and Turkey) have been affected (Figure 1). In Iran 5 cases of MERS-CoV were reported during May and June 2014 (including two additional cases since the last WHO update). All five cases connected to a single hospital in Kerman province and were healthcare associated transmissions. Following increased surveillance and preparedness activities in Kerman province and across the country, there have been no reports of further cases in the affected hospital or in the province or the country to date [7].

Classification and virion structure

The MERS-CoV belongs to Nidovirales Order, Coronaviridae Family, Coronavirinae Subfamily and Beta coronavirus Genus. Nidovirales are membrane-enveloped, Non-segmented and positive-strand RNA viruses which have four distinctive characteristics: 1.an invariant general genomic organization with a very large replicase gene upstream of the structural protein genes; 2.The expression of the replicase-transcriptase polyprotein by means of ribosomal frameshifting; 3.a collection of unique enzymatic activities contained within the replicase-transcriptase protein products; and 4.the expression of downstream genes via transcription of multiple 3'-nested subgenomic mRNAs (this property provided the name for this order) [1]. On the basis of phylogenetic clustering and serologic relationship, coronaviruses have been sorted

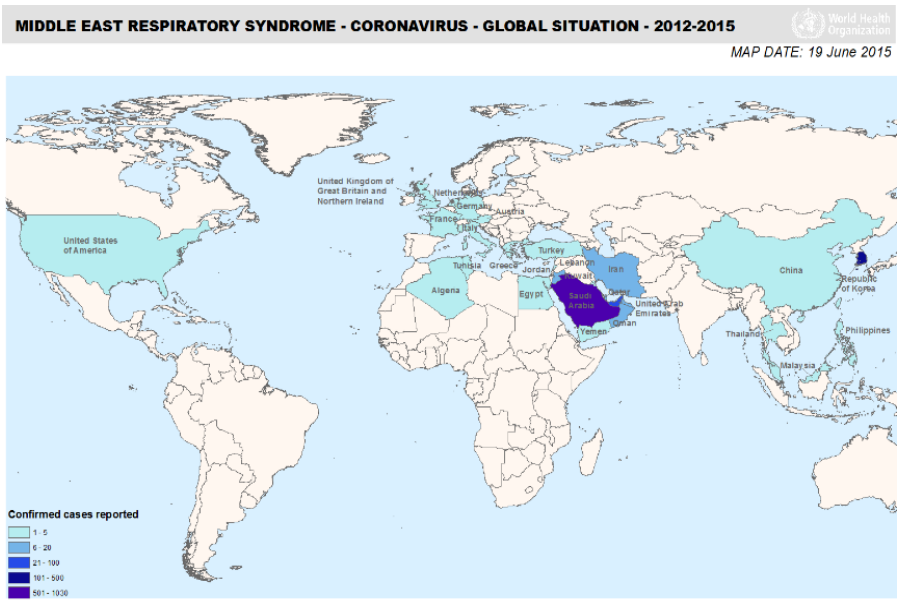

Fig. 1. Global distribution of MERS-CoV according to WHO global alert and response.

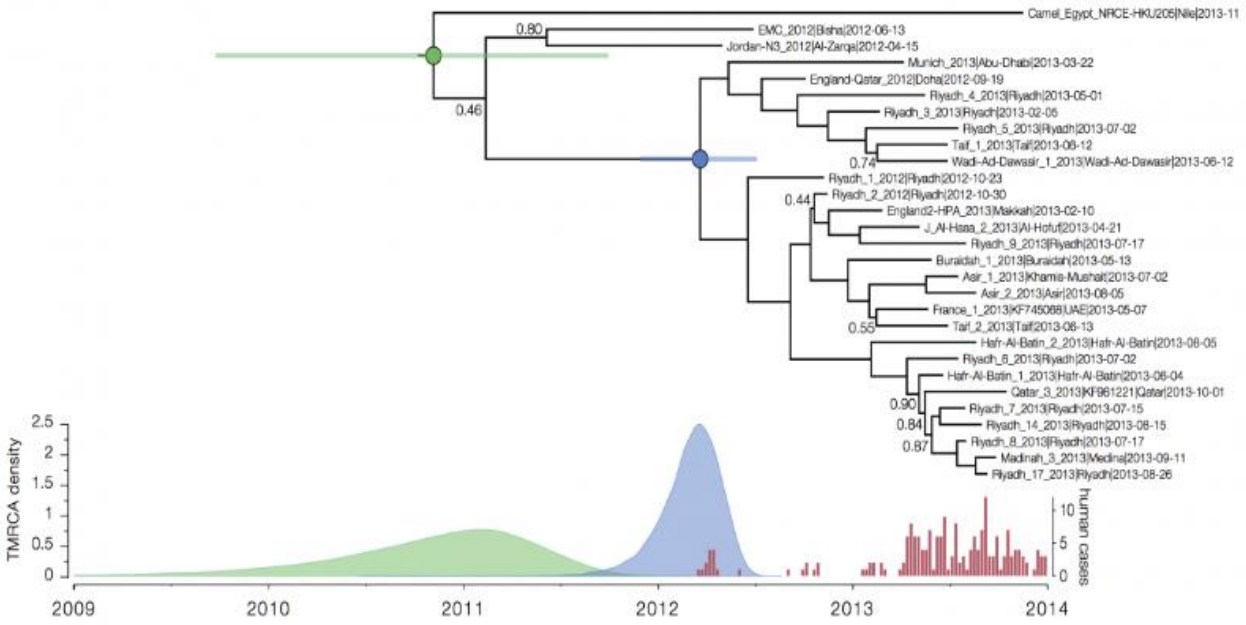

Fig. 2. It shows Time calibrated phylogenetic tree of 28 publically available human MERS-CoV genome sequences and one camel MERS-CoV from Egypt.

into 4 groups Alpha-, Beta-, Gamma- and Delta- coronaviruses. Within the Betacoronavirus Genus there are 4 species including MERS coronavirus, Human coronavirus HKU1, SARS-related coronavirus, Human Coronavirus-EMC and Murine coronavirus is the type species [8]. Figure 2 shows Time calibrated phylogenetic tree of 28 publically available human MERS-CoV genome sequences and one camel MERS-CoV from Egypt. A BEAST phylogeny with posterior probability density estimates of the time of most recent common ancestor (TMRCA) of all sequences (green) and the more recent, predominantly KSA, lineage (blue). Also shown in red is a time series of recorded human cases. Al-Hasa_2_2013 is used as a representative of the nosocomial outbreak in Al-Hasa and Bisha_1_2012 is removed because of its close similarity to Riyadh_1_2012 despite its differing time and location. The numbers by certain nodes are posterior probabilities but only those <0.95 are shown [9].

MERS-CoV seems most closely related to as yet unclassified viruses from insectivorous

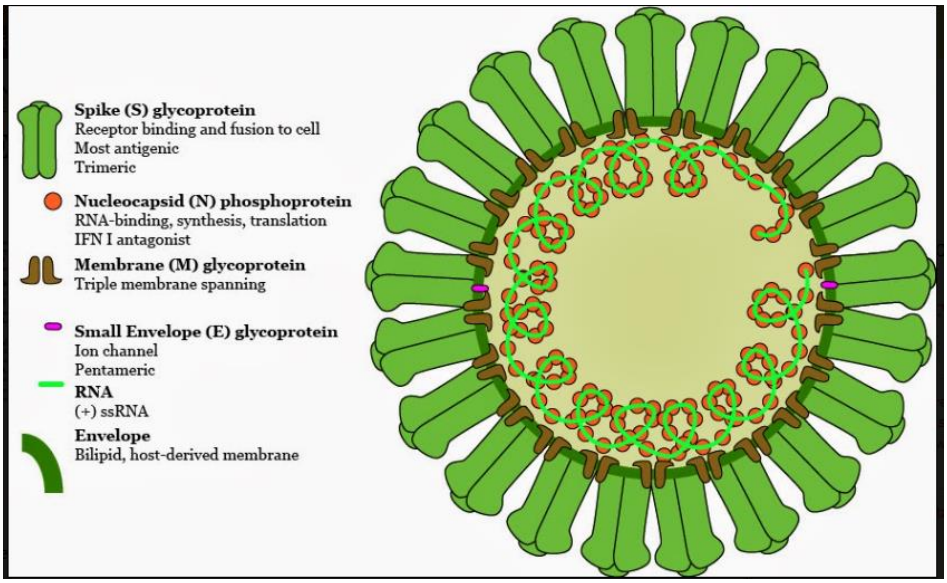

Fig. 3. Schematic of the MERS-CoV. Dr. Ian M Mackay.

Table 1: Number of confirmed cases and deaths of MERS-CoV in the Middle East up to the intervention period.

MONTH AND YEAR	NO. OF LABORATORY CONFIRMED CASES	NO. OF DEATHS
30 NOV. 2012	9	5
25 APR. 2013	17	7
8 MAY. 2013	30	14
17 MAY 2013	40	20
31 MAY 2013	50	30
20 JUN. 2013	64	38
9 JUL. 2013	80	45
13 AUG. 2013	94	47
20 SEP. 2013	130	58
20 JAN. 2014	178	76
27 MAR. 2014	206	86
24 APR. 2014	254	93
9 MAY 2014	536	145
5 FEB. 2015	971	356

European and African bats in the Vespertilionidae and Nycteridae families respectively [10, 11].

Coronavirus's virions are roughly spherical with a moderate degree of pleomorphism [12]. The cube-like or petal-shaped spikes emerge from the virion surface as stalks with bulb like distal termini. Mean particle diameter is 118-136 nm including the contributions of the spikes which projects 16-21 nm from the virion envelope [13, 14]. Coronaviruses have helically symmetric nucleocapsids which consist of a canonical set of 4 major structural proteins: the spike (S), membrane (M) and envelope (E) which located in the membrane envelope and the Nucleocapsid (N) protein which is found in the ribonucleoprotein core [15].

The 128-160 kDa spike glycoprotein(S) consists of trimers of S. S is a class I viral fusion protein which require protease cleavage between the S1 and S2 domains, activated by receptor binding, low PH that mediate membrane fusion leading to virus entry and syncytia formation [16-18]. The S1 domain is extremely variable and often diverging extensively among different isolates of a single coronavirus, in contrast, the S2 domain is highly conserved [1].

The most abundant structural protein in coronavirus is the M protein which gives the virion envelope its shape. The ectodomain of M is modified by glycosylation which is usually N-linked however a subset of Betacoronavirus M-proteins exhibit O-linked Glycosylation that influences both organ tropism and interferon inducing capacity [1].

The E-protein is a small integral membrane protein of 8-12 KDa with limited amounts in virion envelope. In beta- and gamma-coronaviruses E-proteins are palmitoylated on cysteine residues downstream and adjacent to the hydrophobic region [1].

The N-protein is the sole phosphoprotein constituent of the helical Nucleocapsid. Phosphorylation trigger a conformational change in N-protein and it may enhance the affinity of N for viral versus non-viral RNA [1]. Figure 3 demonstrates virion structure.

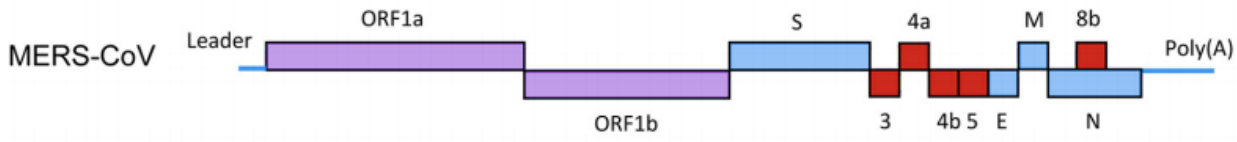

Fig. 4. Genome structure of MERS-CoV. Structural proteins are blue, replicase proteins are purple and unique accessory proteins are red.

Table 2: The known cellular receptors for coronaviruses.

Virus	Receptor
Alpha-coronaviruses (except HCoV-NL63)	APN
HCoV-NL63	ACE2
MHV	mCEACAM1
BCoV , HCo-OC43	N-acetyl-9-o-Acetyl Neuraminic Acid
SARS-CoV	ACE2
MERS-CoV	DPP4

Genome structure, organization and Replication

Genome ranges from 26 to 32 kb which is the largest among all RNA viruses and comprises a basic set of genes in the invariant order 5'-replicase-S-E-M-N-3'. The replicase-transcriptase gene occupying 2/3 of the available coding capacity and is the only protein translated. The products of all downstream ORFs are derived from subgenomic mRNAs. Also there are from one to as many as eight additional ORFs which are accessory genes and fall in any of the intergenic intervals downstream of the replicase gene except between the E and M genes [1, 19-21]. Figure 4 demonstrates genome structure of MERS-CoV.

The interaction between the viral S protein (the variable amino-terminal of the spike protein or S1) and its cognate receptor leads to conformational changes that results in fusion between virion (the conserve half of the spike protein or S2) and cell membrane. The known cellular receptors for coronaviruses are listed in table 2 [1].

The S protein of many coronaviruses are uncleaved in mature virions and require an encounter with a protease at the entry step of infection to separate the receptor binding S1 and fusion S2 components of the spike. Proteolytic activation location is also variable. Following delivery of the viral nucleocapsid to the cytoplasm, translation of replicase gene from genomic RNA occurs. The replicase gene consist of two ORFs: ORF rep1a (polyprotein pp1a) and ORF rep1b (polyprotein 1ab) that after translation, auto-proteolytically processed into mature products from NSP1 to NSP16. NSP3 is papain like protease which carry out the separation of NSP1, NSP2 and NSP3. The main protease Mpro or 3CLpro performs the remaining 11 cleavage events. Expression and assembly of the replicase transcriptase complex sets the stage for viral RNA synthesis resulting in the genomic RNA replication and subgenomic RNA transcription. The immediate outcome of transcription is to enable translation of the proteins that build progeny viruses. The M, S and E proteins are initially inserted to the ER, from there, they transit to the site of virion assembly (the ERGIC). The nucleocapsids composed of progeny genomes encapsidated by N protein coalesce with the envelope components to form virions which bud into the ERGIC then transport to the plasma membrane and released in smooth walled vesicles by exocytosis [1].

Pathogenesis and Pathology

Most coronaviruses spread to susceptible hosts by respiratory or oral-fecal routs of infection with replication first occurring in epithelial cells [1]. While the majority of Middle East respiratory syndrome (MERS-CoV) cases acquired infection through human to human transmission, the primary sporadic cases in clusters are more likely to have been acquired through non-human sources of the virus [22]. Bats are original MERS-CoV host species. DPP4 (dipeptidyl peptidase 4) or CD26 which are expressed in the lower respiratory tracts of humans acts as a functional receptor for MERS-CoV and importantly MERS can use the evolutionary conserved DPP4 protein of pipistrellus bats to infect bat cells. Considering direct contact of humans with bat secreta is rare, there have been intermediate hosts such as camels and goats [23]. Given the synchronized parturition pattern of dormitory camels with birthing in the winter months, an increase of epizootic activity might be expected after some latency during the first half of each year [24]. Also mice, cats, dogs, hamsters and ferrets are able to use DPP4 as a

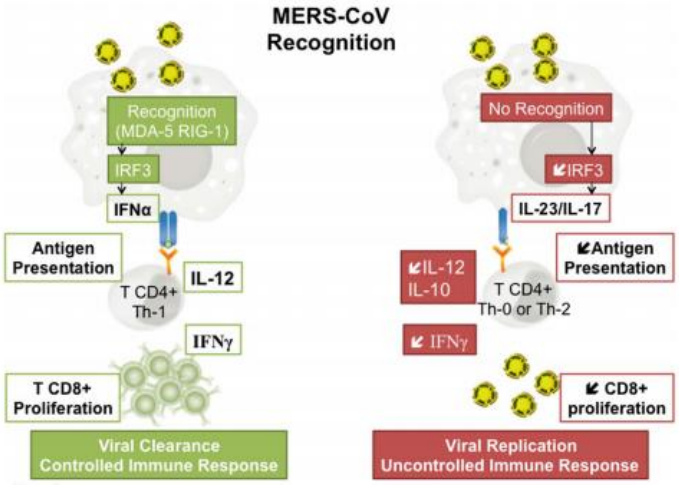

Fig. 5. Key responses to MERS-CoV infection (doi:10.1371/Journal.pone.0088716.g005).

Table 3: Target genes and confirmatory RT-PCR assays for MERS-CoV.

Molecular assay	Target gene	Genome location
Screening assays by real RT-PCR	Upstream of E gene	27,458-27,550
	Nucleocapsid gene N2	29,424-29,477
Confirmation assays by real RT-PCR	ORF 1a gene	11,197-11,280
	ORF 1b gene	18,266-18,347
	Nucleocapsid gene N3	28,748-28,795
Confirmation assays by sequencing RT-PCR	RdRp	15,049-15,290
	Nucleocapsid N protein gene	29,549-29,860

functional receptor for MERS-CoV entry as compared to other animal species [25]. Goat and camel cell lines were shown to be permissive to efficient replication of MERS-CoV [26]. Human to human transmission of MERS-CoV have been reported in several clusters of cases in France, UK, Italy, Jordan, Tunisia, KSA, UAE and Qatar including among family members and health care workers [10].

MERS clinical manifestations and laboratory testing

All MERS-CoV cases reportedly have developed a respiratory disease ranging from mild to severe pneumonia often as companied by acute respiratory distress syndrome (ARDS) , renal failure, pericarditis and disseminated intravascular coagulation (DIC). Clinical manifestations and severity of MERS seems to be more similar to SARS than other coronavirus infections [27].

Literature review shows that viral load peaks at different time points during the progression of disease. For non-SARS-CoV peak viral loads are detected around day 1-2 after onset of disease with apparent clearance of infection over the course of 3 weeks in 50% of healthy children [28]. Also use of upper respiratory specimens for MERS-CoV like nasopharyngeal swabs may not be as sensitive as the use of lower respiratory tract specimens because of higher viral loads [29]. Although the major clinical manifestations presented by MERS patients are associated with the respiratory tract, gastrointestinal symptoms including diarrhea during the course of illness were also observed quite frequently (35%). Other MERS patients specimens such as urine, sera and blood were very rarely tested, not allowing consistent conclusions[30, 31].

Table 4: Testing, sampling and timing for symptomatic and asymptomatic MERS cases.

Patient	***Test***	***Sample***	***Timing***	***Storage and transportation***
Symptomatic	PCR	Sputum Aspirates Lavage Serum	Collect 2 sequential samples	Less than 72 hours, store and ship at 4°C, if longer than 72 hours, store at -80°C and ship on dry ice or liquid nitrogen
	Serology	Serum	Paired sera (1st week of illness and 2-3 weeks later)	
Asymptomatic Contact	PCR	Nasopharyngeal swab Oropharyngeal swab sputum	Within 14 days of last documented contact	
	Serology	Serum	Baseline serum taken within 14days of last documented contact and convalescent serum taken 2-3 weeks later	

According to WHO guidance for health professionals, patients should be evaluate for MERS-CoV if they develop pneumonia and fever with a history of travel to or residence in the Arabian peninsula in the 14 days before illness onset or contact with known confirmed or probable MERS cases in 14 days before illness onset. Additionally the WHO recommends testing for novel coronavirus of persons including health care workers in clusters of acute respiratory infection of unknown etiology requiring hospitalization or where the respiratory infection is unexpectedly severe [32]. According to the MERS case

Table 5: Serological assays for MERS-CoV.

Serological assays	***Antigen***	***Details***	***Reference***
IFA	Whole virus	Vero B4 cells to detect specific IgG and IgM in patient's serum	[37-39]
	Recombinant S and N proteins	Transfected Vero B4 cells expressing recombinant spike or nucleocapsid protein of MERS-CoV For detection of specific IgG and IgM in patient serum (a) Controls of other human pathogenic CoV for differential rIFA should be included. (b) Confirmation by virus plaque reduction neutralization test (PRNT)	
Western Blot	Recombinant S and N proteins	Transfected HEK-293 T cells expressing recombinant spike or nucleocapsid protein of MERS-CoV (a) Confirmation by virus plaque reduction neutralization test (PRNT)	[38]
Protein microarray	Soluble S1	Amino-terminal receptor binding spike domain S1, expressed in HEK-293T cells. For detection of specific IgG and IgM in patient SERUM (a) Controls of other human pathogenic CoV should be included on microarray analysis for differential diagnosis. (b) Confirmation by virus neutralization test	[40]
Neutralization test	PRNT	Vero B4 cells in 24-well plates	[39-43]
	Micro neutralization test	Vero cells monolayers in 96-well micro titer plates	
	Pseudo particle virus	HIV/MERS pseudo particles containing HIV p24 viral protein were used to infect Vero E6 cells in a single well (96-well plate)	

Table 6: Possible dosages and schedule of therapeutic agents for MERS-CoV infection.

Medication	Normal dose Crcl>50 ml/min	Impaired renal function (Crcl=20-50 ml/min)	Hemodialysis (Crcl<20 ml/min)
Oral Ribavirin	2000mg loading dose then 000 mg loading dose then 1200mg q8h for 4 days, then 600mg for 4-6 days	2000 mg loading dose then 600 mg for 4 days, 200 mg for 4-6 days	2000 mg loading dose then 200mg for 4 days, then 200mg
PegIFN-α	1.5mcg/kg once per week		
Oral Lopinavir 400mg/Ritonavir 100mg	100 mg twice daily for 10 days		
Convalescent plasma	300- 500 ml of full plasma (3 – 5 ml/kg) With a rate of 2ml/min for one time in day 2 of ICU admission		

definition mentioned earlier, a confirmed case requires laboratory confirmation by molecular methods including a positive real-time RT-PCR on at least two specific genomic targets or a single positive target with sequencing on a second target (Table 3). Several molecular assays are now in widespread use and a two-step approach of screening and confirmation algorithms have been recommended by WHO and CDC. In both algorithms a screening PCR targeting a region upstream of the E gene is proposed, sometimes combined with nucleocapsid N gene based PCR to enhance sensitivity for specimen screening. For confirmation a second assay with a different set of primers and probes is recommended [33-35].

According to WHO case definition a person with an acute febrile respiratory illness of any severity with positive serological test will be categorized as probable case of MERS-CoV infection and a paired acute and convalescent sera should be tested, ideally combined with molecular testing of respiratory samples. Table 4 demonstrated a summary of testing, sampling and timing for MERS-CoV [36]. Serological assays for MERS-CoV cases are listed in table 5.

Treatment and prevention of MERS-CoV

Possible dosages and schedule of therapeutic agents for MERS-CoV infection are listed in the table 6. The table also includes the possible dosage of pegelated interferon-α (PegIFN-α) that is 50-100 times more effective in vitro for MERS-CoV than SARS-CoV. The long half-life of PegIFN-α and the associated adverse effects calls for extra attention to use of short acting interferon. The use of interferon therapy with ribavirin is not recommended in patients with hepatitis C virus infection and renal dysfunction (Crcl<50 ml/minute) [44].

Using reverse genetic system, a full length infectious cDNA clone of MERS-CoV genome has been successfully constructed into a bacterial artificial chromosome, providing the possibility of developing attended viruses as MERS mucosal vaccine candidates. Experience based on SARS studies suggests that the S protein including RBD of MERS-CoV could be a promising target for the development of vaccines against MERS. As expected recent studies have shown that a recombinant modified vaccinia virus Ankara (MVA) expressing full length S protein of MERS-CoV (MVA-MERS-S) produced high levels of serum antibodies in vaccinated mice that neutralized MERS-CoV infection [45,46]. Another study also demonstrated that S.C. vaccination of a recombinant vaccine targeting the RBD (residues 377-662) of MERS-CoV S fused to FC of human IgG (RBD-FC) protein elicited humoral IgG antibody responses with favorable neutralizing activity and that a truncated Fc-fused RBD protein of MERS-CoV S (S377-588-Fc) induced strong neutralizing antibody responses in vaccinated mice, suggesting that RBD of MERS-CoV is indeed a promising vaccine target [47, 48].

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