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Middle East Respiratory Syndrome (MERS) a Review of an Emerging Disease

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ABSTRACT

The latest emerging disease, Middle East Respiratory Syndrome (MERS) is a viral respiratory illness caused by a member of beta group of corona virus named the Middle East Respiratory Syndrome corona virus (*MERS-CoV*) which was first identified in Saudi Arabia in April 2012. With frequent air travel worldwide, global data as on July 2015 stands at 486 deaths and 1357 confirmed cases across 25 nations, with the possible source, in all the outbreaks, being traced back to the Middle East. Initial presenting symptom of illness is non specific within 14 days of acquiring infection, though the course is more severe in those with chronic debilitating illness leading to respiratory or renal failure. Bat and camel has been implicated as the potential reservoir but confirmation regarding the possible source and route of transmission of infection is yet to be ascertained. No specific treatment or vaccine is currently available. Supportive therapy remains the only modality of treatment. Preventive and control measures play a major role in effective containment of the disease. WHO recommends early detection of cases and isolation, and practice of standard precautions like hand washing, use of masks, gloves, protective eye goggles etc. by the caregivers as well as the healthcare providers.

Keywords: Middle East Respiratory Syndrome, MERS, Corona virus, MERS-CoV, Emerging viral disease.

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INTRODUCTION

When healthcare scientists and disease control experts were busy trying to contain the most dreaded outbreak of Ebola, another emerging disease had started gradually raising its ugly head- MERS or The Middle East Respiratory Syndrome in yet another part of the world. As per World Health Organization, WHO, MERS is a viral respiratory disease which was first identified in Saudi Arabia in April 2012[1]. It is caused by Middle East Respiratory Syndrome corona virus (*MERS-CoV*), also termed Camel flu[1, 2, 3]. The first confirmed case, infected with this hitherto unknown virus, *MERS-CoV*, was reported in Saudi Arabia back in the year 2012. A second case was also reported in the same year in September, in a 49-year-old male living in Qatar who presented with similar flu symptoms, sequencing proved that the virus isolated from the second case was almost identical to that of the first one.[4] World Health Organization (WHO), on May 2013, warned that the novel corona virus, which was till then causing outbreaks primarily in its namesake region, the Middle East, posed "a threat to the entire world."[5] The warning proved very far-sighted till on 30th June 2015, when the global data stood at 1,357 laboratory-confirmed cases of *MERS-CoV*, out of which 486 persons had died[6]. Till date, MERS has swept across 25 countries in the Middle East, Africa, Europe, Asia, and North America.[5] More than 85% of cases that have been diagnosed are from Kingdom of Saudi Arabia (KSA). [Figure 1].

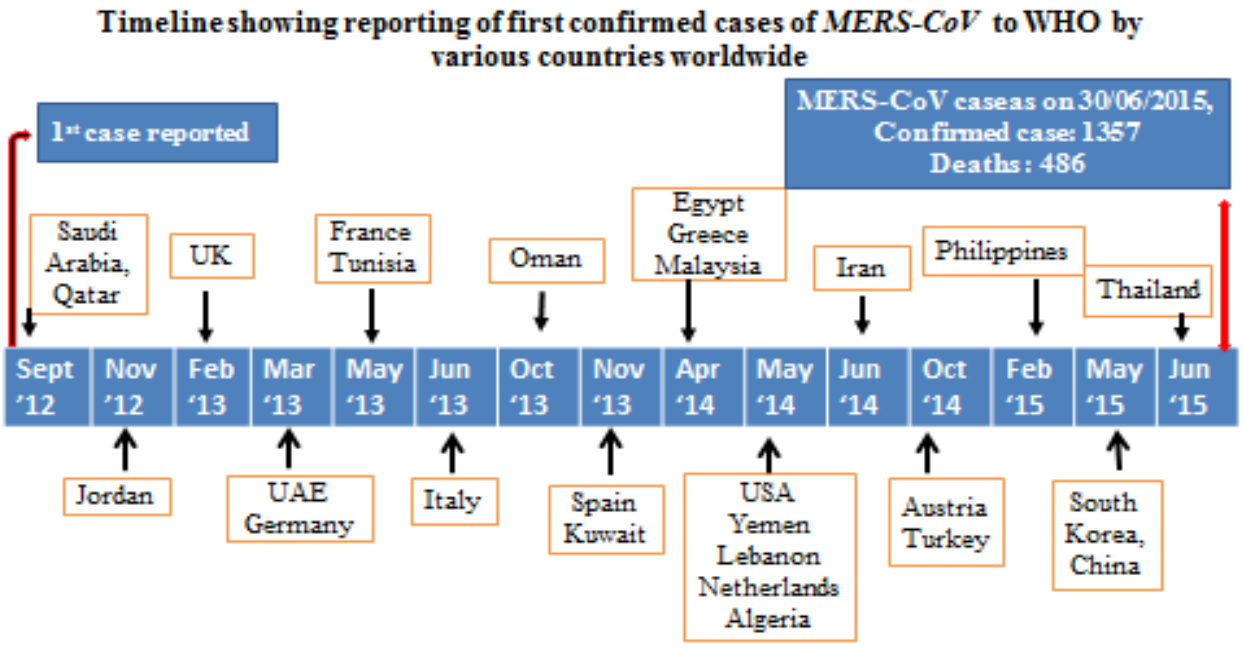

Figure 1: Timeline of first confirmed case of MERS-CoV as reported to WHO by various nations across the world

In Asia, the current outbreak which is occurring in South Korea is the largest outside KSA. The index case here, is a 68 years old male, with a recent history of travel to four Middle Eastern countries and returning to Incheon International Airport on May 4, he was asymptomatic at the time of arrival. He developed symptoms on 11 May and subsequently sought care at 2 out-patient clinics and 2 hospitals, creating multiple opportunities for exposure among healthcare workers and other patients. The case was later hospitalized and confirmed to be due *MERS-CoV* on 20 May and notified to the WHO on the same day. China, on the other hand, informed the WHO on 29 May that a patient isolated at a one of its hospital tested positive for the MERS corona virus. This patient had exposure history and symptoms and had travelled against medical advice from South Korea to Guangdong, China, via Hong Kong (SAR)[7]. On 18th June 2015 Thailand also reported their first MERS confirmed case in a 75 years old Omani male who had travelled to seek medical care there. Later, he and his three family members were isolated and luckily, no more cases were reported.[8] Till date no cases have been reported from India but with the huge number of international travel and a lot of migrant workers this country is also vulnerable.

Virology of MERS-COV

The newly emerged virus, *MERS-CoV* is a member of the beta group of corona virus, Beta corona virus, lineage C. Phylogenetically, *MERS-CoV* genomes are classified into clade A and B. The earliest cases of MERS were of clade A clusters (EMC/2012 and Jordan-N3/2012), whereas new cases which are genetically distinct, are in clade B. *MERS-CoV* is distinct from both the SARS corona virus and the common cold corona virus (HcoV-229E and HcoV-OC43) and also known endemic human beta corona viruses HCoV-OC43 and HCoV-HKU1. Until 23 May 2013, *MERS-CoV* had frequently been referred to as an SARS-like virus, or simply the novel corona virus[1]. Corona viruses are non segmented, single stranded, positive sense, enveloped RNA viruses with a genome of approximate 30kb. They are pleomorphic in shape and measure 120 – 160 nanometres in diameter and are roughly spherical with a helical nucleocapsid symmetry and have a characteristic fringe of surface projections and hence their name. Some corona viruses may even have an inner fringe of short projections. The virions are sensitive to heat, lipid solvents, non-ionic detergents, oxidizing agents and the UV light. They are responsible for the majority of the respiratory tract infections but may also cause gastrointestinal tract, renal, hepatic and nervous system infections. While four of the human corona viruses usually cause only common-cold like illness, the *SARS-CoV* and the *MERS-CoV* are responsible for causing severe and life-threatening human infections[9].

Sources of Infection and Transmission Routes

Phylogenetically, *MERS-CoV* has shown relatedness to bat corona viruses but its origin is still not clear. Recent studies have supported a bat origin for *MERS-CoV*.[10] In one particular study, it has shown that a bat corona virus has a similar binding mode to *MERS-CoV* which initiates the processes of viral entry into human cells.[11] Although bats might be a source of *MERS-CoV*, it is less likely that they are the immediate source for most human cases because human contact with bats is uncommon in this part of the world. Epidemiologic investigations have identified dromedary camels as the likely reservoir host for transmission of *MERS-CoV*[12, 13]. Human-to-human transmission is possible and has been observed in healthcare settings and is also strongly suggested by the presence of clustering of cases but the possible routes of transmission have not been ascertained so far.[14-17] Serologic studies have also shown a low prevalence of *MERS-CoV* antibodies in Saudi Arabian human population as well[18, 19].

Pathogenesis

A host of living beings, ranging from human, primate, porcine, and bat cell lines have been shown to be infected by the MERS-CoV.[20] Targets of infection of the MERS-CoV, as learnt from various studies on ex vivo infections on human lung tissue and human epithelial cell cultures, seem to be type II alveolar cells and non-ciliated lung epithelial cells (Clara cells).[21,22] Endothelial cells were seen to be infected in one case only.[22] Dipeptidyl peptidase 4 (DDP4, also known as CD26 receptor) has been identified as the receptor for the virus by in vitro mass spectrometry analysis of Huh7 cell protein bound to the *MERS-CoV* spike protein.[23] Transfection and localization experiments demonstrated that DPP4 is necessary for infection of a non-permissive cell line and thereby affirming that DPP4 is the receptor for *MERS-CoV*.[23] DPP4 has enzymatic functions, inhibition of which does not affect the entry of the virus in vitro, but the in vivo role of DDP4 enzymatic activity has not been studied so long.[23] DPP4 has various different functions in glucose homeostasis, T-cell activation, neurotransmitter function, and modulation of cardiac signalling.[24] Transcriptional analysis of cells infected by the virus has pinpointed several pathways being modulated during infection.[21] It has been shown to modulate the innate immune response, antigen presentation, mitogen-activated protein kinase (MAPK) and apoptosis pathways. Inhibition of the MAPK pathway has shown to reduce viral replication in culture, giving rise to hope of potential therapeutics. Importantly, several studies show that *MERS-CoV* does not induce an early type I IFN response, this suggests, that *MERS-CoV* may encode for proteins that inhibit sensing of the viral RNA during infection.[20, 22, 25, 26] This modulation of pathways may explain the more lethality of the virus.

Clinical Presentations

The incubation period of MERS can vary from a few days to 14 days[14]. The *MERS-CoV* infected patient may present with cough, high-grade fever and shortness of breath. As per GAR (Global Alert and Response) of WHO, Pneumonia is usually a common sign. Besides that, gastrointestinal symptoms like diarrhoea have also been reported. Respiratory failure requiring mechanical ventilation and support in an intensive care unit are seen in severe illness. Cases of renal failure and septic shock have also been reported. Persons with chronic illness like diabetes, cancer and chronic lung disease, aged people and those with weakened immune systems seem to run a more severe course of illness.[27]

Laboratory Diagnosis of *MERS-COV*

Serological testing for *MERS - CoV*

Immunofluorescence assays: Detection of antibodies to *MERS CoV* can be done using Immunofluorescence assays[28]. These assays when used in conjunction with a serum neutralization test apparently gives good sensitivity and specificity[29].

Protein microarray technology: An assay for antibody detection of *MERS - CoV* using protein microarray technology has also been developed, results of which suggest it to be highly specific.[30]

Enzyme - linked immunosorbent assay (ELISA): US CDC has developed an indirect ELISA for detection of *MERS- CoV* using a recombinant nucleocapsid based protein which can be used as a screening test.

Indirect fluorescent antibody (IFA): test or micro neutralization test. Using the same recombinant nucleocapsid based protein, CDC has developed a whole virus IFA which when used with the indirect ELISA can be used to confirm MERS cases.

Molecular Methods

PCR and sequencing. Polymerase chain reaction (PCR) or more specifically, real - time reverse - transcription polymerase chain reaction (rRT - PCR) based methods are utilized for routine confirmation of cases of *MERS - CoV* infection. Detection of unique sequences of *MERS- CoV* RNA by rRT-PCR followed by confirmation with nucleic acid sequencing when necessary is the ideal method. For routine detection of *MERS - CoV*, three rRT - PCR assays have been developed- (i) an assay targeting upstream of the E protein gene (upE) and (ii) assays targeting the open reading frame 1b (ORF 1b) and (iii) the open reading frame 1a (ORF 1a) [28, 31]. The upE target assay is considered highly sensitive and has been recommended for screening while assay of ORF 1a is considered of equal sensitivity. The assay of ORF 1b is considered less sensitive than the

ORF 1a assay. Targeting the nucleocapsid (N) protein gene of MERS - CoV, the United States Centre for Disease Control and Prevention (US CDC) has developed rRT - PCR assays which can complement upE and ORF 1a assays and can be used for screening and confirmation of MERS infection[32]. Two target sites on the MERS-CoV genome have been identified which are suitable for sequencing to aid confirmation. These target sites are in the RNA-dependent RNA polymerase (RdRp) and (N) genes.[28]

Viral Culture

For the routine diagnostic purpose, viral culture is neither done nor recommended because it calls for expertise and containment facilities. The *MERS-CoV*, though, was seen to grow readily on cell culture lines like Vero cells and LLC-MK2. The infected cells show cytopathic effects like cellular rounding and syncytium formation. Antigen production to detect antibodies in humans via immunofluorescence methods were done by application of this method[1, 33].

Treatment and Vaccine Status

Currently, there is no specific drug or vaccine available to treat or prevent infections caused by the *MERS-CoV*. Given the duration of the virus's presence in the place of its geographical detection and rising number of cases, over the years, being imported to other countries, identification of specific therapeutic and preventive method should be a priority. The general supportive measures remain the mainstay of treatment. Though no in vivo test have been conducted, it has been demonstrated that a variety of therapeutic agents inhibits replication of *MERS-CoV* in cell culture[23, 21]. *In vitro* tests have shown that *MERS-CoV* is sensitive to IFN-β treatment[23]. It has also been demonstrated that *MERS-CoV* replication is inhibited by Ribavirin, which is a known inhibitor of RNA viruses. Both IFN-β and Ribavirin together can inhibit *MERS-CoV* at nanomolar levels[34]. Other inhibitors targeting specific pathways, specifically the MAPK pathway was shown. In VeroE6 cells, *MERS-CoV* replication was shown to be inhibited by MAPK inhibitor - SB203580[21]. Additional therapeutics and vaccinations are in development, with a focus on FDA compounds already in use. Some recent research advances in this area include: Drug testing of US FDA-approved potential formulations at the Small-molecule Inhibitor Leads Versus Emerging and neglected RNA viruses (SILVER) project. Also, an experimental recombinant nano particle vaccine candidate based on the Spike protein that generated important anti-*MERS-CoV* antibodies in a mouse model has been produced by the company Novavax[35]. Immunotherapy, as well as prophylactic use of monoclonal antibodies (e.g. MERS-4, MERS-27 and 3B11) which are capable

of neutralizing infection by the *MERS-CoV*, as shown by some studies also holds promise for future use[36, 37].

Infection Control and Preventive Measures

Presently, there is no effective treatment or vaccines to prevent the infection by *MERS CoV*, therefore, the only other means to limit the spread of *MERS-CoV* infection now are public health measures. In the current outbreak in South Korea it was seen that health care providers have unknowingly helped in dissemination of the disease. Most importantly, eliciting a proper clinical history, especially, travel history from patients with severe respiratory disease cannot be over emphasized, given that, the disease cluster of MERS in South Korea was generated by a primary case imported from the Middle East. WHO, therefore, recommends that health care providers maintain standard precautions consistently, contact precaution and eye precaution while caring for suspected or confirmed cases of MERS, droplet precaution (nasal mask, eye protection such as goggles or face shield, gown, and gloves etc.) while caring for all patients with acute respiratory infection (ARI), airborne precaution when performing procedures which generate aerosol[14] [Figure 2]. Suspected case should be immediately isolated and close contacts identified and monitored for development of symptoms.

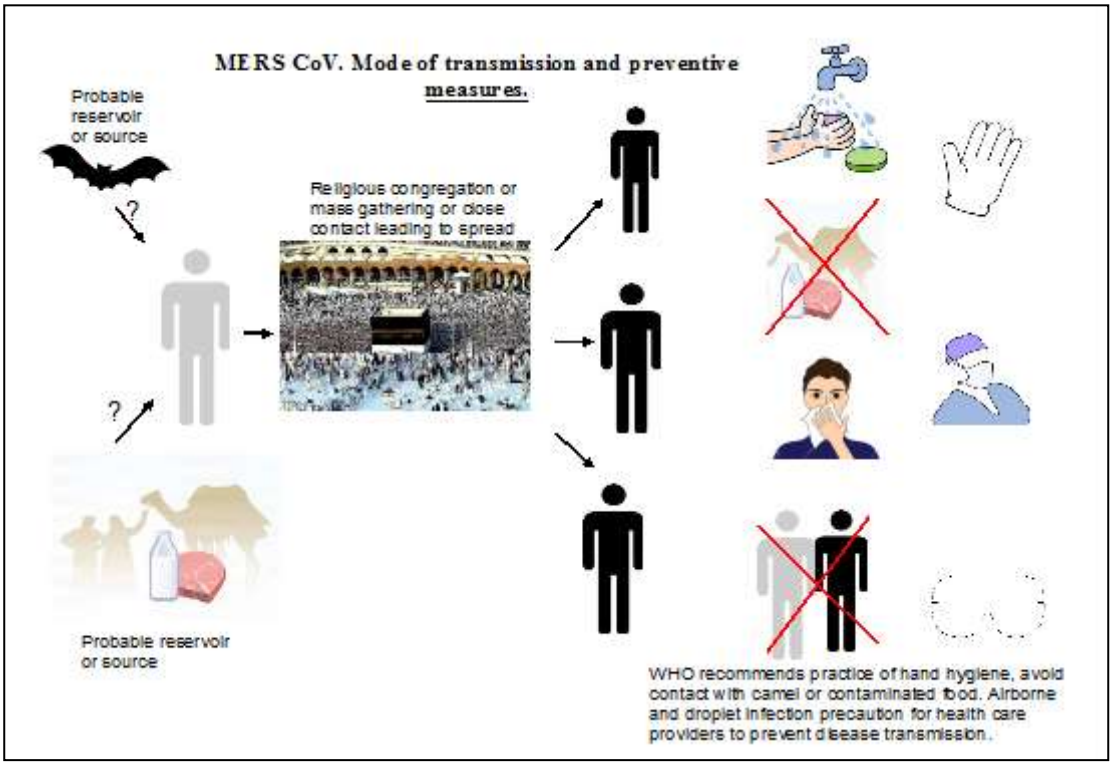

Figure 2: Probable mode of transmission and prevention of *MERS CoV*.

Camels are thought to be related to the sporadic cases which occurs secondary to animal exposure. Therefore, the advice to people at risk of *MERS-CoV* infection by WHO is to avoid contact with camels, practice good hand hygiene, avoid drinking raw milk or eat contaminated food unless it is properly washed, peeled, or cooked.[14] Figure 2. As far as travel is concerned, WHO till now has not restricted travel to Middle East countries with MERS[14]. For the holy pilgrimage to Hajj and Umrah, the Saudi Ministry of Health recommended that people with increased risk of *MERS-CoV* infection and its complications should postpone their trip. These include individual in extreme age group (<12 years, and >65 years), individuals with co morbid conditions like chronic diseases (heart disease, kidney disease, respiratory disease, and diabetes), or with immune deficiency, malignancy, terminal illnesses, and pregnancy[38].

CONCLUSION

A decade after SARS, now *MERS-CoV*, associated with high mortality rates is the subject of global concern[39]. The risk of international disease spread is worrying as KSA is the home of the most holy pilgrimage sites in Islam. Mecca which sees a large mass gathering of approximately two million pilgrims on the annual Hajj and the 'minor' Umrah sees as many as six million pilgrims arrive throughout the rest of the year. Saudi authorities did not report any *MERS-CoV* cases in the run up of the first outbreak in 2012, that is, during Hajj 2013, though, cases have been reported in several countries such as the United Kingdom, Malaysia, Jordan and the United Arab Emirates later[40]. However, following the upsurge in *MERS-CoV* cases in the KSA that began in April 2014[40], and infections by the virus being detected among returning pilgrims from the Umrah[41], proved that the risk from returning pilgrims cannot be ignored. In particular, countries like India from where a sizeable Muslim population make pilgrimage to Mecca and Umrah annually, besides having a lot of its citizens working there as skilled or unskilled workers, needs to be alert and make concrete plans to tackle cases of *MERS-CoV* or any outbreak leading from it. Surveillance and control measures in response to the threat posed by the people returning from the Middle East have to be adapted or developed. But most importantly, further understanding the transmission of the disease and the disease par se is the need of the hour.

REFERENCES

1. Zaki AM, Van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel corona virus from a man with pneumonia in Saudi Arabia. N Engl J Med 2012, 367(19):1814–1820.

2. De Groot RJ, Baker SC, Baric RS, Brown CS, Drosten C, Enjuanes L et al. Middle East Respiratory Syndrome Corona virus (MERS-CoV): Announcement of the Corona virus Study Group. J Virology. 2013; 87(14):7790-7792.
3. Richard Lloyd Parry. Travel alert after eighth camel flu death. .[Internet] The Times, Asia.[Last updated at 12:01 AM, 2015 June 10; cited 2015 June11.] Available from: http://www.thetimes.co.uk/tto/news/world/asia/article4465150.ece
4. RAPID RISK ASSESSMENT Severe respiratory disease associated with a novel corona virus 19 February 2013.[Internet]. European Centre for Disease Prevention and Control, Stockholm, 2013-[cited 2015 June15]. Available from: http://ecdc.europa.eu/en/publications/Publications/novel-corona virus-rapid-risk-assessment-update.pdf.
5. World Health Organization.[Internet] Summary and risk assessment of current situation in Republic of Korea and China. [Cited2015 June4]. Available from: http://www.who.int/csr/disease/corona virus_ infections/risk-assessment-3june2015/en/
6. World Health Organization.[Internet] Emergencies preparedness, response. Middle East respiratory syndrome corona virus (MERS-CoV) – Republic of Korea. Disease outbreak news [cited 2015 June30]. Available from: http://www.who.int/csr/don/30-june-2015-mers-korea/en/
7. World Health Organization. Media Centre.[Internet] Middle East respiratory syndrome corona virus (MERS-CoV) in the Republic of Korea [cited 2015 June10] Available from http://www.who.int/mediacentre/news/situation-assessments/2-june-2015-south-korea/en/
8. World Health Organization.[Internet] Emergencies preparedness, response. Middle East Respiratory Syndrome corona virus (MERS-CoV) – Thailand. Disease outbreak news 20 June 2015 [cited June 28]. Available from http://www.who.int/csr/don/20-june-2015-mers-thailand/en/
9. Joshi RM Middle East Respiratory Syndrome Corona virus (MERSCoV): Perceptions, Predictions, Preventions and the Pilgrimage. Clin Microbial 2013 2:e113.
10. Memish ZA, Mishra N, Olival KJ, Fagbo SF, Kapoor V, Epstein JH, Lipkin WI. Middle East respiratory syndrome corona virus in bats, Saudi Arabia. Emerging Infectious Diseases. 2013; 19(11):1819-1823.
11. Wang Q, Qi J, Yuan Y, Xuan Y, Han P, Wan Y, et al. Bat Origins of MERS-CoV Supported by Bat Corona virus HKU4 Usage of Human Receptor CD26. Cell Host Microbe. 2014 Sep 10; 16(3):328-37.

12. Adney DR, van Doremalen N, Brown VR, Bushmaker T, Scott D, de Wit E, et al. Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels. Emerg Infect Dis. 2014 Dec [date cited 15 June 2015]
13. Hemida MG, Chu DKW, Poon LLM, Perera RAPM, Alhammadi MA, Ng H-Y, et al. MERS corona virus in dromedary camel herd, Saudi Arabia. Emerg Infect Dis 2014 Jul;20(7)1231-1234.
14. World Health Organization. [Internet] Middle East respiratory syndrome corona virus (MERS-CoV) Summary of Current Situation, Literature Update and Risk Assessment [cited 2015 July7]. Available from : http://apps.who.int/iris/bitstream/10665/179184/2/WHO_MERS_RA_15.1_eng.pdf?ua=1
15. Assiri A, McGeer A, Perl TM, , Price CS, Al Rabeeah AA, Cummings DA et al. Hospital outbreak of Middle East respiratory syndrome corona virus. N Engl J Med 2013; 369:407-16.
16. Guery B, Poissy J, el Mansouf L, Sejoune C, EttaharN, LemaireX et al. Clinical features and viral diagnosis of two cases of infection with Middle East Respiratory Syndrome corona virus: a report of nosocomial transmission. Lancet 2013; 381:2265-2272.
17. Gulland A. Two cases of novel corona virus are confirmed in France. BMJ 2013; 346:f3114.
18. Aburizaiza AS, Mattes FM, Azhar EI, , Hassan AM, Memish ZA, Muth D et al. Investigation of anti-middle East respiratory syndrome antibodies in blood donors and slaughterhouse workers in Jeddah and Makkah, Saudi Arabia, fall 2012. J Infect Dis 2014; 209(2) : 243-246.
19. Gierer S, Hofmann-Winkler H, Albuali WH, Bertram S, Al-Rubaish AM, Yousef AA, et al. Lack of MERS corona virus neutralizing antibodies in humans, Eastern Province, Saudi Arabia. Emerg Infect Dis. 2013; 19(2):2034-2036.
20. Zielecki F, Weber M, Eickmann M, Spiegelberg L, Zaki AM, et al. Human cell tropism and innate immune system interactions of human respiratory corona virus EMC compared to SARS-corona virus. J Virol. 2013; 87:5300–5304.
21. Josset L, Menachery VD, Gralinski LE, Agnihothram S, Sova P,Carter VS, et al. Cell host response to infection with novel human corona virus EMC predicts potential antivirals and important differences with SARS corona virus. MBio. 2013 4(3):e00165–13.
22. Chan RWY, Chan MCW, Agnihothram S, Chan LLY, Kuok DIT, Fong FJ et al. Tropism of and innate immune responses to the novel human betacorona virus lineage C virus in human ex vivo respiratory organ cultures. J Virol. 2013; 87(12):6604-14.
23. Raj VS, Mou H, Smits SL, Dekkers DH, Muller MA, Dijkman R et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human corona virus-EMC. Nature 2013;

495(7440):251–4.

24. Hildebrandt M, Reutter W, Arck P, Rose M, Klapp BF. A guardian angel: the involvement of dipeptidyl peptidase IV in psychoneuroendocrine function, nutrition and immune defense. Clin Sci (Lond) 2000; 99: 93–104.
25. Kindler E, Jo´nsdo´ttir HR, Muth D, Hamming OJ, Hartmann R, Rodriguez R et al. Efficient Replication of the Novel Human Betacorona virus EMC on Primary Human Epithelium Highlights Its Zoonotic Potential. MBio 2013; 4(1): e00611–12.
26. Müller MA, Raj VS, Muth D, Meyer B, Kallies S, Smits SL et al. Human corona virus EMC does not require the SARS-corona virus receptor and maintains broad replicative capability in mammalian cell lines. MBio. 2012; 3(6):e00515-12.
27. WHO.[Internet] Middle East respiratory syndrome corona virus (MERS‐CoV) [cited on 2015 July 8]. Available from http://www.who.int/csr/disease/corona virus infections/faq/en/
28. Corman VM, Müller MA, Costabel U, Timm J, Binger T, Meyer B, et al. Assays for laboratory confirmation of novel human corona virus (hCoV-EMC) infections. Euro Surveill. 2012;17(49):pii=20334.
29. Buchholz U, Müller MA, Nitsche A, Sanewski A, Wevering N, Bauer-Balci T, et al. Contact investigation of a case of human novel corona virus infection treated in a German hospital, October-November 2012. Euro Surveill. 2013;18(8):pii=20406.
30. Reusken C, Mou H, Godeke GJ, van der Hoek L, Meyer B, Müller MA, et al. Specific serology for emerging human corona viruses by protein microarray. Euro Surveill. 2013; 18(14):pii=20441.
31. Corman VM, Eckerle I, Bleicker T, Zaki A, Landt O, Eschbach-Bludau M, et al. Detection of a novel human corona virus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. 2012;17(39):pii=20285.
32. Novel Corona virus 2012 Real‐Time RT‐PCR Assay,[Interne] Centers for Disease Control and Prevention.13[cited on 2014 July 25] Available from: http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM355572.pdf
33. Kwok-Hung C, Jasper Fuk-Woo C, Tse H, Chen H, Choi-Yi Lau C, Jian-Piao C et al. Cross-reactive antibodies in convalescent SARS patients' sera against the emerging novel human corona virus EMC (2012) by both immunofluorescent and neutralizing antibody tests. J Infection. 2013; 67(2): 130-140.
34. Falzarano D, de Wit E, Martellaro C, Callison J, Munster VJ, Feldman H. Inhibition of novel β

corona virus replication by a combination of interferon-α2b and ribavirin. Sci Rep.2013;3:1686

35. Coleman, C. M., Liu Y. V., Mu H., Taylor J. K., Massare M., Flyer D. C, et al. Purified corona virus Spike protein nanoparticles induce corona virus neutralizing antibodies in mice. Vaccine. 2014;32(26):3169–3174.
36. Jiang L, Wang N, Zuo T, Shi X, Poon KV, Wu Y et al. Potent neutralization of MERS-CoV by human neutralizing monoclonal antibodies to the viral spike glycoprotein. Sci Transl Med 2014; 6:234ra59.
37. Tang, X.-C., Agnihothram, S. S., Jiao, Y., Stanhope, J., Graham, R. L., Peterson, E. C et al. (2014). Identification of human neutralizing antibodies against MERS-CoV and their role in virus adaptive evolution. Proceedings of the National Academy of Sciences of the United States of America.2014; 111(19): E2018–E2026.
38. Memish ZA, Al-Rabeeah AA. Health conditions of travellers to Saudi Arabia for the pilgrimage to Mecca (Hajj and Umra) for 1434. J Epidemiol Glob Health. 2013;3(2):59–61.
39. The WHO MERS-CoV Research Group -. State of Knowledge and Data Gaps of Middle East Respiratory Syndrome Corona virus (MERS-CoV) in Humans. PLOS Currents Outbreaks. 2013 Nov; 12:5
40. World Health Organization Geneva, Switzerland [Internet]. Middle East respiratory syndrome corona virus (MERS-CoV) summary and literature. [Updated 2014 June 11; cited on 2015 May 12]. Available from: http://www.who.int/csr/disease/corona virus_infections/archive_updates/en/.
41. European Centre for Disease Prevention and Control. Stockholm: ECDC[Internet]. Severe respiratory disease associated with Middle East respiratory syndrome corona virus (MERS-CoV) 16 October 2014.[Cited on 2015 June 14] http://ecdc.europa.eu/en/publications/Publications/mers-cov-severe-respiratory-disease-risk-assessment-16-october-2014.pdf