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MIDDLE EAST RESPIRATORY SYNDROME-CORONAVIRUS (MERS-CoV) IN INDIA AND ABROAD

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ABSTRACT: Middle East Respiratory Syndrome (MERS) is viral respiratory illness that was lately recognized in human. It was first testified in Saudi Arabia in 2012 and has since spread to numerous other countries, as well as the United State. Record people well-known as infected with MERS-CoV developed numerous acute respiratory illnesses, including fever, cough and shortness of breath. Middle East Respiratory Syndrome (MERS) is an illness initiated by a virus (more specifically, a coronavirus) called Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Around 3 to 4 out of every 10 patients reported with MERS have died. The biggest known outbreak of MERS outside the Arabian Peninsula occurred in the Republic of Korea in 2015. The outbreak was allied with a traveler returning from the Arabian Peninsula. MERS-CoV has spread from ill people to others through close contact, like caring for or living with infected person. MERS can affect everyone. MERS patients have ranged in age from younger than 1 to 99 years old. No vaccine available against MERS. The U.S. National Institutes of Health is exploring the probability of emerging one. Antiviral treatment suggested for MERS-CoV infection. Individuals with MERS can seek medical care to help relieve symptoms. For severe cases, current treatment includes care to support vital organ functions.

INTRODUCTION: International travel has increased dramatically over the past six decades; from 25 million in 1950, to 528 million in 1995, 1035 million in 2012 and is expected to reach 1.8 billion in 2030. In 2012, international tourist arrivals in the Middle East were estimated at 52 million [1]. Travellers can be exposed to various infectious agents and may facilitate their spread across borders.

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The importance of travel in the dissemination of respiratory diseases [2] has been demonstrated by the rapid worldwide spread of Severe Acute Respiratory Syndrome (SARS) outbreak in 2003 and the recent pandemic of influenza A (H1N1) in 2009 [3, 4].

The Middle East respiratory syndrome corona virus (MERS-CoV) was isolated for the first time from a Saudi patient with severe pneumonia and a fatal outcome in September 2012. Since then, MERS-CoV has caused an ongoing outbreak in the Arabian Peninsula [5, 6, 7, 8] with sporadic cases imported in European, North African, Southeast Asian countries and USA [9]. Corona viruses are positive sense RNA viruses.

MERS-CoV belongs to Betacoronavirus phylogenetic lineage C that, in addition to MERS-CoV, contains 2 distinct bat-associated CoV species (HKU4 and HKU5). As of 15th May 2014, globally, 572 laboratories confirmed cases of infection with MERS-CoV have officially been reported to WHO, including 173 deaths [6].

Epidemiology: As of 10 March 2016 the World Health Organization (WHO) global case count for MERS was 1,651 laboratory-confirmed cases, including at least 590 deaths (case fatality rate 36%) since the first cases were reported in September 2012 [7].

For cases where information is available, 68% are male and the median age is 54 years. Most cases of MERS world-wide have been reported from, or were acquired in, Saudi Arabia (at least 80%). New infections continue to comprise those acquired in healthcare settings including in a small numbers of cases in healthcare workers, a small number of primary cases, which are thought to have been acquired through contact with camels and/or raw camel milk and some cases for which no clear exposure or source of infection can be identified.

The thirteen cases reported in Saudi Arabia during the past few days back were:

- An asymptomatic healthcare worker linked to two other cases, one of which had frequent contact with camels and consumed their raw milk;
- A man who is a brother of a confirmed case who he denied contact with initially;
- Three men with no clear history of exposure to known risk factors
- A man who had frequent contact with camels and consumed their raw milk; and,
- Five men where the investigations into the history of exposure to known risk factors are ongoing.

Since the beginning of the outbreak, cases of MERS that were acquired from an unknown source in country or from possible zoonotic transmission have occurred in Iran, Jordan, Kuwait, Lebanon, Oman, Qatar, Saudi Arabia, United Arab Emirates (UAE) and Yemen. Imported or import-related cases have been reported from Algeria, Austria, China, Egypt, France, Germany, Greece, Italy, Malaysia, Netherlands, Philippines, Republic of Korea, Thailand, Tunisia, Turkey, United Kingdom (UK) and United States of America (USA).

A recent study found that in healthcare settings, a short period without protective equipment may be sufficient for transmission, with a security guard in the RoK (Republic of Korea) who was within 3 to 6 feet of a fatally-ill patient for 10 minutes without a mask, and without touching the patient, acquiring the infection [8]. The potential for transmission from asymptomatic (but PCR positive) people is currently unclear. However, there is evidence of asymptomatic carriage of the virus. One study found that on day 12 after a first positive test, 30% of asymptomatic or mildly symptomatic persons that had been in contact with a case remained positive for viral RNA in the upper respiratory tract [9]. A case report has found that viral RNA was detectable for over a month after exposure in an asymptomatic health care worker in Jeddah, Saudi Arabia [10]. Transmission to household contacts occurs at low levels, estimated at 5% of household contacts, and low levels of viral RNA may be carried without obvious symptoms, particularly in younger people [11]. Prolonged shedding by some individuals might explain the large outbreaks seen in health care settings.

A recent study based on sequencing of isolates from the RoK and China has found a number of substitutions in the virus, which are now also found in Saudi isolates [12]. The substitutions are likely to have occurred in late 2014, and the authors speculate that the change in the virus may have led to increased transmissibility, and contributed to the size of the outbreak in the RoK. Dromedary camels are the suspected source of infection, but the exact routes of direct or indirect exposure are not fully understood [13]. Evidence of past (over two decades) and current carriage and/or infection has been found in a large number of camels from various regions of the Middle East and elsewhere, in some cases with epidemiological links to human cases, and some with matching sequences to human cases from the same areas [14, 15, 16, 17]. The area of risk for MERS may extend into regions beyond the Middle East [18]. There is no evidence that Australian camels have antibodies to MERS, based on a study of 307 blood samples from 307 wild camels from two different regions [19].

In camels, acute infection is more likely to affect young animals, while older animals are more likely to have evidence of past infection [20]. A large sero-survey in Saudi Arabia found evidence of past MERS Corona virus infection in 2.3% (2/87) of people who work as camel shepherds and 3.6% (5/140) of slaughterhouse workers, and 0.2% (15/10,009) of healthy people in the general population. Similarly, a large sero-survey in Qatar found serological evidence of past infection in healthy camel slaughterhouse workers (7/109 positive) and camel farm workers (12/177 positive) in areas with demonstrated MERS-CoV circulation amongst the camels, and no evidence of past infection in people without contact with camels [21]. These results suggest that individuals with subclinical infection could be the source of infection for cases of MERS who have no contact with camels and no contact with a confirmed case.

They also suggest that the current reported case fatality rate of 35% is over-estimated because mild or asymptomatic cases may not be detected. MERS Corona virus infection can cause severe acute respiratory disease, particularly in people with underlying conditions. People with diabetes, renal failure, chronic lung disease and immune compromised persons are at higher risk of severe disease. The WHO recommends people at high risk of severe disease due to MERS, including those with diabetes, chronic lung disease, pre-existing renal failure, or those who are immune compromised, take appropriate precautions when visiting farms, barn areas or market environments where camels are present.

These measures might include avoiding contact with camels, good hand hygiene, and avoiding drinking raw milk or eating food that may be contaminated with animal secretions or products unless they are properly washed, peeled, or cooked. For the general public, when visiting a farm or a barn, general hygiene measures, such as regular hand washing before and after touching animals, avoiding contact with sick animals, and following food hygiene practices, should be adhered to. In addition to these measures recommended by the WHO, a vaccine for camels may be a useful means of preventing spread amongst camels and to humans. A candidate vaccine based on a highly attenuated vaccinia virus, and known as MVA-MERS-CoV has shown promising results in an early small trial; with the four animals in the treatment group developing neutralizing antibody to MERS-CoV, and viral titers in the treatment group significantly lower than for the control group after MERS-CoV challenge [22].

Virology: Middle East respiratory syndrome corona virus (MERS-CoV) is a lineage C betacoronavirus found in humans and camels that is different from the other human betacoronaviruses (severe acute respiratory syndrome corona virus, OC43, and HKU1) but closely related to several bat corona viruses [8, 23, 24, 25, 26, 27].

Dipeptidyl peptidase 4 (DPP4), which is present on the surfaces of human no ciliated bronchial epithelial cells, is a functional receptor for MERS-CoV [28]. Expression of human and bat DPP4 in no susceptible cells enables infection by MERS-CoV. The DPP4 protein displays high amino acid sequence conservation across different species, including the sequence that was obtained from bat cells.

In a cell line susceptibility study, MERS-CoV infected several human cell lines, including lower (but not upper) respiratory, kidney, intestinal, and liver cells as well as histiocytes [29]. The range of tissue tropism *in vitro* was broader than that for any other known human corona virus. In another study, human bronchial epithelial cells were susceptible to infection [30]. MERS-CoV can also infect nonhuman primate, porcine, bat, civet, rabbit, and horse cell lines [29, 31].

Further study is necessary to determine whether these *in-vitro* findings will translate to broader species susceptibility during *in-vivo* infections [32]. Because of a large increase in cases in Saudi Arabia in the spring of 2014, there was concern that MERS-CoV might have mutated to become more transmissible or virulent.

However, cell culture experiments of viruses isolated during these outbreaks showed no evidence of changes in viral replication rate, immune escape, interferon sensitivity, or serum neutralization kinetics compared with a contemporaneous but phylo-genetically different virus recovered in Riyadh or the original MERS-CoV isolate from 2012 [33].

Genetic Analysis: In an analysis of the full or partial genomes of MERS-CoV obtained from 21 patients with MERS-CoV infection in Saudi Arabia between June 2012 and June 2013, there was sufficient heterogeneity to support multiple separate animal-to-human transfers [34]. Moreover, even within a hospital outbreak in Al-Hasa, Saudi Arabia, there was evidence of more than one virus introduction. By estimating the evolutionary rate of the virus, the authors concluded that MERS-CoV emerged around July 2011 (95 percent highest posterior density July 2007 to June 2012).

Phylo-genetic analysis during the spring of 2014 showed that viruses from patients in Jeddah, Saudi Arabia, were genetically similar, suggesting that the outbreak in Jeddah was caused by human-to-human transmission [33]. 168 specimens that were positive for MERS-CoV during the outbreak in Jeddah, 49 percent came from a single hospital, King Fahd Hospital. Isolates from patients in Riyadh, Saudi Arabia, during the spring of 2014 belonged to six different clade, suggesting that these infections resulted from increased zoonotic activity or transmission from humans in other regions. One cluster of infections observed in a single hospital in Riyadh was associated with a single clade, suggesting nosocomial transmission.

Viruses representing three major genetic clade were examined for their serologic differences by plaque-reduction neutralization and were found to be essentially indistinguishable [35]. An analysis of sequences in MERS-CoV cases during the first half of 2015 reinforced the idea that epidemiologically separate outbreaks (in time and/or place) tend to be caused by viruses of fairly uniform, but distinctive, genetic sequences [36].

Transmission:

Zoonotic Transmission of MERS-CoV: Because most human CoVs originally emerged upon transmission from bats to other animal species and given the phylogenetic relation of MERS-CoV with bat CoVs like HKU4 and HKU5, MERS-CoV most likely originated from bats. Partial genome sequences from viruses closely related to MERS-CoV have been detected in bats from Africa and Europe [37, 38]. Insectivore bats like Pipistrellus are most likely a major reservoir of these group 2c bat CoVs. The identification of a relative small and conserved RdRp fragment from an Egyptian cave bat shown to be identical to the human MERS-CoV EMC isolate [39], however, needs further investigation. Evidence that bats may have served as the original MERS-CoV host species also comes from studies on the receptor usage by MERS-CoV. DPP4 expressed in the lower respiratory tract of humans - acts as a functional receptor for MERS-CoV [40].

Importantly, MERS-CoV can also use the evolutionarily conserved DPP4 protein of Pipistrellus bats to infect cells 40. It remains unclear whether MERS-CoV-like viruses in bats are able to use the DPP4 receptor, although recent investigations revealed that bat DPP4 genes have been subject to significant adaptive evolution, suggesting that the evolutionary lineage leading to MERS-CoV may have circulated in bats for a substantial time period [41]. Three positively selected residues in DPP4 were identified that directly interact with the viral spike protein. Interestingly, recent investigations on the origin of SARS-CoV revealed that closely related SARS-CoV-like viruses in horseshoe bats are able to infect human cells by using the human ACE2 receptor [42].

Therefore, direct trans- mission of bat CoVs to humans, or indirect transmission without requirement of virus adaptation in an intermediate-ate host, is now considered a likely scenario to explain the emergence of novel human CoVs. Considering that direct contact of humans with bats or their secrete may be rare, intermediate hosts that are susceptible to MERS-CoV may be involved in transmit- ting this virus to humans. In case of SARS-CoV, civet cats are thought to have been responsible for the transmission of this virus to humans, although other animal species present at the wet markets in southern China such as ferret badgers were also found to carry a SARS-CoV like virus.

As a consequence, upon detection of MERS-CoV emergence, different animal species commonly found in the Middle East, such as camels and goats, are considered as potential intermediate hosts in the MERS-CoV out- break. Characterization of crucial amino acid residues in DPP4 that are involved in binding the MERS- CoV spike protein revealed that these animal species are more likely able to use

DPP4 as a functional receptor for MERS-CoV entry as compared to other animal species such as mice, cats, dogs, hamsters, and ferrets [43]. Cell lines originating from goats and camels were shown to be permissive to efficient replication of MERS-CoV [44].

Further evidence for the involvement of a specific animal species as an (intermediate) host comes from studies analyzing the host antibody response to MERS-CoV or closely related viruses. Initially, serological studies using samples from different animal species in Oman, Egypt, and the Canary Islands indeed provided clues for the presence of MERS-CoV neutralizing antibodies in dromedary camels [45, 46].

Subsequently, studies in camels in other affected regions, in Jordan and Saudi Arabia, confirmed these findings. Whereas a very high percentage of dromedary camels turned out to have circulating MERS-CoV neutralizing antibodies, other animal species such as sheep, goats, and cows were found negative for virus neutralizing antibodies [47, 48]. These observations have now been supported by more recent studies showing that dromedary camels from a farm in Qatar proved to be positive for MERS-CoV and virus neutralizing antibodies [49].

In addition, the viral sequences obtained from these dromedary camels were almost identical to sequences from two human MERS-CoV cases linked to this farm. Therefore, dromedary camels most likely acquired the virus some time ago from bats and the virus has subsequently spread efficiently between animals in the Middle East region. Dromedary camels are used for racing and beauty contests and are kept in large groups at these festivities, likely promoting subsequent circulation of this virus.

Although MERS-CoV infection in humans is mainly observed in the lower respiratory tract, in camel's nose swabs were found virus positive. Conclusive evidence for the route of transmission from animals to humans, however, is still lacking. In addition, it is not clear whether the virus is introduced multiple times through zoonotic transmission or that human to human transmission is the main driver of the spread of the virus.

Animal-Human Transmission: The source of MERS-CoV and the mode of transmission have yet to be elucidated. However, the continued detection of new human MERS-CoV cases, the low estimated basic reproduction number of the infection (R0), and the detection of multiple distinct MERS-CoV genotypes, suggest the existence of a persistent possibly zoonotic source [50]. There is growing evidence that bats are the original natural reservoir of MERS-CoV and the dromedary camels being a host species for transmission to humans.

1. Bats: Bats have been recognized as natural reservoirs of CoVs and may serve as direct or intermediate hosts for interspecies transmission of SARS-CoVs. Betacoronaviruses were also identified in bats with sequences that are similar to those in the MERS-CoV isolated from humans, supporting the hypothesis that they may be a natural reservoir for MERS-CoV. To date, the strongest direct evidence for this hypothesis is from a short fragment (182 nucleotides in length) of corona virus sequence recovered from a faecal pellet sample from an individual Egyptian tomb bat collected a short distance from the home and work location of the index case-patient in Bisha, Western KSA [39].

However, the European Centre for Disease Prevention and Control (ECDC) had commented that the findings should be interpreted with caution due to the small fragment size of the coronavirus sequence which lied within a conserved region of the genome, and the fact that the finding was made using a newly established assay (a WHO recommended assay yielded negative results) [51]. The agency also pointed out that the corona virus sequence was detected in a faecal pellet and not from the serum, throat swabs, or urine of bats, and hence, the positive findings could also have resulted from something that the bat had eaten; *i.e.* insects that had taken a blood meal from the true reservoir. Given that neither detection of MERS-CoV in bats nor contact of human MERS patients with bats have been reported, further studies are needed to elucidate the role for bats in human infection, as indirect contact (mediated through another intermediate animal vector or fomites) cannot be excluded.

2. Dromedary Camels: Studies had reported the detection of the MERS-CoV and antibodies in various specimens from dromedary camels (*Camelus dromedaries*) in the Middle East (Iran, KSA, Jordan, UAE, Egypt, Kuwait, Qatar and Oman) and African region (Sudan, Somalia, Nigeria, Tunisia and Ethiopia, Kenya and Canary Islands). The present evidence suggests that camels in the Middle East, which originated from the Horn of Africa region, serve as a possible primary source of MERS-CoV infection in humans; serological evidence of the early circulation of MERS-CoV in camels in the African region and the KSA dated back to 1983 and 1992, respectively [52, 20].

However, no autochthonous MERS-CoV infections in humans had been reported in the KSA till 2012 and in Africa to date, suggesting that there might have been silent transmission between camels and humans in these two regions for the past two decades, and the absence of cases in human could be due to poor surveillance; lack of awareness and diagnostic capability for the disease; or a recent mutation in the virus which facilitated its jump from camels (or other animals) to humans.

MERS-CoV infection in animals appears to be restricted to the dromedary camels in the Middle East and African Region. Studies thus far did not find evidence of MERS-CoV infection (acute and past) in the one-hump dromedary camels in the United States (U.S.), Canada and Australia [53, 54]; the two-hump Bactrian camels (*Camelus bactrianus*) in Germany and Mongolia [19, 55] and in other animals such as goats, cows, water buffaloes, sheep, horses, donkeys, mules and chickens in the Middle East [20, 53, 47, 48, 56].

Several phylo-genetic studies and genetic evidence had supported the plausibility of a role for camels in human infection and cross-species transmission between camels and humans [57, 58]. High viral loads had been detected in nasal swabs, conjunctival swabs, rectal swabs, and milk from camels suggesting that droplet contact, fomite and food-borne transmission might be involved. A recent study published by Azhar EI *et al*., reported the isolation of the virus in an air sample collected in a camel barn implicated in a possible camel-to-human outbreak, highlighting the need for further investigation into possible airborne transmission of MERS-CoV [59]. Even though MERS-CoV virus or RNA has not been detected in camel urine to date, the detection of MERS-CoV in urine in human cases suggested that virus shedding in urine is plausible in camels. This, in turn, could be another potential source of food-borne transmission due to the occasional use of camel urine as a traditional medicine in Arabic culture [60]. In a study in Qatar, 13% of lymph node samples taken at a camel slaughterhouse were positive for the virus, suggesting that camel meat might be another source of food-borne transmission [61].

Exposure to dromedary camels was found to be a risk factor in MERS-CoV infection. Serological surveys had found the seroprevalence of MERS-CoV to be higher in healthy camel-exposed individuals such as shepherds and slaughterhouse workers, as compared to the general population in the KSA and individuals without exposure to camels in Qatar [62, 63]. It was proposed that there was a risk of camel workers becoming infected with MERS-CoV, often without being diagnosed, and proceeding to introduce the virus to the general population, where the more severe cases would trigger testing for the virus and result in disease recognition. Notwithstanding these study findings, it was observed that only a minority of the primary cases reported from the KSA had documented camel contact; and other studies had shown an absence of MERS-CoV antibodies in camel abattoir workers in Egypt and the KSA [21, 64, 65, 66].

Younger camels were postulated to play a particular role in zoonotic transmission since they seemed to be more frequently infected and shed more virus than older ones. In a study conducted in Dubai, United Arab Emirates (UAE), from March - June 2014, serological evidence of MERS-CoV infection was found in >96% of all dromedaries >2 years of age [67]. Seroprevalence among dromedaries calves (<1 year of age) was significantly lower but still exceeded 80%.

In addition, RT-PCR testing and virus isolation of nasal swab specimens were only successful among dromedaries <4 years of age (8.3% and 12.1% respectively), particularly in calves (35.3% and 13.6% respectively); while none of the adult dromedaries (>4 years of age) were found positive for the virus, suggesting increased infectivity of calves. The authors recommended that avoiding camels <2 years of age and postponing separation of the calves from the mother until the calves were older could be effective in preventing or controlling the spread of the MERS-CoV infection to humans.

Human to Human Transmission: Human to human transmission of MERS-CoV has been reported in several clusters of cases in France, the United Kingdom, Italy, Jordan, Tunisia, Saudi Arabia, the United Arab Emirates, and Qatar, including among family members and health care workers [24, 68, 25, 69 - 73]. These include a cluster of cases in Saudi Arabia involving 3 family members living within the same house [39] and a family cluster of 3 brothers in Riyadh [71]. A large cluster of 23 confirmed and 2 probable cases has been reported in a hospital in Al- Hasa, Saudi Arabia [24].

The majority of patients experienced severe respiratory diseases and some had acute renal failure, whereas most common symptoms were fever, fever with chills or rigours, cough, shortness of breath, and myalgia. The patterns of spread of MERS-CoV among family or hospital clusters suggest that transmission occurs through droplets or contacts. Differences in receptor expression in the upper and lower respiratory tract of humans could potentially explain limited human to human transmission. Trans-mission appears to occur more readily if the recipient is immunocompromised or has comorbidities, such as diabetes. Since most identified patients had underlying diseases, it is possible that MERS-CoV is a more common infection, at least in Saudi Arabia, and that patients without significant comorbidities develop a mild respiratory disease or remain asymptomatic.

However, spread of MERS-CoV is considered to be relatively inefficient, as two studies indicated that this viral infection does not seem to occur frequently in the normal human population in the Middle East region. Among 130 blood donors sampled in Jeddah in 2012 and 226 abattoir workers sampled in Jeddah and Makkah in October 2012, only 8 reactive sera were seen upon immune fluorescence testing that were all found to be specific for established human CoVs, but not for MERS-CoV [65]. In addition, Gierer *et al*., did not detect MERS-CoV neutralizing antibodies in any of the 268 samples tested that were obtained from persons from the Eastern province of Saudi Arabia [74]. Using independent data sources, different investigators demonstrated that R, the basic reproduction number representing the number of secondary cases per index case in a fully susceptible population, cannot be much above 1, with an upper bound of 1.2 - 1.5 [69, 75].

In the absence of a clear picture of how the virus spreads, intervention strategies may be ineffective. In the case of predominant human to human transmission and absence of approved medication such as vaccines and antiviral, timely identification of new MERS cases followed by their isolation and quarantine may be crucial in controlling the out-break of this emerging CoV. These measures may need to be combined with actions to limit spread and emergence of MERS-CoV from the (intermediate) host.

Transmission Routes: Although camels are suspected to be the primary source of infection for humans, the routes of direct or indirect zoonotic transmission are yet unknown. The majority of primary cases do not report contact with animals [76, 77]. Dromedary camels are ubiquitously present in the Middle East and have been part of desert cultures for centuries with cultural and economic importance. Camel milk and meat, in particular meat from the hump, are commonly consumed while some consider raw organ meet a delicacy [76].

A study in a slaughterhouse in Qatar found MERS-CoV RNA in lymph nodes of slaughtered camels which might be indicative for the presence of the virus in camel meat [78]. However, experimental infection of three camels did not show any evidence for the presence of infectious MERS-CoV in organs and meat [79]. Another route for meat-borne transmission of MERS-CoV is possibly through contamination of the meat during slaughter with respiratory or fecal excreta. In a slaughterhouse in Qatar, 59% of the camels were shedding viral RNA in nasal excretions at the time of slaughter while viral RNA was found in 15% of the fecal swabs. Camel milk, with or without addition of camel urine, is preferably consumed raw as raw milk is believed to have a high nutritional and medicinal value [77].

Raw milk as source of MERS-CoV infection is supported by the observation of the presence of MERS-CoV RNA in raw milk that was collected according to local customs in Qatar. Camel udders are usually not cleaned before milking and hygienic conditions are such that udders and milk can be contaminated with nasal and/or fecal secretions from the dam, saliva of the calves that are used to initiate the letting of the milk, or hands of the milkier [63]. Analysis of camel milk inoculated with MERS-CoV showed that MERS-CoV is stable in milk stored at 22 º C and 48 º C for respectively 48 and 72 h while pasteurization destroyed viral viability [7]. Consumption of raw camel milk has been linked to several human MERS cases in KSA and Qatar [39].

The current WHO advise to avoid contracting MERS-CoV from dromedaries is based on the above observations (people should avoid drinking raw camel milk, camel urine and eating meat that is not thoroughly cooked) but it is clear that the potential for zoonotic droplet and/or aerosol transmission of MERS-CoV is highly neglected both in research and prevention advise.

Advice to Travellers:

- When travelling to areas affected by outbreaks of MERS, if you are around someone who is unwell you should wash your hands often and avoid touching your face. Use a hand sanitiser if soap and water is not available.
- Australians travelling to the Middle East and who are at increased risk of severe disease should avoid contact with camels and their secretions, and avoid drinking raw camel milk. All travelers should practice good hand and food hygiene, particularly where camels are present.
- The WHO advises that if travellers develop an acute respiratory illness severe enough to interfere with usual daily activities while travelling or during the two weeks after their return, they should:
  - seek medical attention, informing the health professional of their recent travel,
  - wash their hands regularly and practice respiratory hygiene (cough etiquette *etc*),
  - And, minimise their contact with others to keep from infecting them.
- Australians travelling to the Middle East or other areas affected by MERS outbreaks to work in healthcare settings (including in healthcare settings in the RoK) should note the advice to healthcare workers on infection control available from the WHO, the CDC and the destination country.

Clinical Features: The clinical manifestations of MERS-CoV infection range from asymptomatic infection to severe pneumonia with acute respiratory distress syndrome, septic shock, and multi organ failure resulting in death [80]. By contrast with SARS, about 75% of patients with MERS had at least one comorbid illness with patients who died more likely to have an underlying condition (86% of patients who died *vs* 42% of recovered or asymptomatic patients). Index or sporadic cases in the first wave in 2013 were older (median age 59 years *vs* 43 years), and more likely to have severe disease requiring admission to hospital (94% *vs* 59%) than were secondary cases. Only secondary cases had mild disease or asymptomatic infection. On the basis of data related to human-to-human transmission in several clusters, the incubation period has been estimated as more than 5 days, but could be as long as 2 weeks [81, 82, 83].

MERS typically begins with fever, cough, chills, sore throat, myalgia, and arthralgia, followed by dyspnoea and rapid progression to pneumonia within the first week, often requiring ventilatory and other organ support. Although most patients with symptomatic disease present with respiratory illness, immuno compromised patients can present with fever, chills, and diarrhoea and later develop pneumonia. Similar to SARS, at least a third of patients with MERS have gastrointestinal symptoms, such as vomiting and diarrhea [81, 84]. Risk factors for development of severe disease, in addition to an immunocompromised state, include comorbidity (*e.g.*, obesity, diabetes, cardiac disease and lung disease) [81, 82, 84].

Concomitant infections and low albumin concentration are predictors of severe illness, and age older than 65 years was associated with mortality in a case series in Saudi Arabia. The few data available about viral dynamics and clinical course suggest that patients with MERS have a shorter time from illness onset to clinical presentation and to a requirement for ventilatory support than do patients with SARS (table), and higher respiratory tract viral loads during the first week of the illness [33].

As for SARS and other severe viral illnesses, common laboratory findings of MERS include leucopenia, particularly lymphopenia [8, 31, 81]. Some patients have a consumptive coagulopathy and high creatinine, lactate dehydrogenase, and liver enzyme concentrations [82]. Co-infection with other respiratory viruses (*e.g.*, parainfl uenza, rhinovirus, influenza A virus[H1N1]pdm09, herpes simplex virus, infuenza B virus) has been reported and nosocomial bacterial infections (including *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Acinetobacter* species, *Candida* species) have occurred in patients receiving invasive mechanical ventilation [8, 85, 86].

Diagnosis: Because lower respiratory tract specimens such as bronchoalveolar lavage fluid, sputum, and tracheal aspirates contain the highest viral loads, [86, 33, 87] they should be collected whenever possible. MERS can be confirmed by detection of viral nucleic acid or by serology. The presence of viral nucleic acid can be confirmed either by positive real-time reverses transcription PCR on at least two specific genomic targets or by a single positive target with sequencing of a second positive PCR product [88].

Available real-time reverse transcription PCR tests include an assay targeting RNA upstream of the E gene (upE) and assays targeting open reading frames 1b (ORF 1b) and 1a (ORF 1a). The assay for the upE target is highly sensitive and is recommended for screening; the ORF 1a assay is of equal sensitivity. The ORF 1b assay is less sensitive but is useful for confirmation. These assays have not shown cross-reactivity with other respiratory viruses including human coronaviruses. Two target sites on the MERS-CoV genome suitable for sequencing to aid confirmation are in the RNA-dependent RNA polymerase (*RdRp*; present in *ORF 1b*) and *N* genes [88]. In MERS cases confirmed by PCR, serial sampling for PCR testing from the upper and lower respiratory tracts and other body parts (*e.g.*, serum, urine, and stool) are recommended to understand viral replication kinetics and to guide infection control. Respiratory samples should be collected at least every 2 - 4 days to confirm viral clearance after two consecutive negative results are obtained.

For confirmation of infection by antibody detection, paired serum samples should be collected 14 - 21 days apart with the first taken during the first week of illness. A positive screening assay (ELISA, immune-fluorescence assay) should be followed by a confirmatory (neutralisation) assay. Single samples might also be valuable for identification of probable cases and should be collected at least 14 days after the onset of symptoms [14, 46, 89]. Serological results should be carefully interpreted because they might be confounded by cross-reactivity against other coronaviruses [90].

Treatment: No specific drug treatment exists for MERS and supportive treatment is the mainstay of management. Evidence-based recommendations for treatment provide the basis for decision making in clinical settings (panel) [91]. MERS-CoV is readily inhibited by type 1 interferon's (IFN-α and especially IFN-β) in cultured cells, [92, 93] and IFN-α2b combined with ribavirin can lessen lung injury and reduce lung titers when administered to rhesus macaques within 8 h of virus inoculation [94]. This combination was tested in severely ill patients, showing an improvement in survival at 14 days but not 28 days, possibly a result of administration in the advanced stages of disease [92, 95].

Several drugs inhibit MERS-CoV in cell culture, including ciclosporin and mycophenolic acid [96, 97]. Other compounds (chloroquine, chlorpromazine, loperamide, and lopinavir) inhibit virus replication (effective concentration 3-8 μmol/L) *in-vitro*, [98, 99] although whether these drugs will be useful in patients is unknown. MERS-CoV-specific peptide fusion inhibitors, which function similarly to the HIV drug enfuvirtide, diminish virus replication in cultured cells, providing a novel approach to MERS treatment [100].

Human monoclonal neutralising antibodies and convalescent sera from recovered patients might be useful for treatment if delivered in a timely fashion [91, 101, 102], an exploratory post-hoc meta-analysis of studies of SARS and severe influenza showed a significant reduction of mortality following antibody treatment compared with placebo or no treatment [103]. Systemic corticosteroids have been used empirically in some patients to dampen immune pathological host responses, although no survival benefit has been reported [95]. Steroids should be used cautiously, if at all, because their use was associated with worsened outcomes in patients infected with SARS-CoV during the 2002 03 epidemic. More data are needed from animal studies and carefully done clinical and virological studies of priority treatments such as convalescent plasma and interferons (ideally in randomized clinical trials if sufficient numbers of patients are available). At present, clinical management of patients with severe disease largely relies on meticulous intensive care support and prevention of complications [91].

Prevention: Recommendations for prevention of MERS are available from WHO, the US Centers for Disease Control and Prevention, and the Saudi Ministry of Health [104, 105]. The main infection prevention and control measures are droplet precautions (wearing a surgical mask within 1 m of patients) and contact precautions (wearing gown and gloves on entering patients' rooms and removing them on leaving). Droplet precautions should be added to the standard precautions when providing care to all patients with signs of acute respiratory infection [104]. Eye protection should be used when health-care workers care for probable or confirmed patients.

Public Health England, US Centers for Disease Control and Prevention, and Saudi Ministry of Health [104] recommendations for management of known or suspected MERS-CoV infection include the use of personal protective equipment such as gowns, gloves, eye protection (goggles or face shield), and respiratory protection equivalent to a fit-tested National Institute for Occupational Safety and Health-certified disposable N95 filtering face piece respirator. Patients with MERS should be placed in negative pressure rooms or in rooms in which room exhaust is filtered through high-efficiency particulate air filters. Airborne precautions with at least six air changes per hour should be applied in treatment rooms when performing aerosol-generating procedures [106, 107]. These recommendations are evidence based and have proven to be effective in hospitals in affected countries.

Camels infected with MERS-CoV can develop rhinitis or show no signs of infection and might shed virus through nasal and eye discharge and faeces. The virus can also be found in raw milk from infected camels. MERS-CoV is stable in camel breast milk for extended periods of time [108] thus, pasteurization or cooking is recommended to destroy the virus. Raw urine should not be used for medicinal purposes [105, 109].

Because signs of disease are non-specific, it is not possible to know whether an animal in a farm, market, race track, or slaughterhouse is excreting MERS-CoV without virological testing. Camel farm workers, slaughterhouse workers, market workers, veterinarians, and those handling camels at racing facilities should practice good personal hygiene, including frequent hand washing after touching animals, avoiding touching eyes, nose, or mouth with hands, and avoiding contact with sick animals.

Consideration should also be given to wearing protective gowns and gloves while handling animals, especially if camels have signs of upper respiratory tract disease [105]. The Saudi Government issues updated health guidelines for pilgrims [82].

Although MERS-CoV did not cause severe community-acquired pneumonia in any of the 38 hospital-admitted pilgrims investigated during the 2013 Hajj [12], good infectious disease surveillance and control measures are essential to prevent major outbreak of MERS during mass gatherings [106, 110].

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) - Saudi Arabia: Between 6 and 13 December 2016 the National IHR Focal Point of Saudi Arabia reported ten (10) additional cases of Middle East Respiratory Syndrome (MERS) including two (2) fatal cases. Three (3) deaths among previously reported MERS cases were also reported.

Details of the Cases: [112]

1. A 72-year-old male national living in Taif city, Taif Region. He developed symptoms on 9 December and was admitted to hospital on 10

December. The patient who has comorbidities, tested positive for MERS-CoV on 12 December. He has a histo+

2. Nine year of contact with camels and consumption of their raw milk in the 14 days prior to the onset of symptoms. Currently the patient is in critical condition admitted to ICU but not on mechanical ventilation. The Ministry of Agriculture has been informed and investigation of camels is ongoing.

3. A 64-year-old female national living in Buridah city, Qassim Region. She developed symptoms on 3 December and was admitted to hospital on 9 December. The patient who has comorbidities, tested positive for MERS-CoV on 10 December. Investigation of history of exposure to the known risk factors is ongoing. Currently the patient is in critical condition admitted to ICU on mechanical ventilation.

4. A 59-year-old male national living in Mahayl Assir city, Assir Region. He developed symptoms on 28 November and was admitted to hospital on 8 December. The patient who has no comorbidities, tested positive for MERS-CoV on 10 December. He has a history of contact with camels and consumption of their raw milk in the 14 days prior to the onset of symptoms. Currently the patient is in stable condition admitted to a negative pressure isolation room on a ward. The Ministry of Agriculture has been informed and investigation of camels is ongoing.

5. A 60-year-old male national living in Mahayl Assir city, Assir Region. He developed symptoms on 28 November and was admitted to hospital on 4 December. The patient who has comorbidities, tested positive for MERS-CoV on 6 December. He has a history of contact with camels and consumption of their raw milk in the 14 days prior to the onset of symptoms .The patient was in critical condition admitted to ICU on mechanical ventilation. He passed away on 6 December. The Ministry of Agriculture has been informed and investigation of camels is ongoing.

6. A 49-year-old male non-national living in Jeddah city, Jeddah Region. He developed on 30 November and was admitted to hospital on 6 December. The patient who has no comorbidities, tested positive for MERS-CoV on 7 December. The patient has no comorbid conditions. Investigation of history of exposure to the known risk factors is ongoing. Currently the patient is in stable condition admitted to a negative pressure isolation room on a ward.

7. A 53-year-old male non-national living in Riyadh city, Riyadh Region. He developed symptoms on 4 December and was admitted to hospital on 7 December. The patient who has no comorbidities, tested positive on 8 December. Investigation of history of exposure to the known risk factors is ongoing. Currently the patient is in critical condition admitted to ICU on mechanical ventilation.

8. A 56-year-old male national living in Riyadh city, Riyadh Region. He developed symptoms on 3 December and was admitted to hospital on 6 December. The patient who has comorbidities, tested positive for MERS-CoV on 7 December. Investigation of history of exposure to the known risk factors is ongoing. The patient was in stable condition admitted to a negative pressure isolation room on a ward. His conditions deteriorated and he passed away on 10 December.

9. A 24-year-old male national living in Hofouf city, Al Ahssa Region. He developed symptoms on 24 November and was admitted to hospital on 3 December. The patient who has no comorbidities, tested positive for MERS-CoV on 5 December. Investigation of history of exposure to the known risk factors is ongoing. Currently the patient is in stable condition admitted to a negative pressure isolation room on a ward.

10. A 78-year-old male national living in Riyadh city, Riyadh Region. He developed symptoms on 27 November and was admitted to hospital on 3 December. The patient who has comorbidities, tested positive for MERS-CoV on 5 December. Investigation of history of exposure to the known risk factors is ongoing. Currently the patient is in stable condition admitted to a negative pressure isolation room on a ward.

11. A 58-year-old male national living in Afif city, Riyadh Region. He developed symptoms on 3 December and was admitted to hospital on 4 December. The patient who has no comorbidities, tested positive for MERS-CoV on 5 December. Investigation of history of exposure to the known risk factors is ongoing. Currently the patient is in stable condition admitted to a negative pressure isolation room on a ward.

Contact tracing of household and healthcare contacts is ongoing for these cases.

The National IHR Focal Point for the Kingdom of Saudi Arabia also notified WHO of the deaths of 3 MERS-CoV cases that were reported in a previous DON published on 19 December 2016 (case numbers 4, 5, and 9). Globally, since September 2012, WHO has been notified of 1864 laboratory-confirmed cases of infection with MERS-CoV including at least 659 related deaths.

WHO Risk Assessment: [112] MERS-CoV causes severe human infections resulting in high mortality and has demonstrated the ability to transmit between humans. So far, the observed human-to-human transmission has occurred mainly in health care settings.

The notification of additional cases does not change the overall risk assessment. WHO expects that additional cases of MERS-CoV infection will be reported from the Middle East, and that cases will continue to be exported to other countries by individuals who might acquire the infection after exposure to animals or animal products (for example, following contact with dromedaries) or human source (for example, in a health care setting). WHO continues to monitor the epidemiological situation and conducts risk assessment based on the latest available information.

WHO Advice: [112] Based on the current situation and available information, WHO encourages all Member States to continue their surveillance for acute respiratory infections and to carefully review any unusual patterns. Infection prevention and control measures are critical to prevent the possible spread of MERS-CoV in health care facilities. It is not always possible to identify patients with MERS-CoV early because like other respiratory infections, the early symptoms of MERS-CoV are non-specific. Therefore, health-care workers should always apply standard precautions consistently with all patients, regardless of their diagnosis. Droplet precautions should be added to the standard precautions when providing care to patients with symptoms of acute respiratory infection; contact precautions and eye protection should be added when caring for probable or confirmed cases of MERS-CoV infection; airborne precautions should be applied when performing aerosol generating procedures.

Until more is understood about MERS-CoV, people with diabetes, renal failure, chronic lung disease, and immune compromised persons are considered to be at high risk of severe disease from MERS-CoV infection. Therefore, these people should avoid close contact with animals, particularly camels, when visiting farms, markets, or barn areas where the virus is known to be potentially circulating. General hygiene measures, such as regular hand washing before and after touching animals and avoiding contact with sick animals, should be adhered to.

Food hygiene practices should be observed. People should avoid drinking raw camel milk or camel urine, or eating meat that has not been properly cooked. WHO does not advise special screening at points of entry with regard to this event nor does it currently recommend the application of any travel or trade restrictions.

CONCLUSION: The awareness of the disease and the easy access to a more developed health care system could explain the higher incidence of MERS-CoV diagnosis in Saudi Arabia compared to other countries in Africa where the disease is likely to be overlooked. Larger scale serological screening of human populations in areas where MERS-CoV is endemic in dromedary camels should be considered. More extensive screening of bats in Saudi Arabia and East Africa, especially the Egyptian tomb bat, needs to be considered. Screening dromedary camel populations in Africa (Sahara desert and surrounding areas), and East Asia (Pakistan, Afghanistan, and Iran) will help better delineate the geographical distribution of dromedaries involvement.

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