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Review Article

Spatiotemporal Distribution and Associated Risk Factors for Middle East Respiratory Syndrome-Coronavirus In Dromedary Camels: Review

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ABSTRACT

The Middle East respiratory syndrome (MERS) is caused by Coronaviruses(CoV). Dromedary camels are likely to be a natural host of MERS, and transmission between camels is clearly documented. The first evidence of dromedaries being the reservoir of MERS-CoV came from serological studies. MERS-CoV was found circulating in dromedary camels during the last 20 years and neutralizing antibodies were detected in camels.High levels of MERS-CoV antibodies have been observed in dromedaries in the Middle East and Africa.Serological follow-up of dromedary dams and their calves has shown a typical pattern of juvenile infection.Differences in virus susceptibility and pathogenicity between animals of different species could be explained by a distinct tissue distribution of dipeptidyl phosphatase 4, the MERS-CoV receptor. Detection of MERS-CoV in dromedaries is performed to understand the epidemiology and evolutionary dynamics of the virus and to reduce the risk of human transmission. Sero-prevalence reports spatiotemporal distribution of MERS-CoV in dromedary camels among countries on the world where it was null in North America in 2005 and Australia in 2014. But it ranges from 29-100% in other studied countries. It was 100% in Saudi arabia, United Arab Emirates, Oman, Jordan. Protective experimental immunizations in dromedaries have already started using a modified vaccinia virus Ankara (MVA) vaccine expressing the MERS-CoV spike protein.

Keywords: Middle east respiratory syndrome; Coronavirus; Mers-cov; Mers coronavirus; Dromedary camels; Epidemiology

INTRODUCTION

The MERS is among viral diseases that affect camels. It affects mainly the respiratory system, and caused by Middle East respiratory syndrome Coronavirus (MERS-CoV). The virus belongs to the family Coronaviridae, single-stranded RNA of positive polarity. MERS-CoV, a member of the Betacoronavirus genus lineage C, was first identified in Saudi Arabia in 2012.

The virus specific antibodies have been detectedin the serum of dromedary camels across Northern Africa, including Tunisia, Egypt, Sudan, Ethiopia, Nigeria, Kenya and Somalia, andacross the Arabian Peninsula, including Jordan, Saudi Arabia, Qatar,Oman and United Arab Emirates[1-4].Genomic and epidemiologic studies comparing MERS-CoV sequences from household clustersand camels, and of dromedary farms and human contacts in UAE [5,6], and of patients with corresponding MERS-CoV positive camels in Saudi Arabia demonstrate camels as potential source of human infection [7].

A vaccine expressing the MERS-CoV spike protein confer mucosal immunity in dromedary camels with serum neutralizing antibodies and reduction of excreted infectious virus and viral RNA transcripts in vaccinated animals [8]. Surveillance and epidemiological studies reveals that infected dromedary camels serve as a reservoir with spill-over human infections via close contacts[9-14].

Dromedary camels assumed as the only reservoir for MERS-CoV until 2015where studies in Qatar on 15 healthy alpacas (*Vicugnapacos*) in 20 herd that shared a barn with dromedaries were 100%seropositive to viruses[15]. An otherstudyshowed similar to dromedary camels, infected alpacas didn't develop fever, but unlike dromedary camels, none of the alpacas had any observable nasal discharge over the course of infection. All infected animals could mount neutralizing antibodies to MERS-CoV[16]. That viruse could infect bat cell lines derived from six species as well as pig, camel, sheep, nonhuman primates, and human cell lines. The transmission and infection nature of MERS-CoV is via the respiratory secretions; coughing or droplet nuclei of an infected person[17]. Its cellular receptor, later identified as the DPP4

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receptor, conserved across many mammalian species and various tissues like lung and kidney epithelium[18].

The exact source of MERS-CoV and how it is transmitted to humans is not known. Since, the role of camels and other animals in the epidemiology of MERS-CoV and the route of transmission to humans remains unclear. Thus, further investigation of MERS-CoV transmission within and among species is necessitates a better understanding of the role of potential reservoirs during an outbreak. However, it is still unclear whether camels are the natural reservoir of the virus and the only source of human infection. Clearly, transmission from camels to humans does take place, and camel exposure is a risk factor for humaninfection but such transmission is not efficient and infectionis not directly proportional to exposure.The aims of this review is to summarize the spatiotemporal distribution and associated risk factors for MERS-CoV occurrence in camels globally.

ETIOLOGY OF MIDDLE EAST RESPIRATORY SYNDROME-CORONAVIRUSES

The MERS is caused by Coronaviruses. CoVs are enveloped, single-stranded positive-sense RNA viruses displaying a large genome of 26 kb to 32 kb. The viruse is a putative member of a new species[19]within the order Nidovirales, family Coronaviridae, subfamily Coronavirinae, genus Betacoronavirus, subgroup2c[20].

The coronaviruse has two phylogenetic clades, clade A (earliest case) and clade B (new case)[21]. Basedon genotype and serological characteristicsthe viruses are classified within 4 groups: Alphacoronaviruses (αCoVs), Betacoronaviruses (βCoVs), Gammacoronaviruses, and Deltacoronaviruses[22,23]. MERS-CoV had frequently been referred to as a SARS-like virus,"SaudiSARS"or the novel coronavirusuntil 2013[24]. or Human Coronavirus Erasmus Medical Center/2012(HCoV-EMC/2012)is the name of a novel strain of coronavirus isolated from the sputum of the first person to become infected with what was later named Middle East respiratory syndrome coronavirus, or MERS-CoV[19,25,26].

OCCURENCES OF MIDDLE EAST RESPIRATORY SYNDROME–CORONAVIRUSES IN CAMEL

The global population of camels is estimated to be about 30 million, 95% of these being dromedary camels[27]. Dromedary camels inhabit the Middle East region, North and East Africa and North-western parts of Asia. Of the total of 28.5 million dromedary camels worldwide, 77% are in Africa, the largest camel populations found in Somalia (6.2 million), Sudan (4.8million), Kenya (3 million) and Ethiopia (2.3 million). Only 4% are in the Arabian Peninsula. The highest density of camels by land area in the Arabian Peninsula is found in Qatar and UAE[27].

The first evidence of dromedaries as reservoir of MERS-CoV came from serological studies. High levels of MERS-CoV antibodies have been observed in dromedaries in the Middle East and Africa[1-4,28,29]. Serological follow-up of dromedary dams and their calves has shown a typical pattern of juvenile infection. Maternal antibodies against MERS-CoV in dromedary calves generally disappear between 4 and 8 months of age, permitting infection to occur during the sero negative period;young infected dromedaries then develop antibodies that persist for a long time[30,31]carried out study in different parts of Africa, have encompassed diverse geographical and ecological variables, study findings may well be relevant in regions such as Saudi Arabia where zoonotic MERS remains a recurrent threat. Furthermore, it is not clear that transmission of MERS-CoV to humans is absent in Africa. Another study has reported evidence of humans with MERS-CoV seropositivity in Kenya[32]. Further studies are needed to assess whether or not zoonotic MERS-CoV transmission occurs in Africa and epidemiological data provide identification of situations of highest risk. Better understanding of the risk factors and virus transmission dynamics of MERS-CoV within camels is important in responding to the global health threat posed by MERS-CoV[33]. However, in a few cases, MERS-CoV has been isolated at the age of 20 days or even at younger, indicating that maternal antibodies are not necessarily protective. A plausible hypothesis could indeed be that young camels that lack antibodies have a high probability of being infected and in turn, expose the mothers to infection or re-infection [34]. The longitudinal study conducted by Meyer et al.[34] on natural MERS-CoV infections in camels confirms assumptions from preliminary cross-sectional studies in camels[3,30,35]. MERS-CoV infection appears to predominantly affect young, immunologically naive animals. Serum antibodies might not have been sufficient to mediate protective immunity in the respiratory tract because dams and calves were sporadically infected even as maternal antibodies peaked at day 7 postparturition[34]. Findings of virus isolation from calves but not dams are in line with earlier observations of reduced viral load in seropositive camels on reinfection [36,37] indicating that neutralizing antibodies might not provide sterile immunity but could still reduce the viral replication level. The predominance of infection in young animals is better explained by the absence of immunity than by other factors, such as social group density, because the number of newborn camels in our study was negligible compared with the overall size of the herd at the farm. Moreover, young camels were not kept in a contiguous group but in small compartments, where they had more contact with their mothers than with other young animals. Calves are likely to have been infected through fomites or through adult animals shedding low quantities of virus.

Camel breeding, even if involving a small number of newborn animals, should be classified as a risk for human acquisition of MERS-CoV. The greatest risk should be assumed for the time after the fourth month of life until the first wave of natural infections, which should occur during the first year of life in camels raised in MERS-CoV–endemic regions. Measures for the prevention of infection, such as personal protective equipment, hand hygiene, and environmental sanitation, as applied on the farm in our study, should be sufficient for protection, given that no human MERS-CoV illnesses occurred among staff and only 2 of 300 workers with regular contact with camels had detectable MERS-CoV–specific IgG antibodies. Because persons with underlying disease and the elderly show the most severe outcomes of MERS-CoV infection, these groups should generally avoid farms where camel calves are being raised.

So far, the specific source of infection for young dromedaries is not known, although it is likely to be from other dromedaries. Extensive investigations in other animal species, including rodents, ticks, horses and small ruminants, have not demonstrated other reservoirs of infection to date. Their host range is very wide and includes both mammalian and avian species. Coronaviruses can cause acute and chronic respiratory, enteric, neurological and hepatic diseases in their hosts[38].

MERS-CoV has been found in dromedary camels in several countries in the Middle East and Africa. Highpercentage of

seropositive dromedaries were foundin Arabian Peninsula and Africa. Serological tests showed a higher incidenceof virus infections in adult than young camels[39]. Also, young dromedaries (≤ 2 years) had lower viral load than theadult ones, which indicates a higher risk of infection for humans during the reproductiveseason (spring) when a number of immunocompetent camels increased. The diseases acquired in dromedaries at age of less than one year and becomes a source of infection to humans, but not known how the virus is transmitted from camels to humans (FAO, 2017).

In an attempt to investigate the time frame of MERS-CoV introduction to dromedary camel population, multiple studies screened stored serum samples. All 151 dromedary camel serum samples obtained in 2003 from UAE (100%) were seropositivity. Archived serum samples, obtained from dromedary camels in Saudi Arabia from1992 to 2010 had high seropositivity ranging from 72 % to 100 %[30]. From Egypt, 189 stored dromedary camel serum samples collected in 1997, and from Sudan and Somalia, collected between 1983 and 1984, were tested, and 81% have neutralizing antibodies to virus[4].

Serum samples collected from 105 dromedary camels living in the Canary Islands, a Spanish archipelago located just off the southern coast of Morocco, between 2012 and 2013, 14 % have antibodies against MERS-CoV[40]. In an attempt to screen feral camels in Australia, 307 dromedary camels' sera from two different locations were sampled between December 2013 and June 2014. All tested negative for specific viruseantibodies[41].

MERS-CoV is a zoonotic virus transmitted from animals to humans. The origins of the virus are not fully understood but, it is believed that originated in bats and transmitted to camels sometime in the distant past[17]. MERS-CoV is a zoonotic virus, and dromedary camels are a reservoir host[5,42,43]. Bats are a likely original reservoir; coronaviruses similar to MERS-CoV have been identified in bats[44], but epidemiologic evidence of their role in transmission is lacking. Infection of other livestock species with MERS-CoV is possible[45]; however, attempts to inoculate goats, sheep, and horses with live MERS-CoV did not produce viral shedding[16], and no epidemiologic evidence has implicated any species other than dromedaries in transmission. Sporadic zoonotic transmission from dromedaries has resulted in limited human-to-human transmission chains, usually in healthcare or household settings[46-49].

Transmission following exposure to camel feces may be biologically plausible, although no epidemiologic evidence indicates the likelihood of such transmission. Similarly, although transmission following consumption of raw camel milk may be biologically plausible, epidemiologic studies have not consistently identified milk consumption as a unique risk factor for MERS-CoV infection or illness, independent of other direct or indirect camel exposures [11,50]. No epidemiologic evidence supports transmission associated with camel urine or meat.Thus, further investigation of MERS-CoV transmission within and among species is necessitates a better understanding of the role of potential reservoirs during an outbreak[51]. However, it is still unclear whether camels are the natural reservoir of the virus and the only source of human infection. Clearly, transmission from camels to humans does take place, and camel exposure is a risk factor for human infection but such transmission is not efficient and infectionis not directly proportional to exposure. but camels are a major reservoir host for the viruse and an animal source of infection in humans[51]. Strains of viruse identical to human strains have been isolated from camels in several countries, including Egypt, Oman, Qatar, and Saudi Arabia[46,52,53].

The strongest evidence of camel-to-human transmission of MERS-CoV comes from a study in Saudi Arabia whereviruse isolated from a man with fatal infection and his camelswere identical; showing that viruse can infect dromedary camels and transmitted to humans by close contact.

SPATIOTEMPORALDISTRIBUTIONS AND SPREADOF THE DISEASES

At the end of January 2020, a total of 2519 laboratory-confirmed cases of MERS, including 866 associated deaths (case-fatality rate: 34.3%) were reported globally. The majority of these cases were reported from Saudi Arabia (2121 cases), including 788 related deaths with a case-fatality rate of 37.1% (WHO, 2020).

Since September 2012 and as of 19 February 2020, **2,527 cases confirmed; including 904 case fatalities** 2494 cases of MERS-CoV, including 912 deaths, have been reported by health authorities worldwide. Up to July18 2018, a total of 2229 laboratory-confirmed cases of MERS, including **827 case fatalities** deaths (case–fatality rate: 37.1%) in 27 countries were reported to WHO worldwide, with most being reported in Saudi Arabia (1854 cases with 717 deaths) were reported globally; (WHO, 2018).Epidemiologic studies have provided evidence of endemic MERS-CoV infection among dromedaries in the Greater Horn of Africa as far back as 1983[54] and in Saudi Arabia as far back as 1992-1993[30].

Multiple surveillance studies explored the extent of MERS-CoV infection in dromedaries. Presence of specific MERS-CoV antibodies in dromedary camels' sera was used as an indicator of previous exposure to the virus, while the presence of MERS-CoV RNA material in nasal secretions, usually identified through RT-PCR, indicated current infection and active viral shedding. Serum samples from 303 dromedary camels from Saudi Arabia were screened in 2013 and found to have high seropositivity of 72 % to MERS-CoV[30].

All serum samples from 50 dromedary camels in Oman were positive for MERS-CoV specific antibodies[55]. Similar results were reached from a larger study conducted in the United Arab Emirates (UAE), where 500 dromedary camels' sera screened in 2013 revealed 96% seropositivity[3].

In Africa, a study assessed the geographic distribution of MERS-CoV among dromedaries by investigating serum samples from Nigeria, Tunisia, and Ethiopia[56]. In Nigeria, serum samples collected between 2010 and 2011 from 358 adult dromedaries distributed over 4 provinces were tested, and 94 % were positive for MERS-CoV antibodies. In Tunisia, 48.5 % of 204 serum samples of dromedaries collected from three provinces tested positive for MERS-CoV[56]. In Ethiopia, 96.3 % of the serum samples collected between 2011 and 2013 from 188 dromedaries from three regionswere positive for MERS-CoV antibodies.

An increase in seropositivity rate with age was observed which confirms the trend observed inEthiopia in a previous study[9]. Thus a higher virus RNA detection rate in young animals compared with older animals which could be related to a lack of prior immunity as published in previous studies in Saudi Arabia[57,58]. Young animals were naïve and more susceptible to virus infection[34,58].

Sero-prevalence reports spatiotemporal distribution of MERS-CoV in dromedary camels among countries on the world (Table 1). It

was null in North America[2,59]. But it ranges from 29-100% in other studied countries. It was 100% in Saudi Arabia, United Arab Emirate, Oman and Jordan(Table 1).

RISK FACTORSFOR THE OCCURRENCE AND SPREAD OF MIDDLE EAST RESPIRATORY SYNDROME-CORONAVIRUS

Host risk factors

Middle East Respiratory Syndrome-Coronavirus pinpointed a zoonotic introduction of a novel coronavirus probably originating from bats into human populations[32]. The viruse was found circulating in dromedary camels from last 20 year[60], and neutralizing antibodies were detected in camels[61]. Dromedary camels are the primary animal host for MERS-CoV and the only species from which antibodies specific to the virus detected serologically among livestock [35,62].

The first study on domestic animal host shows IgG antibodies specific to MERS-CoV in dromedary camel herds in Oman and the Canary Islands. One hundred percent of the camels tested in Oman (n=50) and 14 percent (n=105) of Spanish camels were positive for the viruse antibodies. Several species of domestic animals in various countries including Oman, Egypt, Jordan, and Saudi Arabia were screened for antibodies against viruse, including sheep, goats, cattle, and buffalo, but all were negative[9,20,35,62].

Coronavirus RNA sequences found in bat fecal samples are closely related to MERS-CoVsequences[63]. The viruse grows readily in

Table 1: Spatiotemporal distribution of MERS-CoV in dromedary camels among countries on the world (Sero-prevalence reports).

Year	Countries	Number examined	Prevalence (%)	References
1983-1997	Sudan and Somalia	189	81	Muller et al. (84)
1992-2010	Saudi Arabia	264	87	Alagailiet al. (17)
1992-2013	Kenya	774	30	Cormanet al. (1)
1993	Saudi Arabia	131	90	Hemidaet al. (2)
2003	UAE	151	100	Meyer et al (3)
2005	UAE	33	91	Alexandersenet al. (59)
2005	North America	6	0	Alexandersenet al. (59)
2009, 2013	Tunisia	204	54	Reuskenet al. (14)
2010-11	Nigeria	358	94	Reuskenet al. (14)
2011-13	Ethiopia	188	97	Reuskenet al. (14)
2012-13	Canary-Islands	105	14	Reuskenet al. (28)
2012-13	Oman	50	100	Reuskenet al. (28)
2012-13	Saudi Arabia	310	90	Hemidaet al. (35)
2012-2015	Pakistan	565	55.8	Saqibet al. (125)
2013	Egypt	110	94	Pereraet al. (62)
2013	Jordan	11	100	Reuskenet al. (28)
2013	Qatar	14	79	Haagmanset al. (10)
2013	Saudi Arabia	206	95	Alagailiet al. (30)
2013	UAE	182	96	Meyer et al. (3)
2013	Oman	76	7%	NowotnyandKolodziejek (124)
2013	Egypt	52	92	Chu et al. (113)
2013	Saudi Arabia	5	100%	Memish et al. (63) Azharet al. (12)
2013	Kenya	335	46.9%	Deem et al. (115)
2013-14	Saudi Arabia	21	100%	Hemidaet al. (2)
2014	Australia	2	0%	Hemidaet al. (2)
2014-2015	Iraqi	18	85%	Thwinyet al. (126)
2014-2016	Saudi Arabia	584	70.9%	Kasemet al. (7)
2014-2016	Egypt	2541	71.2%	Ali et al. (65)
2015	UAE	376	29%	Yusofet al. (128)
2015	Burkina Faso	525	84.6%	Miguelet al. (31)
2015	Ethiopia	632	99.4%	Miguelet al. (31)
2015	Morocco	343	100%	Miguelet al. (33)
2015-2017	Saudi Arabia	689	56.4	Kasemet al. (7)
2016	Saudi Arabia	171	84.21%	Harrathand Abu Duhier (118)
2018	Israel	71	71.8%	Harcourt et al. (117)
2019	UAE	11	82%	Lau` et al. (120)

bat-derived cell lines and unlikely to be immediate source for most human cases because human contact with bats is uncommon. Theviruse excreted in the nose of dromedaries seems to be much higher than that of other animal species described so far, suggesting a more prominent role of dromedaries in the transmission of MERS-CoV to humans[64].

Sex: male camels showed a higher positivity (83.5%) to viruse antibodies than the female camels (66.5%). For MERS-CoV RNA the male camels showed 20% positivity while female camels exhibited 4.9%[7]. A previous study stated that there was no difference in the seroprevalence rates between female camels (82.7%) and males (85.1%), while viruse RNA level was higher in females (7.1%) than in males (2.6%)[65]. This variation is due to the difference in sample numbers and the age of animals included in both studies

Another point highlighted by the study on camel function as a risk factor is the function of camels which is also related to sex. Camels raised for milking show the highest sero-prevalence followed by camels raised for their meat and also camels used for transport activities have the lowest seroprevalence[58]. The higher seropositivity rate in females bred for milking could be related to the high viral RNA detection rates in younger animals, e.g. calves[34].

A plausible hypothesis could indeed be that young camels that lack antibodies have a high probability of being infected and in turn, expose the mothers to infection or re-infection. The lower seropositivity rate in camels bred for their meat or for transport activities, which are mostly males, could also be linked with the fact that males are often separated from the herd (the two sexes are only mixed during the reproduction activities) and have thus fewer contacts with other camels (i.e. females and calves)[31].There is convincing evidence that dromedary camels are host animals for the strain of MERS-CoV that infects humans. Whether camels are indeed the reservoir for MERS-CoV or whether they function as a vehicle for the virus from a yet unidentified animal reservoir to humans remains to be established.

Age: adult camels had a higher seroprevalence of MERS-CoV antibodies (86.6%) compared to young camels less than 2 years (57.7%) in Saudi Arabia[7]. While young animals less than 2 years showed a high positivity (15.4%) to MERS-CoV RNA compared to adult animals, previous studies have shown high seropositivity in adults compared to juvenile camels that exhibited a high infection rate[14,57].

Two hundred and three samples from live dromedary camels in Saudi Arabia were collected in 2013 and found to have high seropositivity (72 %) to MERS-CoV[30]. Seropositivity was higher among adults dromedary camels (two years and older) compared to juvenile dromedary camels (less than two years of age), 95 % vs. 55 % respectively [59]. In the same study, 202 dromedary camels' nasal swabs were tested for the presence of MERS-CoV RNA material using RT-PCR; 25% were positive. In other words, one-fourth of the tested dromedary camel population was shedding the virus and was potentially infectious. Of those shedding the virus, 71% were juvenile and 29% were adult dromedary camels older than two years.

Biology of the pathogen as a risk factor

Middle East Respiratory Syndrome-Coronavirus viruses can be recovered from the full-length Complementary DNA (cDNA) clone, using susceptible Vero A66 and Huh-7 cells, with titers of around 106 plaque-forming unit/ml (p.f.u./ml) at 72 h posttransfection (h.p.t.). The recovered viruses can be cloned by three rounds of plaque purification, and their phenotypic and genotypic properties can be determined. MERS-CoV rescued from both cell lines induce a Clear Cytopathic Effect (CPE), characterized by the induction of cell fusion, which was more apparent in Huh-7 cells[66].

In both cell lines, viral mRNAs could be readily detected at 7 h Post-Infection (PI) and reached maximum levels around 13 h p.i. Viral RNA levels remained more or less constant until 24 h p.i. in Vero cells, whereas the amount isolated from Huh7 cells declined due to the more rapid development of cytopathology in this cell line between 13 and 24 h p.i. After the peak of viral RNA, accumulation had been reached, the titer of virus released from MERS-CoV infected Vero cells increased steadily from ~5×10^5 to ~5×10^7p.f.u./ml. The bulk of the viral progeny is released significantly earlier from Huh7 cells, although the final titers at 24h p.i. are comparable to that obtained from Vero cells[67].

Differences in virus susceptibility and pathogenicity between animals of different species could be explained by a distinct tissue distribution of DPP4, the MERS-CoV receptor. DPP4 distribution in the respiratory tract was similar among llamas and pigs but differed from that of dromedary camels[64]. In contrast, DPP4 was barely detected in the respiratory tract of sheep, probably accounting for the lack of infection reported here. These results are in concordance with those reported that MERS-CoV experimentally inoculated sheep showed no clinical disease and that only small amounts of virus were detected in nasal swab samples.DPP4 (also named CD26) has been identified as the receptor for MERS-CoV[68]. All HCoV receptors identified to date are exopeptidases, although their proteolytic activity is not necessary for the virus to bind to the receptors, nor for them to enter the host cell[69,-71].

A comparative analysis of HCoV receptor expression across the respiratory tract of humans may provide clues regarding differences in pathobiology between HCoVs. In cell lines and ex vivo lung cultures, DPP4has expressed in type I and II alveolar cells, ciliated and non-ciliated bronchial epithelium, bronchial submucosal glands, endothelium, alveolar macrophages and leukocytes[72]. This largely corresponds with viral tropism in ex vivo human lung cultures, which show infection of non-ciliated cells in bronchi, bronchioles, endothelial cells and type I and II pneumocytes, but rarely in alveolar macrophages[26,73,74]. Remarkably, the binding site of DPP4 is different in different species, explaining why not all animals can be infected with MERS-CoV.

Environmental risk factors

Lifestyle: Surprisingly, there was no observed difference between nomadic and sedentary herds in the seropositivity rate or virus RNA positive rate[31]. Two hypotheses may explain this pattern. Firstly, the sedentary lifestyle is found in animal production systems where animals live at high density in 'commercial' farms. In such situations, the virus may be introduced more easily to the herd with animals being bought from other sources and the virus once introduced will amplify to infect most of the susceptible animals, since they are in close contact with each other. The virus appears to have a density-dependent transmission pattern.

In contrast to this, nomads are long-distance travelers who connect to different regions. Consequently, they have multiple opportunities to come into contact with other camel populations

during their travels, or through indirect contacts with water points and thus increasing the probability of encountering animals shedding MERS-CoV. In supportof these interpretations, the lowest seroprevalence was found for the mixed lifestyle which is associatedwith medium herd sizes and relatively small range movements[31].

Herd size: The role of camel density in shaping the large spatial scale (i.e. national) variation pattern in seropositivity and virus RNA detection rates is supported by the identification of a herd size effect on serological prevalence. Higher seropositivity rate was found in large or medium size herds as compared with small herds, suggesting that the transmission of the virus is density dependent. More studies are now necessary to better describe the virus transmission dynamics within herds and between herds, with mechanistic models accounting for a disease transmitted through close contact and the possibility of re-infections.Such a model would allow determining the minimum size of a camel herd required for the MERS-CoV to persist in that herd without 'fadeouts': i.e. critical community size[2].

Seasonal difference: MERS-CoV spread among dromedary camels shows that the virus produces acute epidemics in calves, often born in Spring[17,75]. Such outbreaks may cause an increase in the number of primary cases and increased opportunities for subsequent transmission, multiplying the number of admissions of MERS-CoV cases to hospitals with the possibility of further triggering hospital outbreaks as previously reported[27,49,76-78] but not find evidence to explain a seasonal pattern on human-to-human transmission [79].

Transmission

The exact source of MERS-CoV and how it is transmitted to humans is unknown. Initial investigations have indicated that MERS-CoV originated from bats; sequences related to MERS CoV have been found in several bat species[48]. Limited, non-sustained human-to-human transmission in health-care settings continue to occur, primarily in the Kingdom of Saudi Arabia, due to the non-specificity of MERS symptoms resulting in late diagnosis of MERS. Even though, it is unclear how the virus is transmitted from camels to humans, the WHO advises avoiding contact with camels and to eat only fully cooked camel meat, pasteurized camel milk, and to avoid drinking camel urine (WHO, 2016). A speculative model of how humans, camels, and bats may interact to acquire and spread MERS-CoV is given by[29].

The world today is watching the evolution of the situation in China with concern and fear, where at the end of 2019 an increase was registered in patients with a respiratory infection infected by a new coronavirus. This has now been identified with the acronym COVID-19, pinpointed in the city of Wuhan. The appearance of a new infectious disease is always a complex situation, especially if it is an epidemic of significant extension or severity. The cases increased rapidly in Wuhan and Hubei Province, and they extended in smaller numbers and with limited transmission chains throughout China. Imported cases and secondary cases have been reported in more than 24 countries. On January 30, 2020, WHO declared this epidemic as a Public Health Emergency of International Concern. The COVID-19 virus has been identified and sequenced genetically[80,81]. It is related to other coronaviruses that circulate in bats (including the SARS coronavirus), leading to the belief that its natural reservoir is probably these flying mammals. The intermediate host, which is probably another mammal, has not yet been identified. The point of contact with humans could be a live animal market in Wuhan, which today is shut down[82,83]. Itis possible that this virus went unnoticed for several weeks in a city of 11 million inhabitants and at the beginning of the flu season, until the alert was given due to the increase in severe cases (pneumonia) and it was possible to isolate and identify the coronavirus COVID-19 in several patients. The jump of a virus from animals to humans (spillover) is common among coronaviruses. This happened with SARS in 2002–2003 and with MERS since 2012. It has been shown that the 2019-nCoV virus is transmitted easily from person to person, as groups of intrafamily cases and transmission to health personnel have been identified. The transmission capacity, which is usually estimated using the so-called basic reproduction number or R0, is a controversial variable of this new disease. An R0 value less than 1 indicates a low extension capacity of an infectious disease, while R0 values greater than 1 indicate the need to use control measures to limit estension. Reliable estimates place the R0 value of the COVID-19 in 1.4–2.5, similar to theR0 ofthe coronavirus SARS at the beginning of the epidemic (2.2–3.7). This value was reduced to anR0 of 0.67–1.23 at the end of the epidemic. By contrast, the coronavirus MERS has always remained at lower R0 values (0.29–0.80)[84]. It seems that the COVID-19 could be more easily transmitted than SARS. However, there is a need to exercise caution. The R0 value indicates the transmission potential of an infectious disease. A higher R0 does not mean a more extensive disease. The flu, for example, whose R0 value ranges around 1.3 each year, infects millions of people worldwide. Neither does the R0 indicate the transmission rate either. R0 is also an average value: there are people who, although infected, will not transmit the disease to anyone, while others may transmit it to many more people. These individuals, called «super-spreaders», were protagonists oftwo extraordinary events during the SARS epidemic in Toronto (Canada) and MERS in Seoul (South Korea) when, from one patient who was a «super-spreader», dozens of patients, visitors and health personnel from two hospitals were infected. Control measures, such as those used in China, can significantly reduce the R0 of a disease. In this initial phase of the COVID-19 epidemic, its R0 value is being estimated from multiple assumptions and using complex mathematical models.As epidemiologists, some of us approach these mathematical models with circumspect: a popular saying states «All the models are wrong, but some are useful». This saying also applies to another controversial parameter appearing at the start of all epidemics: the number of real cases. Current statistics, without entering into discussions about the Chinese authorities' communication policy or transparency, probably reflect a bias towards the most severe cases which are the most likely to have reached out to the health system. Numbers for mild cases and asymptomatic cases are likely to be lower than reality. In recent weeks the detection capacity (RT-PCR test) of infected patients in the epidemic zone has increased, and this fact could partly explain the increase in case numbers, although many patients may still be undiagnosed. This possibility leads to the discussion about the estimation of the fatality rate of this disease, which currently stands at around 2.0%, with more than 40,000 cases and 1000 deaths (WHO, 2020). The mortality rate for SARS was around 10%, so the disease caused by COVID-19 seems, for now, to be less severe.

Recent research shows the human to human transmission route of SARS-CoV-2, these studies data shows a person who has been visited Wuhan city market their family members also find infected by this virus[85]. According to WHO guideline person to person

transmission occurred via direct contact or droplet spreading by the infected individual via coughing and sneezing. Likewise[86], has suggested the presence of this dangerous virus in fecal swab and blood that indicates the multiple transmission routes of infection. It's has been already shown that the transmission of SARS-CoV and MERS-CoV occurred through nosocomial transmission and considered airborne pathogens.

Risk for camel-to-camel or camel-to-human transmission may be influenced by crowding, mixing of camels from multiple sources, transportation, and characteristics of live animal markets[28]. Phylogenetic modeling has provided supportive evidence that long-term MERS-CoV evolution has occurred exclusively in camels, with humans acting as a transient and usually terminal host[87].

Pathogenesis

A very important stage after transmission is the binding of coronavirus to host cell receptors. It is noteworthy that SARSCoV-2 share the same cellular receptor with SARS-CoV genera. The spike protein of coronavirus from all four families, guides to coronavirus entry into the host cell[88]. Corona viruses entre into the host cell by a two-step process: first host cell receptor recognized for viral attachment and fuse viral and host cell membrane. The spike protein is present in two very different forms pre-fused (before fusion to host) and post-fused (after fusion to host cell).

The pre-fused spike protein displays a homo-trimer structure with three receptor binding S1 receptor binding side and resting at the top of trimeric S2[89-91]. The post-fusion structure is a coiled-coil structure with contained only S2[92,93]. The virus invasion may have two pathways (a) the ACE-2 receptor (b) using the integrin receptor.Angiotensin-Converting Enzyme-2 (ACE-2) receptor presents the cell membrane of the cells of the lungs, heart, and kidney. ACE-2 is expressed by type I and type II alveolar epithelial cells. Among them, type II is shown more than 80% ACE-2 receptor.

Men had a higher level of ACE-2 receptor rather than women. This enzyme considers as the main entry point for coronavirus[92,94]. SARS-CoV-2 can also fuse directly to the cell surface in the detection Beta-CoV receptor reveals that human cells expressing ACE-2 receptor have a crucial role to play in binding SARS-CoV-2, Spike (S) glycoprotein, and ACE-2 host receptor[95].

A 30 % difference in the S1 unit of S protein sequence between SARS-CoV and SARS-CoV-2. The RGD-motif of S protein, which is different in sequence from SARS-CoV and MERS-CoV, shows tightly binding to lung cells. It has long been known that SARSCoV is primarily a respiratory disease, so it also needed protease from the respiratory tract such as trans-membrane protein serine-2 (TMPRSS-2) and HAT[96-98](Figure 1). The TMPRSS-2 and HAT both activate the binding affinity of the S-protein cleavage trimer.

Some studies support that the S-protein and ACE-2 increase the affinity 10 to 20-fold for SARS-CoV-2[99,100]. After binding SARSCoV-2 to host cell receptor, it required a serial activation of kinase and protease like activities for the internalization of the virus. The phagocytosis mechanism is complex, where the interconnected, and cross-activation of proteins take participates inside the cells. TMPRSS-2 and HAT cleaves pattern for S-fragments differ from each other; HAT cleaves S protein mainly at R667, where TMPRSS-2 cleaves at multiple sites, both cleavages enhance the cell-virus fusion[97]. The infection of the target cell by SARSCoV-2 occurred due to S-pseudotypedvirions, which is less sensitive to cathepsin inhibitor when the target cell expresses TMPRSS-2[97,98]. Pseudo virions are still producing by SARS-CoV-2; still, TMPRSS-2, rely on endosomal cathepsin for the entry. Meanwhile, other accessory proteins may be involved in viral binding and invasion, such as cathepsin[101]2005) and clathrin(Figure 2), while potential molecules facilitated an

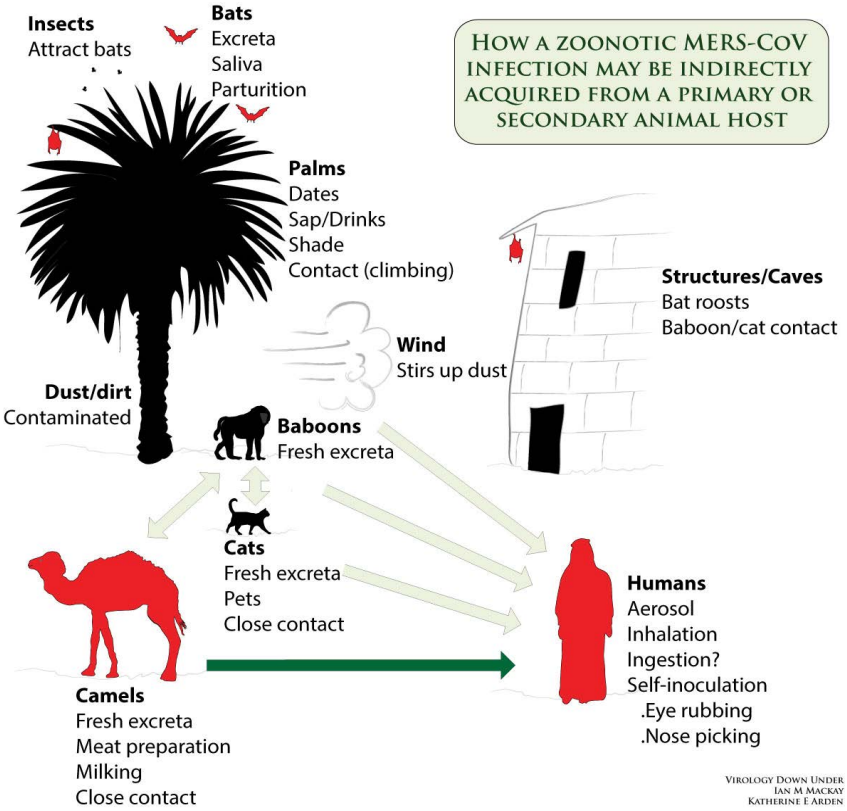

Figure 1: A speculative model of how humans, camels, and bats may interact to acquire and spread MERS-CoV. Source: [29].

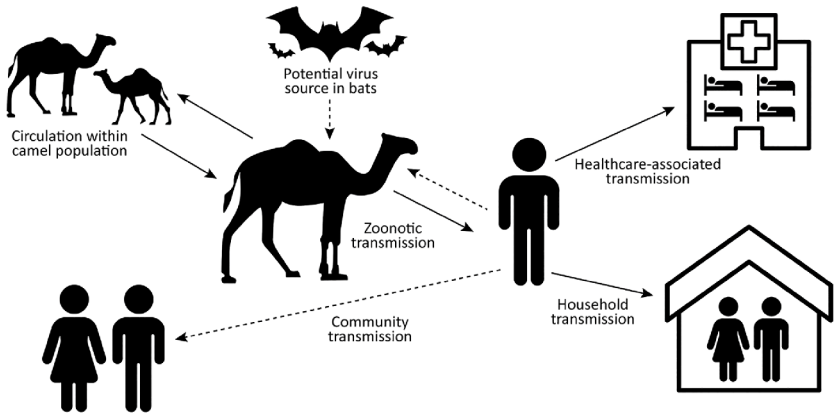

Figure 2: Summary of Middle East respiratory syndrome coronavirus transmission pathways. Solid lines indicate known transmission pathways; dashed lines indicate possible transmission pathways for which supporting evidence is limited or unknown [51].

uncertain membrane invasion of SARS-CoV-2[102-104]. SARS-CoV-2 cell entry Depends on ACE-2 and TMPRSS2, and it is Blocked by a Clinically Proven Protease Inhibitor. The lack of a complete understanding of the phagocytosis mechanism that is critical for SARS-CoV-2 to the host's which pathway involvement.

A recent study shows that Ca^{+2} ions increase infectivity and entry into MERS-CoV and Rubella virus cells [68,105] because of the presence of negatively charged peptide on fusion protein (S-protein). Studies show that the spike protein of coronavirus has evolutionary changes and obtain some features for its adaptations in human host cells [106,107]. In some research article it has been showing the involvement of Ca^{+2} ions play a significant role in which is several receptorbased events and initiates internalization of pathogenicity of the virus by altering the actin filaments and cytoskeleton arrangements through affecting the actions of several proteins[108]. When a virus binds an integrin receptor association ($\alpha5\beta1$), a serial activation of kinase activates that contributes to the internalization is needed. The binding of virus or virus particle induce Ca+2 response inside the host cell that lead cellular response. The integrin α5-subunit and β1-subunit provide a docking site for various kinases, such as β-subunit Focal Adhesion Kinase (FAK)and α-subunit Talin Adapter Proteins[109]. The tyrosine kinase FAK plays a vital role as a key mediator of the integrin signaling event controller. RGD motif of spike protein (S-Protein) interaction to integrin stimulates FAK tyrosine phosphorylation result's in FAK signaling activate, meanwhile at time stimulated FAK promotes phospholipase C-γ (PLC-γ) activities that directly participate in the generation and catalyzed of inositol triphosphate 3 (IP3) and Diacylglycerol (DAG).Thus, the PLC-γ activates coronin, found as an actinbinding protein within cytosol and thus further stimulation of PLC-γ these proteins proceed IP3 and this diffuse towards Endoplasmic Reticulum (E.R.) and binds to Inositol Triphosphate-3 Receptor (IP3R), present at E.R. this results in immobilization of Ca^{+2} into the cytosol[110,111].

Phagocytosis is a complex mechanism by interconnected and cross-activation of intracellular proteins. Moreover, in this complex mechanism, cytosolic proteins of the host also play a role in virus engulf, one of the best proteins talin, which a ubiquitous cytosolic protein that docks the 5-subunit of integrin and acts as a substrate for the Ca+2 activated protease, called calpain. Thus, an increase in Ca+2 concentration in host cells leads to re-armament or deform the actin filaments by binding on actin that provide an intact binding between actin filaments and help into the invasion of the virus into lung cells(Figure 2). So, this fact can be possible; the concentration of Ca+2 into lung cells also increases the binding and entry of SARS-CoV-2 inside the cell, and that plays a vital role in the pathogenicity of the virus. Nonetheless, if the concentration of Ca+2 ions in lung cells reduces, this may be a step towards reducing the degree of coronavirus infection.

PREVENTION AND CONTROL OF THE DISEASE

Protective experimental immunizations in dromedaries have already started using a modified vaccinia virus Ankara (MVA) vaccine expressing the MERS-CoV spike protein[112]. Preliminary data showed a significant reduction in excretion of infectious virus and viral RNA in small numbers of vaccinated and challenged dromedaries compared to controls. Protection is correlated with the presence of serum neutralizing antibodies againstMERS-CoV.

In spite of frequent reports of nosocomial infection of MERS-CoV, human-to-human transmission is not sustainable and MERS is considered to be a zoonotic disease. To date, WHO has declared that the overall transmission patterns of MERS remain unchanged, i.e. multiple introductions from animals to humans and secondary transmission in healthcare settings (WHO, 2017). Therefore, identification of the zoonotic sources of MERS-CoV might guide control strategies at the human–animal interface to stop future human infection. If the spillover process of MERS-CoV from animals to humans could be stopped, we may be able to put an end to further nosocomial outbreaks in the Middle East and beyond. Available serological studies have indicated that the seropositivity of MERS-CoV neutralizing antibodies is much lower in juvenile than in adult camels, suggesting that MERS-CoV infection in camels may target young animals[113-116]. In agreement with the serological findings, the detection rate of MERS-CoV RNA in the nasal and/or rectal swabs of juvenile camels was higher than in those of adult camels [117]. In addition, a recent study found that MERS-CoV mainly targeted camels of less than 4 years of age, particularly calves, and the infection in juvenile camels manifested as an acute, epidemic and time-limited infection. Thus, delaying the social separation of calves or avoiding contact with camels aged less than 4 years might be a simple but effective measure to reduce spillover of MERS-CoV from camels to humans[118]. Although there is no evidence of sustained human-tohuman transmission of MERS-CoV, nosocomial infection may sometimes lead to MERS outbreaks. The MERS outbreak in the Republic of Korea, the largest MERS outbreak ever recorded outside of Saudi Arabia, was a result of nosocomial transmission: a single exported case

with a travel history in the Middle East resulted in 185 laboratory-confirmed human infections in Korea and one in China, with 36 deaths (WHO, 2015). The outbreak pattern in Korea was similar to the hospital outbreaks that occurred in the Middle East, which were attributed to failures of infection prevention and control in healthcare settings[119].

Enhancing infection prevention and control awareness and implementation measures is critical to preventing the possible spread of MERS-CoV in health-care facilities. It is not always possible to identify patients with MERS-CoV early because some have mild or non-specific symptoms. For this reason, it is important that all health-care facilities establish and implement clear triage policies for rapid screening and assessment of potential MERS-CoV cases and all cases with acute respiratory symptoms. It is also important for health-care workers to apply standard precautions consistently with all patients, regardless of their diagnosis, in all work practices all of the time. Droplet precautions should be added to the standard precautions when providing care to any patient with symptoms of acute respiratory infection (WHO, 2017)[118-120].

Health-care facilities that provide care for patients suspected of or confirmed to be infected with MERS-CoV should take appropriate measures to decrease the risk of transmission of the virus from an infected patient to other patients, health-care facility workers (medical and service personnel) and visitors[121-125]. These measures involve interventions at the patient-carer interface and other general measures such as linen management, cleaning and disinfection and waste management. Contact precautions and eye protection should be added when caring for probable or confirmed cases of MERS-CoV infection and airborne precautions should be applied when performing aerosol-generating procedures. Hospital cleaning staff should also be informed of and trained to take proper precautions when cleaning rooms of MERS-CoV patients (WHO, 2017).

MVA-specific antibodies that cross-neutralize camelpox virus are another very important advantage of this vaccine since outbreaks of camelpox still occur in dromedaries. Another approach would be to add a MERS-CoV component to the already existing attenuated camelpox vaccine Ducapox. Since Ducapox has been used in the Middle East for many years, the acceptance of such a vaccine can be anticipated[126-128]. However, it is important for the success of a vaccine to adhere strictly to the exact time of vaccination since the window of the disappearance of maternal antibodies and appearance of antibodies as a result of infection is narrow. Therefore, early diagnosis, prompt isolation of suspected cases and timely contact tracing of case contacts are key strategies to prevent nosocomial transmission.

CONCLUSION

Collaboration between human and animal health sectors in affected countries is essential to understanding the risk of transmission of MERS-CoV between animals and humans, whether there is any seasonal variation in the circulation of the virus in animals and the natural reservoir(s) of MERS-CoV. Given limited knowledge of mechanisms of MERS-CoV transmission, current precautions to prevent zoonotic transmission, such as recommendations to avoid consumption of raw camel milk and meat, are prudent despite the lack of epidemiologic evidence linking these exposures to MERS-CoVinfec-tion. Such precautionary recommendations, while appropriate in the context of limited knowledge, should not be interpreted as evidence of an epidemiologic association with MERS-CoV transmission. It is important to work towards limiting the spread of infection in animal populations (through development of vaccines and better management of infected animals/herds) so as to reduce the opportunity for further human exposure. In addition, a better understanding of transmission in health-care settings, especially the exposures that result in human-to-human transmission, the potential role of asymptomatic infected health-care workers and the possible role of environmental contamination, is urgently needed.

Continuous epidemiologic and virologic monitoring is required to determine other exposures resulting in transmission and to assess for the possibility of improved virus fitness and adaptation. The disease is widely distributed in high camel population rearing areas in the world indicating the need for action via planned vaccination. Until additional evidence is available to further refine recommendations to prevent MERS-CoV transmission, continued use of existing precautionary recommendations is necessary.

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