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Cross host transmission in the emergence of MERS coronavirus

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Coronaviruses (CoVs) able to infect humans emerge through cross-host transmission from animals. There is substantial evidence that the recent Middle East respiratory syndrome (MERS)-CoV outbreak is fueled by zoonotic transmission from dromedary camels. This is largely based on the fact that closely related viruses have been isolated from this but not any other animal species. Given the widespread geographical distribution of dromedaries found seropositive for MERS-CoV, continued transmission may likely occur in the future. Therefore, a further understanding of the cross host transmission of MERS-CoV is needed to limit the risks this virus poses to man.

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Introduction

Coronaviruses are positive strand RNA viruses that cause disease in humans and animals. Through cross-host transmission events these viruses may substantially broaden their host range. Animal-to-human zoonotic transmissions led to worldwide spread of viruses such as hCoV-OC43 and NL63, that mainly cause common cold like symptoms in humans. The recent zoonotic introduction of two more pathogenic viruses, severe acute respiratory syndrome (SARS) CoV and Middle East respiratory syndrome (MERS)-CoV, on the other hand revealed the damage these viruses can do to human society. Evidence that SARS-CoV emerged through zoonotic transmission came from genetic analyses of virus isolates from infected humans and palm civets during the SARS epidemic [1]. The virus underwent rapid adaptation in both hosts, primarily in the receptor binding domain of the viral spike protein, to allow more efficient infection of human cells. Subsequent surveillance studies identified at least 60 novel bat coronaviruses worldwide, including ancestors of SARS-CoV. Now there is substantial evidence that Chinese horseshoe bats are natural reservoirs of SARS-CoV like viruses [2,3]. These viruses were isolated from bat fecal samples and they use the same host viral receptor, angiotensin converting enzyme 2 (ACE2), from humans, civets and Chinese horseshoe bats to enter cells [4••]. Recent studies further document the capacity of this virus to jump the species barrier [5]. Currently, twelve years after the outbreak several potential scenarios are revealed that might explain the emergence of SARS-CoV. Although adaptation of the virus that led to a pathogenic variant may have primarily taken place in the civet cat, it may also have occurred first in humans and spread subsequently to civet cats. Alternatively, recombination events between viruses present in bats may occur relatively frequently, leading to variants in bats that are already pathogenic for humans [5]. In this review we summarize the current evidence found so far for cross host transmission of MERS-CoV, a virus that was identified three years ago.

MERS-CoV like viruses in bats

Shortly after the isolation and characterization of MERS-CoV from a patient in Saudi Arabia in 2012, it was realized that also in this case bats could potentially serve as a host for this virus. Different from SARS, only few isolated cases were seen early on in 2012, suggesting a spillover directly from an animal reservoir, potentially bats. Phylogenetic analysis of the replicase gene of coronaviruses indeed showed that MERS-CoV is most closely related to Tylonycteris bat coronavirus HKU4 and Pipistrellus bat coronavirus HKU5, which prototype two species in lineage C of the genus Betacoronavirus [6]. In addition, MERS-CoV could be grown in bat cells [7]. This is highly remarkable, as most other prototype CoVs, including human, bovine, feline, or porcine CoVs, are normally unable to do so. Follow up studies revealed that MERS-CoV uses a phylogenetically conserved cell surface amino peptidase, dipeptidyl peptidase 4 (DPP4), also known as CD26, as a functional receptor [8••]. This multifunctional protein plays a major role in glucose metabolism by its degradation of incretins and is also implicated in T-cell activation, chemotaxis modulation, cell adhesion, and apoptosis. Consistent with the capacity of MERS-CoV to replicate in bat cells, expression of human and bat (*Pipistrellus pipistrellus*) DPP4 in non-susceptible

human COS-7 cells enabled infection by MERS-CoV [8[dot][dot]]. Other bat CoVs also appear to utilize this receptor to enter cells. The spike protein of CoV HKU4 binds to DPP4, however, it does not mediate viral entry into human cells because of the inability to become activated by human endogenous proteases [9,10]. In fact, two residue differences between the two spikes account for their different capabilities to mediate viral entry into human cells [11], revealing a potential adaptation of MERS-CoV to become more pathogenic in humans. Although these studies provide clues about the origin MERS-CoV, to date no close relative has been identified in bats that is capable of using DPP4. Studies in Saudi Arabia to investigate the presence of MERS-CoV in bats were unable to recover additional sequences beyond a 190-nt fragment from the conserved polymerase gene in a single specimen [12]. The subsequent characterization of an African bat virus closely related to MERS-CoV showed that this bat virus roots the phylogenetic tree of MERS-CoV [13,14]. However, spike gene sequences obtained from bat CoVs characterized thus far are still phylogenetically distantly related to MERS-CoV. Theoretically the initial host switch from bats may have taken place in Africa. In the absence of hard evidence that bats act as the reservoir of current MERS-CoV zoonotic transmissions, subsequent studies focused on other animal species. Phylogenetic analysis of DPP4 indicated that human, macaque, horse, and rabbit DPP4 cluster together with bovine and porcine DPP4, which are somewhat more distantly related (Figure 1). Small animals, including ferret and mice, are more divergent with respect to the DPP4 virus binding region [15], which has consequences for MERS-CoV binding and capacity to infect cells [16]. In fact the presence of a glycosylation site in the virus binding domain of DPP4 prohibits MERS-CoV infection of these small animal species [17]. On the other hand several studies demonstrated the potential of MERS-CoV to infect cells of different species *in vitro* [16,18]. Further experimental evidence is needed to prove their *in vivo* susceptibility to MERS-CoV. Besides receptor functionality, the distribution of the DPP4, presence of enzymes able to cleave the spike protein and cause fusion are potentially critical in the host range of MERS-CoV. Innate and specific host responses may negatively affect the outcome of the infection resulting in the inability of the virus to spread efficiently.

## Dromedaries as source for zoonotic transmission

Some of the earliest observations suggested contact of MERS patients with livestock, including dromedary camels and goats [19–22]. Data provided by the Food and Agriculture Organization of the United Nations (FAO) for the period 2012–2014 show that cows, dromedary camels, goats and sheep are the main sources of meat and milk in the MERS-CoV affected countries [23]. Through numerous serologic studies in these animals in the Arabian Peninsula it became clear that the genetic

diversity in MERS-CoV in people could only be explained by multiple independent, geographically structured, zoonotic events [20,24]. Sheep, goats, cattle, dromedary camels, horses and poultry have been analyzed for the presence of MERS-CoV specific antibodies [25–30] but serologic evidence for MERS-CoV infection has only been found in dromedaries.

In August 2013, dromedaries were implicated for the first time as possible source for human infection based on the presence of MERS-CoV neutralizing antibodies in dromedaries from Spain (Canary Islands) and Oman [31[dot][dot]]. Since then, MERS-CoV specific antibodies have been detected in camels across the Middle East and the African continent and in sera taken up to 30 years ago, suggesting a geographically widespread and longstanding enzootic circulation in camels. Levels of seropositivity observed in adult dromedaries in Asia and Africa was generally >80% with exception of the Canary islands and Tunisia were seropositivity rates of 14% and 54% respectively were reported [25,27–30,31[dot][dot],32[dot][dot],33,34,35[dot],36[dot],37[dot],38,39[dot],40,41]. A recent study suggested that seropositivity at the Canary Islands is solely due to exposure of camels >20 years ago in Africa [42], however a different study described the presence of MERS-CoV neutralizing antibodies in a 2-year old camel that was born on the Canary Islands [31[dot][dot]].

Subsequently, MERS-CoV genomes were detected in nasal swabs of dromedaries in ARE, Egypt, Iran, Israel, KSA, Kuwait, Oman, Pakistan and Qatar [29,32[dot][dot],38,39[dot],40,41,43–48,49[dot],50[dot]], and the virus was isolated from dromedaries in ARE, Egypt, KSA and Qatar [38,40,51,52[dot]]. Phylogenetic analysis of MERS-CoV genomes shows that viruses isolated from humans and dromedary camels are highly similar (Figure 2). Besides nasal swabs, MERS-CoV RNA has been found in oral, bronchial and anal swabs, milk and feces as well as in lymph nodes, turbinate, olfactory epithelium, larynx, pharynx, trachea and lung lobs [46,50[dot],53[dot],54]. Sampling of the nasal cavity seems to be the best option for sampling live camels [46]. In one study in which lung tissue with lesions was sampled in a slaughterhouse in KSA, MERS-CoV RNA was detected in 61.5% of the samples [50[dot]]. The percentage positive nasal swabs varied from 1.6% to 59% in various studies but direct comparison of shedding rates is difficult as they will be dependent on the number of animals sampled, their age distribution, sampling season, origin of the camels and connectivity with other camels from different origins. However the majority of studies show that calves (≤2 year) with primary infections will most likely play a bigger role in MERS-CoV epidemiology than older animals for which less frequent shedding is observed and who demonstrated higher rates of seroconversion [26,29,34,38,40,50[dot]]. A higher rate of virus isolation was observed for calves than subadults (2–4 years of age) implying an increased infectivity of animals <2 years [40]. Infectious virus seems to be excreted for only a

Figure 1
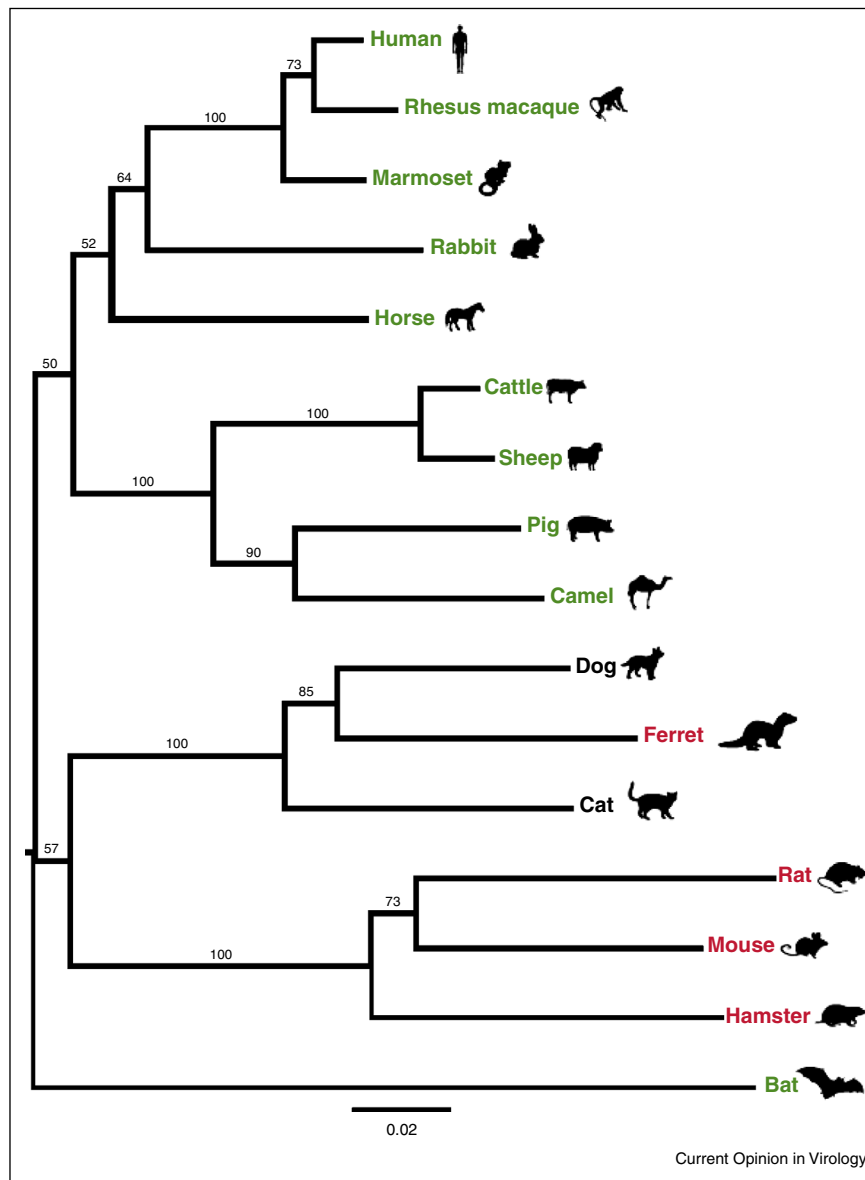
Phylogenetic tree based on a DPP4 fragment containing the S1 binding region (residues 246–504 of 16 different species). Sequence alignment was performed by using ClustalW in the MEGA5.0 software package (www.megasoftware.net), and the trees were constructed by using the neighbor-joining method with P distances (gap/missing data treatment; complete deletion) and 1000 bootstrap replicates as in MEGA version 5.0. Known MERS-CoV susceptible species (green), non-susceptible species (red) and unknown (black).

limited period of time (up to 7 days) in the field and under experimental conditions while in the latter situation excretion of viral RNA could be detected for up to 35 days [40,53•]. Mild disease of the upper respiratory tract or no overt disease has been observed in young and adult camels naturally infected with MERS-CoV [38,39•,48,49•,50•]. Experimental infections indicated that MERS-CoV in adult dromedaries gives a mild respiratory disease with purulent nasal discharge [53•]. A study in mother–calf pairs in Dubai showed that MERS-CoV causes acute infections in dromedaries and it was concluded that persistent infection in adult animals is unlikely [40]. The detection of MERS-CoV RNA in camels with pre-existing antibodies in a herd in KSA and an observed lack of correlation between viral RNA loads and levels of neutralizing antibodies in camels for slaughter in Qatar might indicate limited immune-protection and a potential for re-infection despite previous exposure [38,46] Indeed, circulation up to 2–4 months of the same virus strains at the herd level has been demonstrated [40,46].

Figure 2
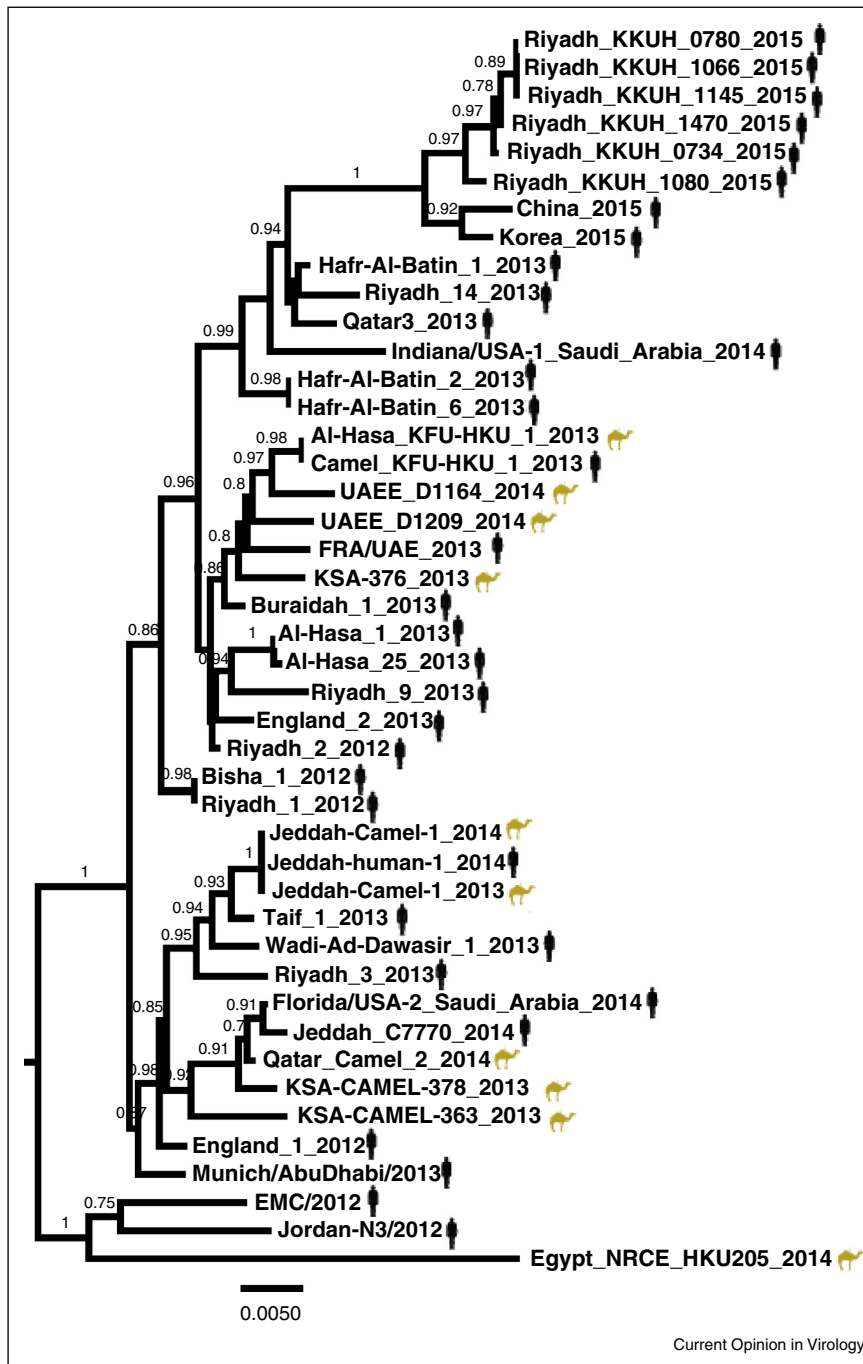
Phylogenetic analysis of Middle East respiratory syndrome coronaviruses (MERSCoVs). Complete genome sequences of human and camel MERS-CoV isolates were aligned by using ClustalW, and a phylogenetic tree was constructed by using the PhyML method in Seaview 4 (all 3 software packages can be found at http://pbil.univ-lyon1.fr/software/seaview) and was visualized in FigTree version 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/). Values at branches show the result of the approximate likelihood ratio; values <0.70 are not shown. MERS-CoV isolates from dromedary camels are shown in camel silhouette and the human MERS-CoV isolates are shown in human silhouette. Scale bar indicates nucleotide substitutions per site.

Camel-to-human transmission of MERS-CoV

Accumulating evidence shows that dromedary camels are a reservoir for human infections. Analysis of an outbreak associated with a barn in Qatar found both dromedaries and humans to be infected with nearly identical strains of MERS-CoV. Although this was the first temporal association between camel and human infection, it could not be concluded whether the people on the farm were infected

by the camels or *vice versa*, or if a third (wildlife) source was responsible [32••]. Further support for camels as a reservoir came from a nation-wide study in KSA that found widespread circulation of different genetic variants of MERS- CoV in camels, with geographic clustering of human and camel MERS-CoV sequences [29]. A case study with concurrent dromedary and human infection on a barn in KSA found evidence for one-directional camel-to-human transmission; while the dromedaries showed declining RNA viral loads and increasing antibody titers, the owner was hospitalized with acute MERS [48,49•,55]. A more recent study of risk factors for primary MERS-CoV illness in humans indicated that direct exposure to dromedary camels during the 2 weeks before disease onset was associated with MERS-COV illness [56••]. In addition, several studies suggest that persons in regular contact with camels have higher seropositivity rates than persons not in contact with camels. An overall seroprevalence of 0.15% was observed in a cross-sectional study in >10 000 persons in KSA while 3.6% of 140 slaughterhouse workers were seropositive [57••]. In a study in Qatar, MERS-CoV antibodies were detected in healthy persons with daily, occupational contact with dromedaries but not in persons without such contact [58••]. Four out of five dedicated camel slaughterers were seropositive at a location with massive exposure potential as 59% of the camels for slaughter was positive for MERS-CoV RNA at two time points [46,58••]. Other studies did not yield serologic evidence of infection in persons with occupational exposure to dromedaries [49•,59–61]. However, only one of those studies documented actual MERS-CoV circulation in dromedaries in one herd during contact with a small group of 7 persons [59].

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 [62,63]. Dromedary camels are ubiquitously present in the Middle East and have been part of desert cultures for centuries with cultural and economic importance [62]. Camel milk and meat, in particular meat from the hump, are commonly consumed while some consider raw organ meet a delicacy [62]. 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 [46]. However, experimental infection of three camels did not show any evidence for the presence of infectious MERS-CoV in organs and meat [53•]. 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 was shedding viral RNA in nasal excretions at the time of slaughter while viral RNA was found in 15% of the fecal swabs [36•]. 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 [63]. 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 initiated the letting of the milk, or hands of the milker [54]. Analysis of camel milk inoculated with MERS-CoV showed that MERS-CoV is stable in milk stored at 22 °C and 4 °C for respectively 48 and 72 h while pasteurization destroyed viral viability [64]. Consumption of raw camel milk has been linked to several human MERS cases in KSA and Qatar [49•]. 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.

Conclusions

There is very strong evidence that dromedary camels are a reservoir for human infections. Although camels are suspected to be the primary source of infection for humans and MERS-CoV genomes from humans and dromedary camels have been shown to be highly similar, routes of direct or indirect zoonotic transmission are yet unknown. Therefore, detailed case control studies are needed to unravel the exact routes of transmission. By doing so preventive measures can be taken to reduce the zoonotic transmission of MERS-CoV. In addition, efforts to block or reduce virus excretion from MERS-CoV infected dromedary by vaccination in combination with the development of therapeutics to treat MERS patients need to be further evaluated. The use of the evolutionarily conserved DPP4 protein as a viral receptor suggests that not only dromedary camels but also other animal species may be potential targets of this virus. Further experimental studies in different animal species and serosurveillance studies in livestock animals should continue to monitor the spread of MERS-CoV in the Middle East but also in other areas such as Africa and Asia.

Acknowledgments

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest
•• of outstanding interest

1. Song HD, Tu CC, Zhang GW, Wang SY, Zheng K, Lei LC, Chen QX, Gao YW, Zhou HQ, Xiang H *et al.*: **Cross-host evolution of severe**

acute respiratory syndrome coronavirus in palm civet and human. *Proc Natl Acad Sci U S A* 2005, **102**:2430-2435.

2. Lau SK, Woo PC, Li KS, Huang Y, Tsoi HW, Wong BH, Wong SS, Leung SY, Chan KH, Yuen KY: **Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats**. *Proc Natl Acad Sci U S A* 2005, **102**:14040-14045.

3. Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H *et al.*: **Bats are natural reservoirs of SARS-like coronaviruses**. *Science* 2005, **310**:676-679.

4. Ge XY, Li JL, Yang XL, Chmura AA, Zhu G, Epstein JH, Mazet JK,
•• Hu B, Zhang W, Peng C *et al.*: **Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor**. *Nature* 2013, **503**:535-538.
First isolation of a coronavirus from bats.

5. Menachery VD, Yount BL Jr, Debbink K, Agnihothram S, Gralinski LE, Plante JA, Graham RL, Scobey T, Ge XY, Donaldson EF *et al.*: **A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence**. *Nat Med* 2015 http://dx.doi.org/10.1038/nm.3985.

6. van Boheemen S, de Graaf M, Lauber C, Bestebroer TM, Raj VS, Zaki AM, Osterhaus AD, Haagmans BL, Gorbalenya AE, Snijder EJ, Fouchier RA: **Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans**. *MBio* 2012:3. pii:e00473-12.

7. Müller MA, Raj VS, Muth D, Meyer B, Kallies S, Smits SL, Wollny R, Bestebroer TM, Specht S, Suliman T *et al.*: **Human coronavirus EMC does not require the SARS-coronavirus receptor and maintains broad replicative capability in mammalian cell lines**. *MBio* 2012:3. pii: e00515-12.

8. Raj VS, Mou H, Smits SL, Dekkers DH, Müller MA, Dijkman R,
•• Muth D, Demmers JA, Zaki A, Fouchier RA *et al.*: **Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC**. *Nature* 2013, **495**:251-254.
Identification of the receptor MERS-CoV uses to infect cells.

9. Wang Q, Qi J, Yuan Y, Xuan Y, Han P, Wan Y, Ji W, Li Y, Wu Y, Wang J *et al.*: **Bat origins of MERS-CoV supported by bat coronavirus HKU4 usage of human receptor CD26**. *Cell Host Microbe* 2014, **16**:328-337.

10. Yang Y, Du L, Liu C, Wang L, Ma C, Tang J, Baric RS, Jiang S, Li F: **Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus**. *Proc Natl Acad Sci U S A* 2014, **111**: 12516-12521.

11. Yang Y, Liu C, Du L, Jiang S, Shi Z, Baric RS, Li F: **Two mutations were critical for bat-to-human transmission of Middle East respiratory syndrome coronavirus**. *J Virol* 2015, **89**:9119-9123.

12. Memish ZA, Mishra N, Olival KJ, Fagbo SF, Kapoor V, Epstein JH, Alhakeem R, Durosinloun A, Al Asmari M, Islam A *et al.*: **Middle East respiratory syndrome coronavirus in bats, Saudi Arabia**. *Emerg Infect Dis* 2013, **19**:1819-1823.

13. Ithete NL, Stoffberg S, Corman VM, Cottontail VM, Richards LR, Schoeman MC, Drosten C, Drexler JF, Preiser W: **Close relative of human Middle East respiratory syndrome coronavirus in bat, South Africa**. *Emerg Infect Dis* 2013, **19**: 1697-1699.

14. Corman VM, Ithete NL, Richards LR, Schoeman MC, Preiser W, Drosten C, Drexler JF: **Rooting the phylogenetic tree of middle East respiratory syndrome coronavirus by characterization of a conspecific virus from an African bat**. *J Virol* 2014, **88**:11297-11303.

15. Raj VS, Smits SL, Provacia LB, van den Brand JM, Wiersma L, Ouwendijk WJ, Bestebroer TM, Spronken MI, van Amerongen G, Rottier PJ *et al.*: **Adenosine deaminase acts as a natural antagonist for dipeptidyl peptidase 4-mediated entry of the Middle East respiratory syndrome coronavirus**. *J Virol* 2014, **88**:1834-1838.

16. van Doremalen N, Miazgowicz KL, Milne-Price S, Bushmaker T, Robertson S, Scott D, Kinne J, McLellan JS, Zhu J, Munster VJ: **Host species restriction of Middle East respiratory syndrome coronavirus through its receptor, dipeptidyl peptidase 4**. *J Virol* 2014, **88**:9220-9232.

17. Peck KM, Cockrell AS, Yount BL, Scobey T, Baric RS, Heise MT: **Glycosylation of mouse DPP4 plays a role in inhibiting Middle East respiratory syndrome coronavirus infection**. *J Virol* 2015, **89**:4696-4699.

18. Eckerle I, Corman VM, Müller MA, Lenk M, Ulrich RG, Drosten C: **Replicative capacity of MERS coronavirus in livestock cell lines**. *Emerg Infect Dis* 2014, **20**:276-279.

19. Albarrak AM, Stephens GM, Hewson R, Memish ZA: **Recovery from severe novel coronavirus infection**. *Saudi Med J* 2012, **33**:1265-1269.

20. Drosten C, Seilmaier M, Corman VM, Hartmann W, Scheible G, Sack S, Guggemos W, Kallies R, Muth D, Junglen S *et al.*: **Clinical features and virological analysis of a case of Middle East respiratory syndrome coronavirus infection**. *Lancet Infect Dis* 2013, **13**:745-751.

21. Buchholz U, Muller MA, Nitsche A, Sanewski A, Wevering N, Bauer-Balci T, Bonin F, Drosten C, Schweiger B, Wolff T *et al.*: **Contact investigation of a case of human novel coronavirus infection treated in a German hospital, October–November 2012**. *Euro Surveill* 2013, **18**:20406.

22. Reuss A, Litterst A, Drosten C, Seilmaier M, Bohmer M, Graf P, Gold H, Wendtner CM, Zanuzdana A, Schaade L *et al.*: **Contact investigation for imported case of Middle East respiratory syndrome, Germany**. *Emerg Infect Dis* 2014, **20**:620-625.

23. FAO FAOSTAT 2012–2014. 2015 [cited 2015 0827]; Available from: http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QA/E.

24. Cotten M, Watson SJ, Kellam P, Al-Rabeeah AA, Makhdoom HQ, Assiri A, Al-Tawfiq JA, Alhakeem RF, Madani H, AlRabiah FA *et al.*: **Transmission and evolution of the Middle East respiratory syndrome coronavirus in Saudi Arabia: a descriptive genomic study**. *Lancet* 2013, **382**:1993-2002.

25. Reusken CB, Ababneh M, Raj VS, Meyer B, Eljarah A, Abutarbush S, Godeke GJ, Bestebroer TM, Zutt I, Muller MA *et al.*: **Middle East Respiratory Syndrome coronavirus (MERS-CoV) serology in major livestock species in an affected region in Jordan, June to September 2013**. *Euro Surveill* 2013, **18**:20662.

26. Hemida MG, Perera RA, Wang P, Alhammadi MA, Siu LY, Li M, Poon LL, Saif L, Alnaeem A, Peiris M: **Middle East Respiratory Syndrome (MERS) coronavirus seroprevalence in domestic livestock in Saudi Arabia, 2010 to 2013**. *Euro Surveill* 2013, **18**:20659.

27. Perera RA, Wang P, Gomaa MR, El-Shesheny R, Kandeil A, Bagato O, Siu LY, Shehata MM, Kayed AS, Moatasim Y *et al.*: **Seroepidemiology for MERS coronavirus using microneutralisation and pseudoparticle virus neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt June 2013**. *Euro Surveill* 2013, **18**:20574.

28. Alexandersen S, Kobinger GP, Soule G, Wernery U: **Middle East respiratory syndrome coronavirus antibody reactors among camels in Dubai, United Arab Emirates, in 2005**. *Transbound Emerg Dis* 2014, **61**:105-108.

29. Alagaili AN, Briese T, Mishra N, Kapoor V, Sameroff SC, de Wit E, Munster VJ, Hensley LE, Zalmout IS, Kapoor A *et al.*: **Middle East respiratory syndrome coronavirus infection in dromedary camels in saudi arabia**. *MBio* 2014, **5** e00884-14.

30. Meyer B, Garcia-Bocanegra I, Wernery U, Wernery R, Sieberg A, Muller MA, Drexler JF, Drosten C, Eckerle I: **Serologic assessment of possibility for MERS-CoV infection in equids**. *Emerg Infect Dis* 2015, **21**:181-182.

31. Reusken CB, Haagmans BL, Muller MA, Gutierrez C, Godeke GJ,
•• Meyer B, Muth D, Raj VS, Smits-De Vries L, Corman VM *et al.*: **Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study**. *Lancet Infect Dis* 2013, **13**:859-866.
First report describing the presence of antibodies against MERS-CoV in dromedary camels.

32. Haagmans BL, Al Dhahiry SH, Reusken CB, Raj VS, Galiano M,
•• Myers R, Godeke GJ, Jonges M, Farag E, Diab A *et al.*: **Middle
East respiratory syndrome coronavirus in dromedary camels:
an outbreak investigation**. *Lancet Infect Dis* 2014, **14**:140-145.
Study describing similar MERS-CoVs in dromedary camels and humans.

33. Meyer B, Muller MA, Corman VM, Reusken CB, Ritz D, Godeke GJ,
Lattwein E, Kallies S, Siemens A, van Beek J *et al.*: **Antibodies
against MERS Coronavirus in Dromedary Camels, United Arab
Emirates, 2003 and 2013**. *Emerg Infect Dis* 2014, **20**:552-559.

34. Reusken CBEM, Messadi L, Feyisa A, Ularamu H, Godeke GJ,
Danmarwa A, Dawo F, Jemli M, Shamaki SM, Woma DY *et al.*:
**Geographic distribution of MERS coronavirus among
dromedary camels, Africa**. *Emerg Infect Dis* 2014, **20**:1370-1374.

35. Corman VM, Jores J, Meyer B, Younan M, Liljander A, Said M,
• Gluecks I, Lattwein E, Drexler BJB, Bornstein JFS *et al.*: **MERS
coronavirus anibodies in dromedary camels from Kenya,
1992–2013**. *Emerg Infect Dis* 2014, **20**:1319-1322.
Serological study in dromedary camels providing evidence that these
animals had antibodies to MERS-CoV already long time back ago.

36. Muller MA, Corman VM, Jores J, Meyer B, Younan M, Liljander A,
• Bosch BJ, Lattwein E, Hilali M, Musa BE *et al.*: **MERS coronavirus
neutralizing antibodies in camels, Eastern Africa, 1983–1997**.
Emerg Infect Dis 2014, **20**:2093-2095.
See annotation to Ref. 35••.

37. Hemida MG, Perera RA, AlJassim RA, Kayali G, Siu LY, Wang P,
• Chu K, Perlman S, Alnaeem MAAA *et al.*: **Seroepidemiology of
Middle East respiratory syndrome (MERS) coronavirus in
Saudi Arabia (1993) and Australia (2014) and characterisation
of assay specificity**. *Euro Surveill* 2014, **19**:20828.
Serological study in dromedary camels providing evidence that in Aus-
tralia these animals are negative for antibodies to MERS-CoV.

38. Hemida MG, Chu DKW, Poon LLM, Perera RAPM, Alhammadi MA,
Ng H, Siu LY, Guan Y, Alnaeem A, Peiris M: **MERS coronavirus in
dromedary camel herd, Saudi Arabia**. *Emerg Infect Dis* 2014,
20:1231-1234.

39. Chu DKW, Poon LLM, Gomaa MM, Shehata MM, Perera RAPM,
• Zeid DA, El Rifay AS, Siu LY, Guan Y, Webby JR *et al.*: **MERS
coronaviruses in dromedary camels, Egypt**. *Emerg Infect Dis*
2014, **20**:1049-1053.
Identification of MERS-CoV in dromedary camels.

40. Wernery U, Corman VM, Wong EY, Tsang AK, Muth D, Lau SK,
Khazanehdari K, Zirkel F, Ali M, Nagy P *et al.*: **Acute middle East
respiratory syndrome coronavirus infection in livestock
Dromedaries, Dubai, 2014**. *Emerg Infect Dis* 2015, **21**:1019-1022.

41. Yusof MF, Eltahir YM, Serhan WS, Hashem FM, Elsayed EA,
Marzoug BA, Abdelazim AS, Bensalah OK, Al Muhairi SS:
**Prevalence of Middle East respiratory syndrome coronavirus
(MERS-CoV) in dromedary camels in Abu Dhabi Emirate,
United Arab Emirates**. *Virus Genes* 2015, **50**:509-513.

42. Gutiérrez c, Tejedor-Junco M, González M, Lattwein E,
Renneker S: **Presence of antibodies but no evidence for
circulation of MERS-CoV in dromedaries on the Canary
Islands, 2015**. *Eurosurveillance* 2015, **20**:30019.

43. OIE, WAHID. Weekly disease information 2014. 2014 [cited 2015
0830]; Available from: http://www.oie.int/wahis_2/public/wahid.php/
Reviewreport/Review?page_refer=MapFullEventReport&reportid=
16411.

44. ProMED-mail. MERS-COV-Eastern Mediterranean (79): Qatar
(OIE), Kuwait (suspected) animal reservoir, camel, Archive
Number: 20140611.2533756. 2014 [cited 2015 0830]; Available
from: http://www.promedmail.org/direct.php?id=2533756.

45. Nowotny N, Kolodziejek J: **Middle East respiratory syndrome
coronavirus (MERS-CoV) in dromedary camels, Oman, 2013**.
Euro Surveill 2014, **19**:20781.

46. Farag E, Reusken C, Haagmans B, Mohran K, Raj VS, Pas S,
Voermans J, Smits SL, Godeke GJ, Alhajri MM *et al.*: **High
proportion of MERS-CoV shedding dromedaries at
slaughterhouse with potential epidemiological link to human
cases, Qatar 2014**. *Infect Ecol Epidemiol* 2015, **5**:28305.

47. Smits SL, Raj VS, Pas SD, Reusken C, Mohran KA, Farag E, Al-
Romaihi HE, AlHajri MM, Haagmans B, Koopmans M: **Reliable

typing of MERS-CoV variants with a small genome fragment**.
J Clin Virol 2015, **64**:83-87.

48. Azhar E, El-Kafrawy S, Farraj S, Hassan A, Al-Saeed M, Hashem A,
Madani T: **Evidence for camel-to-human transmission of MERS
coronavirus**. *N Engl J Med* 2014, **370**:2499-2505.

49. Memish Z, Cotten M, Meyer B, Watson S, Alsahafi A, Al
• Rabeeah A, Corman V, Sieberg A, Makhdoom H, Assiri A *et al.*:
**Human infection with MERS coronavirus after exposure to
infected camels, Saudi Arabia, 2013**. *Emerg Infect Dis* 2014,
20:1012-1015.
Evidence for dromeadry camel to human transmission of MERS-CoV.

50. Khalafalla AI, Lu X, Al-Mubarak AI, Dalab AH, Al-Busadah KA,
• Erdman DD: **MERS-CoV in upper respiratory tract and lungs of
dromedary camels, Saudi Arabia, 2013–2014**. *Emerg Infect Dis*
2015, **21**:1153-1158.
Study describing the presence of MERS-CoV in the upper respiratory
tract of naturally infected dromedary camels.

51. Raj VS, Farag E, Reusken CB, Lamers MM, Pas SD, Voermans J,
Smits SL, Osterhaus AD, Al-Malawi N, Al Romaihi HE *et al.*:
**Isolation of MERS coronavirus from a dromedary camel, Qatar
2014**. *Emerg Infect Dis* 2014, **20**:1339-1342.

52. Chan RW, Hemida MG, Kayali G, Chu DK, Poon LL, Alnaeem A,
• Ali MA, Tao KP, Ng HY, Chan MC *et al.*: **Tropism and replication
of Middle East respiratory syndrome coronavirus from
dromedary camels in the human respiratory tract: an in-vitro
and ex-vivo study**. *Lancet Respir Med* 2014, **2**:813-822.
Evidence that MERS-CoV isolated from dromedary camels replicates in
human respiratory tissues *ex vivo*.

53. Adney DR, van Doremalen N, Brown VR, Bushmaker T, Scott D, de
• Wit E, Bowen RA, Munster VJ: **Replication and shedding of
MERS-CoV in upper respiratory tract of inoculated dromedary
camels**. *Emerg Infect Dis* 2014, **20**:1999-2005.
Study describing the presence of MERS-CoV in the upper respiratory
tract of experimentally infected dromedary camels.

54. Reusken CB, Farag E, Jonges M, Godeke GJ, El-Sayed AM,
Pas SD, Raj VS, Mohran KA, Moussa HA, Ghobashy H *et al.*:
**Middle East respiratory syndrome coronavirus (MERSCoV)
RNA and neutralising antibodies in milk collected according to
local customs from dromedary camels,Qatar, April 2014**. *Euro
Surveill* 2014, **19**:20829.

55. Drosten C, Kellam P, Memish ZA: **Evidence for camel-to-human
transmission of MERS coronavirus**. *N Engl J Med* 2014,
371:1359-1360.

56. Alraddadi BM, Watson JT, Almarashi A, Abedi GR, Turkistani A,
•• Sadran M, Housa A, Almazroa MA, Alraihan N, Banjar A *et al.*: **Risk
factors for primary Middle East respiratory syndrome
Coronavirus illness in humans, Saudi Arabia, 2014**. *Emerg
Infect Dis* 2016, **22**:49-55.
First study analysing risk factors for primary MERS-CoV infection provid-
ing evidence for the important role of camel contact in the transmsssion of
the virus.

57. Müller MA, Meyer B, Corman VM, Al-Masri M, Turkestani A, Ritz D,
•• Sieberg A, Aldabbagh S, Bosch BJ, Lattwein E *et al.*: **Presence of
Middle East respiratory syndrome coronavirus antibodies in
Saudi Arabia: a nationwide, cross-sectional, serological
study**. *Lancet Infect Dis* 2015, **15**:629.
Detection of antibodies to MERS-CoV in humans with camel contact.

58. Reusken CB, Farag E, Haagmans B, Mohran K, Godeke GJ,
•• Raj VS, Alhajri F, Al-Marri SA, Al Romaihi HE, Al-Thani M *et al.*:
**Occupational exposure to dromedaries and risk for MERS-
CoV infection, 2013–2014**. *Emerg Infect Dis* 2015, **21**:1422-1425.
Detection of antibodies to MERS-CoV in humans with camel contact.

59. Hemida MG, Al-Naeem A, Perera RA, Chin AW, Poon LL, Peiris M:
**Lack of middle East respiratory syndrome coronavirus
transmission from infected camels**. *Emerg Infect Dis* 2015,
21:699-701.

60. Memish ZA, Alsahly A, Masri MA, Heil GL, Anderson BD, Peiris M,
Khan SU, Gray GC: **Sparse evidence of MERS-CoV infection
among animal workers living in Southern Saudi Arabia during
2012**. *Influenza Other Respir Viruses* 2014, **9**:64-67.

61. Aburizaiza AS, Mattes FM, Azhar EI, Hassan AM, Memish ZA,
Muth D, Meyer B, Lattwein E, Muller MA, Drosten C:

Investigation of anti-middle East respiratory syndrome antibodies in blood donors and slaughterhouse workers in Jeddah and Makkah, Saudi Arabia, fall 2012. *J Infect Dis* 2014, **209**:243-246.

62. Gossner C, Danielson N, Gervelmeyer A, Berthe F, Faye B, Kaasik Aaslav K, Adlhoch C, Zeller H, Penttinen P, Coulombier D: Human–dromedary camel interactions and the risk of acquiring zoonotic Middle East respiratory syndrome coronavirus infection. *Zoonoses Public Health* 2016, **63**:1-9.

63. Shamsia S: Nutritional and therapeutic properties of camel and human milks. *Int J Genet Mol Biol* 2009, **1**:52-58.

64. van Doremalen N, Bushmaker T, Karesh WB, Munster VJ: Stability of Middle East Respiratoy syndrome coronavirus in milk. *Emerg Infect Dis* 2014, **20**:1263-1264.