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detected in the saliva of humans with hemorrhagic CHIKV infection and in a macaque CHIKV hemorrhagic model [4]. The requirement for hemorrhagic disease is likely due to virus entering the oral cavity via gingival bleeding, and a similar pathway to infection is also plausible. It will be important to determine the relationship between hemorrhagic CHIKV infections and compromised immunity in saliva-based transmission.

Gardner *et al.* [4] suggested the finding may not be broadly applicable to humans because transmission was observed only in highly immunodeficient mice and not in wild-type mice. Nonetheless, the study is potentially of great significance, particularly in the context of severe hemorrhagic disease and immunodeficiency. Many countries most affected by CHIKV have high numbers of immunocompromised patients, particularly due to HIV infection, but also due to other infections, other morbidities, intrinsic immunodeficiency, or even extreme age. In addition to increased susceptibility to CHIKV infection, many of these patients likely have oral lesions, which could also promote transmission and infection via saliva. The impact within this patient population could be highly significant. CHIKV infections can spread extremely rapidly through communities and we wonder whether transmission via saliva can contribute to this rapid spread. Further studies are required to rigorously test for CHIKV transmission via saliva, but this preliminary study suggests that we need to seriously consider this mode of transmission.

Numerous viruses are spread orally, most notably Epstein–Barr virus (EBV) and cytomegalovirus (CMV). EBV is highly infectious via the oral route, which is reflected in the common name given to EBV infections – the ‘kissing disease’. In addition to kissing, other high risk activities for EBV transmission include sharing toothbrushes and eating utensils with an infected individual. The risk of transmission by saliva is particularly high among children and close family.

HIV and hepatitis B and C viruses are typically transmitted by blood or sexual contact but have also been detected in saliva. Hepatitis B transmission can occur through saliva [5], but there are no established reports of transmission via saliva for HIV or hepatitis C [6]. Ebola virus is present in the saliva of infected individuals and although it seems likely that transmission via saliva does occur, the significance of this route of transmission is unclear, particularly as the virus is inactivated rapidly in saliva [7]. A number of viruses can be transmitted through open sores in the mouth, with saliva as the vehicle for transmission. Of course, many respiratory viruses can also be transmitted by saliva. Thus, the significance of viruses in saliva for transmission varies greatly from virus to virus, from highly transmissible to not at all.

There are few examples of non-vector based arbovirus transmission under natural conditions, with most cases being by intranasal or aerosol route of infection [8]. To our knowledge, this is the first report of likely arbovirus transmission via saliva. This mode of transmission has not been considered to date in models assessing CHIKV spread. Although it is unlikely that measuring CHIKV in saliva will have broad diagnostic value, its presence in the saliva may have prognostic value, particularly in relation to disease severity. The possibility of aerosol transmission also needs to be considered. It is intriguing to consider the possibility that CHIKV in the saliva could be related to the neurological complications reported in a minority of CHIKV cases [2]: could salivary CHIKV lead to central nervous system (CNS) infections via olfactory neurons, as has been described for a number of other viruses [9]? More study is required to assess the significance of CHIKV in the saliva, particularly in immunocompromised patients. This needs urgent attention as it will have a major influence on efforts to counter the spread of this emerging virus. The possibility of saliva-mediated transmission of other arboviruses also needs to be reassessed.

¹Institute for Glycomics, Griffith University, Queensland, Australia

*Correspondence: s.mahalingam@griffith.edu.au (S. Mahalingam).

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Spotlight Deciphering MERS-CoV Evolution in Dromedary Camels

Lin Du¹ and Guan-Zhu Han^{1,*}

The emergence of the Middle East respiratory syndrome coronavirus (MERS-CoV) poses a potential threat to global public health. Many aspects of the evolution and transmission of MERS-CoV in its animal reservoir remain unclear. A recent study provides new insights into the evolution and transmission of MERS-CoV in dromedary camels.

The initial case of the Middle East respiratory syndrome coronavirus (MERS-CoV) was reported in 2012. Since then,

MERS-CoV has been thought to represent a potential threat to global public health. As of December 22, 2015, a total of 1621 MERS cases from 26 countries have been reported, resulting in 584 (36.0%) deaths (<http://www.who.int/emergencies/mers-cov/en/>). Accumulating evidence suggests that dromedary camels serve as an intermediate reservoir for human MERS-CoV infections. However, much remains unclear about the prevalence and evolution of MERS-CoV in dromedary camels, as well as the routes of MERS-CoV transmission. A recent study in *Science* by Sabir *et al.* [1] reports an extensive surveillance for MERS-CoV in dromedary camels and provides new insights into the evolution and transmission of MERS-CoV.

Coronaviruses (CoVs) are classified into four genera, that is, *Alphacoronavirus*, *Betacoronavirus* (further into four clades A–D), *Gammacoronavirus*, and *Deltacoronavirus*. MERS-CoV belongs to the clade C of *Betacoronavirus*. Sabir *et al.* [1] provide a snapshot of the CoV diversity in dromedary camels. They found that 25.3% of camels were positive for CoVs and identified three different CoV species, including MERS-CoV, β 1-HKU23-CoV (*Betacoronavirus* clade A), and camelid α -CoV (*Alphacoronavirus*). The co-infection of MERS-CoV and camelid α -CoV appears to be frequent. The high prevalence of these CoVs (12.1% and 19.8% for MERS-CoV and camelid α -CoV, respectively) indicates that they are enzootic in dromedary camels.

Sabir *et al.* [1] found at least five lineages of MERS-CoV in Saudi Arabian camels, though there might be some problems in defining viral lineages (some of the viral lineages they defined are not phylogenetically monophyletic). This result suggests multiple lineages have been co-circulating, a prerequisite for recombination to occur. As with other positive-sense RNA viruses, recombination did occur among MERS-CoV strains. Sabir *et al.* [1] identified at least six recombination events, indicating recombination is frequent in MERS-CoV.

Most surprisingly, one lineage (designated lineage 5 by Sabir *et al.*), which is associated with the recent Riyadh outbreak and the human infections in South Korea and China, has a recombinant origin. This recombinant lineage originated between December 2013 and June 2014, but has quickly become predominant in Saudi Arabian camels since November 2014. It raises a potential link between recombination and increased pathogenicity, which merits further investigation.

Phylogenetic approaches have been extensively used as a tool for tracing the origin, evolution, and epidemiology of MERS-CoV. Because different genome regions of recombinants have different evolutionary histories, ignoring recombination could seriously compromise phylogenetic analysis [2]. Therefore, recombination analysis should be performed before phylogenetic analysis of MERS-CoV

There are four possible routes of MERS-CoV transmission in humans and camels, namely camel-to-human, human-to-human, camel-to-camel, and human-to-camel (Figure 1). The former three routes have been well accepted [3,4]. In the Sabir *et al.* study [1], the high prevalence again suggests that MERS-CoV is enzootic in dromedary camels and could be transmitted among camels. However, the human-to-camel transmission is theoretically possible but largely ignored [5], because of the obsession of animals as sources of human viruses. Recent studies reveal influenza A virus can be transmitted from human to a

range of animal species (especially swine), a phenomenon known as reverse zoonosis [6]. For another CoV, severe acute respiratory syndrome-associated coronavirus (SARS-CoV), reverse zoonosis has been reported in cats and pigs [7]. When inspecting Sabir *et al.*'s phylogenetic trees of MERS-CoV (Figures 1, S2, and S3 in [1]), we found many viruses of camel origin fell within the diversity of human viruses. There are two possible explanations for this phylogenetic pattern: (i) It is artifact due to sampling bias, if many camel viral strains are not sampled. If this is the case, we must miss substantial diversity of MERS-CoV and should expand our surveillance in camels to fully capture the MERS-CoV diversity. (ii) MERS-CoV could be transmitted from humans to camels, which means that humans serve as a source of MERS-CoV for camels. The role of camels in generating and maintaining MERS-CoV diversity has been repeatedly emphasized [1]. If human-to-camel transmission occurs, it requires us to refine our concept of the definition of a 'breeding ground' for MERS-CoV, which has important practical implications for developing vaccination strategies and control measures.

Both MERS-CoV and SARS-CoV belong to the *Betacoronavirus* genus, but they belong to different clades, and it will be interesting to compare the transmission pattern between SARS-CoV and MERS-CoV. Phylogenetic analysis of SARS-CoV indicates two independent transmission events occurred from animals to humans

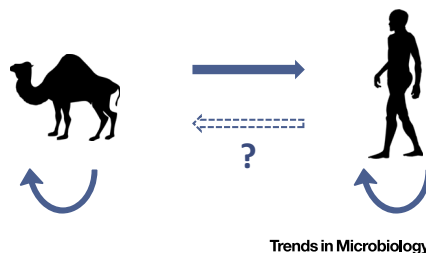


Figure 1. Four Possible Routes for MERS-CoV Transmission. The well accepted human-to-human, human-to-camel, and camel-to-camel are labeled in solid arrows. The possible and ignored human-to-camel transmission is labeled in a dashed arrow. The camel and human images courtesy of Steven Traver and T. Michael Keeseey.

[8]. In contrast, the phylogenetic tree of MERS-CoV from Sabir *et al.* [1] suggests tens of independent transmission events (Figures 1, S2, and S3 in [1]). It follows that the barrier of human-animal transmission is relatively lower for MERS-CoV. It is of great importance to understand the mechanisms underlying the difference of transmission pattern between SARS-CoV and MERS-CoV, with the implications for assessing the emergence risk of other CoVs.

Although the study of Sabir *et al.* provides important insights into the evolution and transmission of MERS-CoV in dromedary camels, several outstanding questions arise: (i) What is the evolutionary and pathogenic significance of recombination in MERS-CoV? Comparing the pathogenicity of parental and recombinant strains might help resolve this question. (ii) Does human-to-camel transmission occur in MERS-CoV? The phylogenetic analysis of Sabir *et al.* suggests this possibility. Also, there are some other clues for this type of transmission [5], but rigorous experimental evidence is still needed. (iii) Are there other intermediate hosts

for MERS-CoV? To date, other than camels, sheep, cows, and chickens have been detected for MERS-CoV infections. But none of them show serological evidence of MERS-CoV infection [9]. Nevertheless, more animal species should be sampled. (iv) What are the mechanisms underlying the difference of interspecies transmission between SARS-CoV and MERS-CoV? Both ecological and molecular mechanisms [e.g., the usage of different receptors, angiotensin converting enzyme 2 (ACE2) for SARS-CoV and dipeptidyl peptidase 4 (DPP4) for MERS-CoV] might contribute to this difference.

In summary, Sabir *et al.*'s study reveals the extensive diversity of MERS-CoV in dromedary camels. Further studies and surveillance, as conducted by Sabir *et al.* [1], will help improve our understanding of the evolution, ecology, and human-animal interface of MERS-CoV.

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¹Jiangsu Key Laboratory for Microbes and Functional Genomics, Jiangsu Engineering and Technology Research Center for Microbiology, College of Life Sciences, Nanjing Normal University, Nanjing, Jiangsu 210046, China

*Correspondence: guanzhu@email.arizona.edu (G.Z. Han). <http://dx.doi.org/10.1016/j.tim.2015.12.013>

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