DOI : 10.5958/2277-8934.2022.00014.5 *Vol 29 No 2, p 105-110*

MERS-COV IN DROMEDARY CAMELS: SEQUENCE-BASED COMPARISON OF ANTIGENICITY AND PATHOGENICITY OF STRUCTURAL AND NON-STRUCTURAL PROTEINS

Sabry M. El-Bahr[1,2], Mahmoud Kandeel[1,3], I.F. Albokhadaim[1], M. Al-Nazawi[1] and Hafiz Ahmed[4]

[1]Department of Biomedical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa 31982, P.O. 400, Saudi Arabia

[2]Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Alexandria 21523, Egypt

[3]Department of Pharmacology, Faculty of Veterinary Medicine, Kafr Elsheikh University, Egypt

[4]GlycoMentra Inc., 1450 South Rolling Road, Baltimore, MD 21227, USA

ABSTRACT

The goal of this researcher was to map the camel MERS-CoV structural and non-structural proteins and track changes in antigenicity and pathogenicity from 2013 to 2018. This is critical for developing effective diagnostic and control methods. Therefore, the sequences of MERS-CoV structural proteins such as Spike (S), Membrane (M), Nucleocapsid (N) and Envelope (E) and nonstructural proteins, comprising polyprotein-ab (polyab) and Open reading frame-3 (ORF3) were retrieved. The amino acid sequences of each of these proteins were analysed to estimate their antigenic and pathogenic properties from 2013 to 2018. The antigenicity profiles showed variations in antigenicity minimum, maximum, range, and average from 2013 to 2018. MERS-CoV proteins' maximum antigenicity score was declining in the examined time frame. In 2013, the maximum score was 0.86, and by 2018, it had dropped to 0.6. This resulted in a greater range of antigenicity with the onset of MERS-CoV in 2013, with a range of 0.56, which eventually decreased to 0.22 in 2018. The net effect on the mean value of antigenicity score revealed that MERS-CoV antigenicity decreased gradually. Between 2013 and 2018, the mean antigenicity score of structural and non-structural proteins decreased. Non-structural proteins, on the other hand, had the greatest alterations, with a mean value of 0.52 in 2013 and 0.42 in 2018, representing a loss of 19.2 per cent of their antigenicity score. In contrast, the structural protein showed a mean value of 0.55 in 2013 and 0.53 in 2018, i.e. lost only 3.6% of its antigenicity score. Given that ORF3 was nonantigenic, therefore, all changes in antigenicity changes were attributed to polyab protein. Pathogenicity score has decreased from 0.81 in 2013 to 0.78 in 2018. Further studies are required to map these changes on the virus-host cell interaction level.

Key words: Antigenicity, camels, MERS-CoV, pathogenicity, proteins

MERS-CoV was first identified in Saudi Arabia in 2012 in a patient with severe pneumonia (Zaki *et al,* 2012). Initially the virus was termed as "human coronavirus Erasmus Medical Centre (EMC) or EMC-CoV". This novel coronavirus later was termed as Middle East respiratory syndrome coronavirus (MERS-CoV) according to the announcement of the Coronavirus Study Group of the International Committee on Taxonomy of Viruses (ICTV) (de Groot *et al*, 2013). The MERS-genome CoV is 30 kb (30,119nt) long and encodes four structural proteins (Spike, Envelope, Membrane, and Nucleocapsid) as well as 16 nonstructural proteins (van Boheemen *et al,* 2012).

MERS-CoV infection has a 35.4 per cent fatality rate, and new cases and deaths have continued till the end of 2018 (WHO, 2018). According to current research, many coronaviruses that are phylogenetically similar to MERS-CoV, such as BatCoV-HKU4, BatCoV-HKU5, and other MERS-related coronaviruses, are considered to have originated in bats (Woo *et al,* 2012; van Boheemen *et al,* 2012; Corman *et al,* 2014; Anthony *et al,* 2017). The BatCoV-HKU4 has also been discovered to be capable of activating the MERS-CoV cellular receptor, bolstering the bat origin theory (Wang *et al,* 2014). There is, however, no direct evidence that MERS-CoV may be isolated from bats (Xu *et al,* 2019). Because camel isolates are nearly identical to human isolates and many domestic camels are MERS-CoV seropositive, dromedary camels are thought to be the animal reservoir for MERS-CoV (Xu

SEND REPRINT REQUEST TO SABRY MOHAMED EL-BAHR email: selbahar@kfu.edu.sa; sabryelbahr@hotmail.com

et al, 2019). Camels in the Middle East and northern Africa contain antibodies to MERS-CoV or a virus that is quite similar to it, according to serological evidence dating back to the 1980s (Alagaili *et al,* 2014; Corman *et al,* 2014; Müller *et al,* 2014). These findings are not applicable to Bactrian camels or dromedaries from other countries, such as Australia and Kazakhstan (Hemida *et al,* 2014a). While these findings support the theory that camels can transmit illnesses to humans, initial MERS cases may not have had any interaction with camels. Furthermore, because the MERS-CoV receptor, dipeptidyl peptidase 4, is highly conserved in mammals, other mammalian hosts may be involved (van Doremalen *et al,* 2017). In this study, the changes in MERS-CoV antigenicity and pathogenicity were be evaluated from 2013 to 2018. The results were further analysed in the light of structural and non-structural protein components, as well as, the specific proteins used in the analysis.

Materials and Methods

Retrieval of input protein data and analytical programs

The sequences of the input proteins of interest (polyab, ORF3, S, M, N, and E) were retrieved from the GenBank at NCBI.

Prediction of the antigenic properties of MERS-CoV proteins

The amino acid sequences of proteins of interest (polyab, ORF3, S, M, N, and E) were utilised for antigenic property analysis, and the VaxiJen v2.0 server (Doytchinova and Flower, 2007) with a threshold of 0.4 was employed (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). A summary of antigenicity statistics for MERS-CoV 2013-2018 is presented in Table 1. The antigenicity score for each of the proteins of interest: polyab, S, M, N, and E was recorded. The algorithm was used to analyse the antigenic property after inserting a threshold value of 0.4.

Prediction of pathogenic and antigenic properties

Using the protein sequences of each chosen MERS-CoV isolate, we used the MP3 program (http://metagenomics.iiserb.ac.in/mp3/application.php) with default settings to estimate pathogenicity scores (Gupta *et al,* 2014). The hybrid findings (SVM+HMM) were taken into account, and the SVM scores were employed in the analysis. The pathogenic score for each of the proteins of interest (polyab, ORF3, S, M, N, and E) was recorded (Table 2). For each isolate, the average scores of all these proteins were also computed.

Statistical analysis

All data handling and presentation were done by MS excel and GraphPad Prism software. The results were presented in Mean±SD or, in some cases, the mean + range is provided. Descriptive statistics were used to express changes in each isolate parameter.

Results and Discussion

MERS-CoV is an emerging infectious virus that is exceedingly dangerous to humans and is zoonotic in nature. Effective vaccination and diagnostic methods for this virus are still mostly unknown. Gaps in our understanding of these pathogens' protective immunity and antigenicity offer obstacles to the development of vaccines and diagnostics. Respiratory sickness caused by the MERS-CoV virus continues to grow for unnoticeable reasons. Since SARS-CoV-2 has emerged, it has become clear that effective therapies against extremely deadly human coronaviruses developed quickly. Despite this fact, the efforts to develop efficient vaccines and diagnostics against MERS-CoV are still in their primary stages. There has been a surge in R&D efforts to produce diagnostic, preventive, and therapeutic solutions for SARS-CoV-2, which has caused millions of affections. In contrast, MERS-CoV with more than 1,700 acetate cases of sickness and 600 deaths in 27 countries has less attention in developing vaccines and diagnostics.

Table 1. Summary of antigenicity statistics for MERS-CoV 2013-2018.

Year	2013	2014	2015	2016	2017	2018
Antigenicity scores (Mean ± SD)	0.5271 ± 0.1305	0.503 ± 0.1091	0.4891 ± 0.09007	0.4911 ± 0.08907	0.4941 ± 0.08299	0.4911 ± 0.07723

Table 2. Summary of pathogenicity statistics for MERS-CoV 2013-2018.

Year	2013	2014	2015	2016	2017	2018
Pathogenicity scores (Mean ± SD)	0.8115 ± 0.5868	0.7722 ± 0.6045	0.7227 ± 0.6039	0.7529 ± 0.5104	0.7775 ± 0.5217	0.776 ± 0.531

A summary of MERS-CoV antigenicity and its statistics during 2013-2018 is provided in Fig 1. The number of data entries each year ranged from 12-84 with the highest hits in 2014 followed by 2015. Surprisingly, the antigenicity profiles shoed variations in antigenicity minimum, maximum, range, and average from 2013 to 2018. MERS-CoV proteins' maximum antigenicity score decreased. In 2013, the maximum score was 0.86, and by 2018, it had dropped to 0.6. (Fig 2). This resulted in a greater range of antigenicity with the onset of MERS-CoV in 2013, with a range of 0.56, which eventually decreased to 0.22 in 2018. (Fig 1A, Fig 2). The net effect on the mean value of antigenicity score revealed that MERS-CoV antigenicity decreased gradually. In 2013, the antigenicity score was 0.5271 ± 0.1305 which decreased to 0.4911 ± 0.077 in 2018. As the initial data showed declining antigenicity of MERS-CoV, we further investigated the changes in antigenicity in the protein components of MERS-CoV (Fig 3). Both structural and non-structural proteins showed declining mean antigenicity scores from 2013-2018. However, the greater changes were with NS, which showed a mean value of 0.52 in 2013 and 0.42 in 2018, i.e. lost about 19.2% of its antigenicity score. In contrast, the structural protein

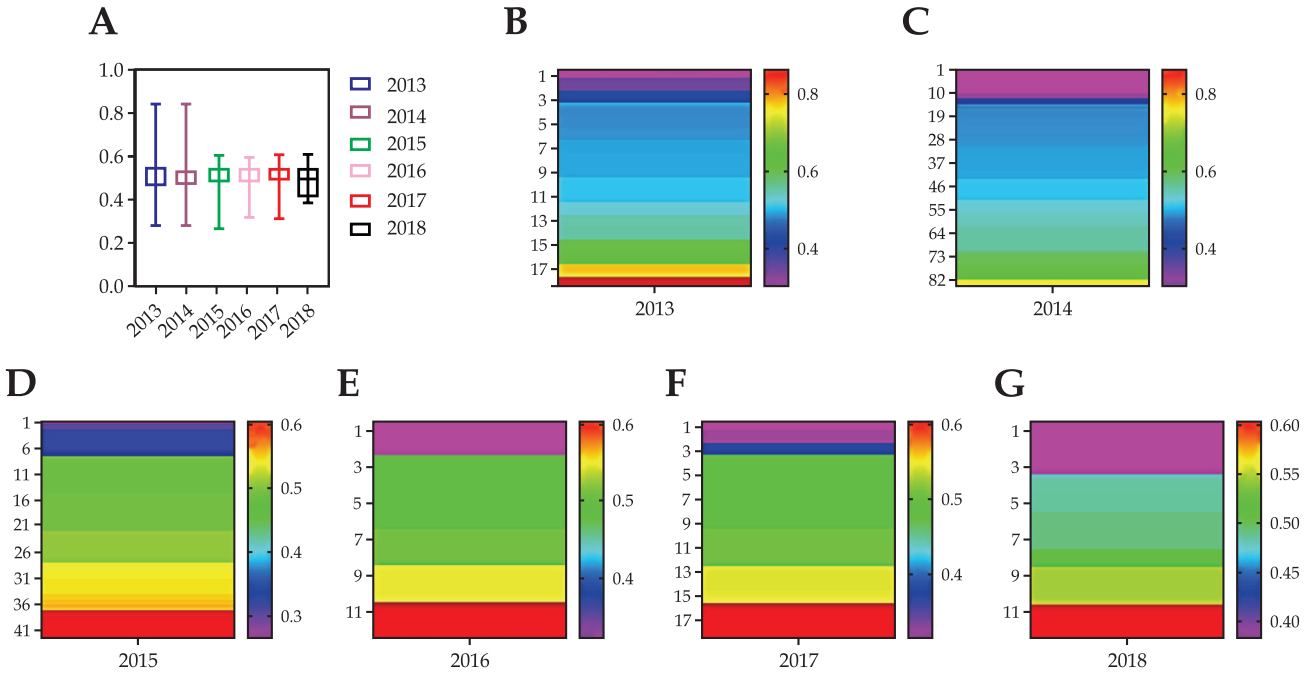

Fig 1. MERS-CoV antigenicity scores. (A) Box and whiskers plot showing the range of antigenicity with cross-line at mean values (B) Heat map representation of antigenicity score in the year 2013. A colour scale from red to pink (right panel) to imply the estimated antigenicity score. (C) The same plot as in B for 2014 (D) 2015 (E) 2016 (F) 2017 (G). 2018.

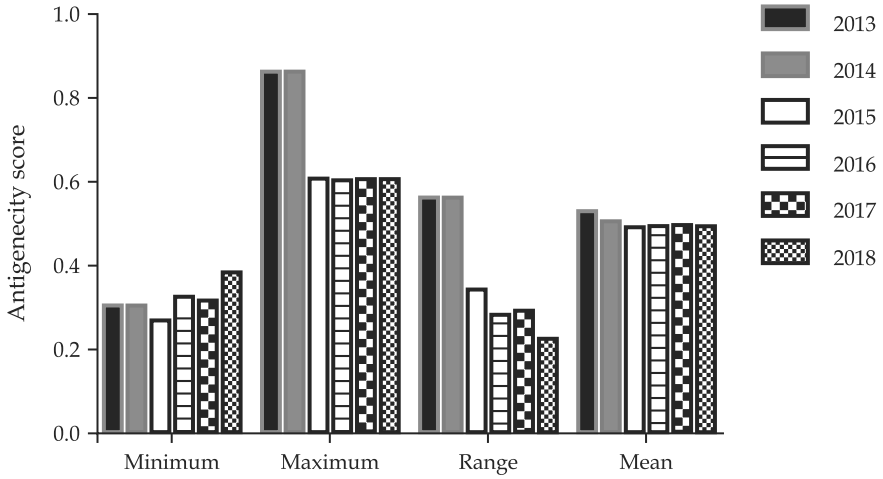

Fig 2. The mean and range for MERS-CoV 2013-2018.

showed a mean value of 0.55 in 2013 and 0.53 in 2018, i.e. lost only 3.6% of its antigenicity score. In this study, we used only two NS proteins in all procedures, polyab and ORF3. Given that ORF3 was nonantigenic, therefore, all changes in antigenicity were attributed to polyab. The analysis of MERS-CoV pathogenicity scores from 2013 to 2018 demonstrated a moderate decline. Pathogenicity score has decreased from 0.8115 ± 0.5868 in 2013 to 0.776 ± 0.531 in 2018. The range of pathogenicity of viral proteins was broad in the early years of MERS-CoV emergence, but it narrowed in subsequent years (Fig 4). The changes in the pathogenicity of MERS-CoV proteins were further analysed according to the protein components, comprising S, N, E, M and Polyab (Fig 5). Among these proteins, polyab and E proteins showed a gradual decrease in pathogenicity.

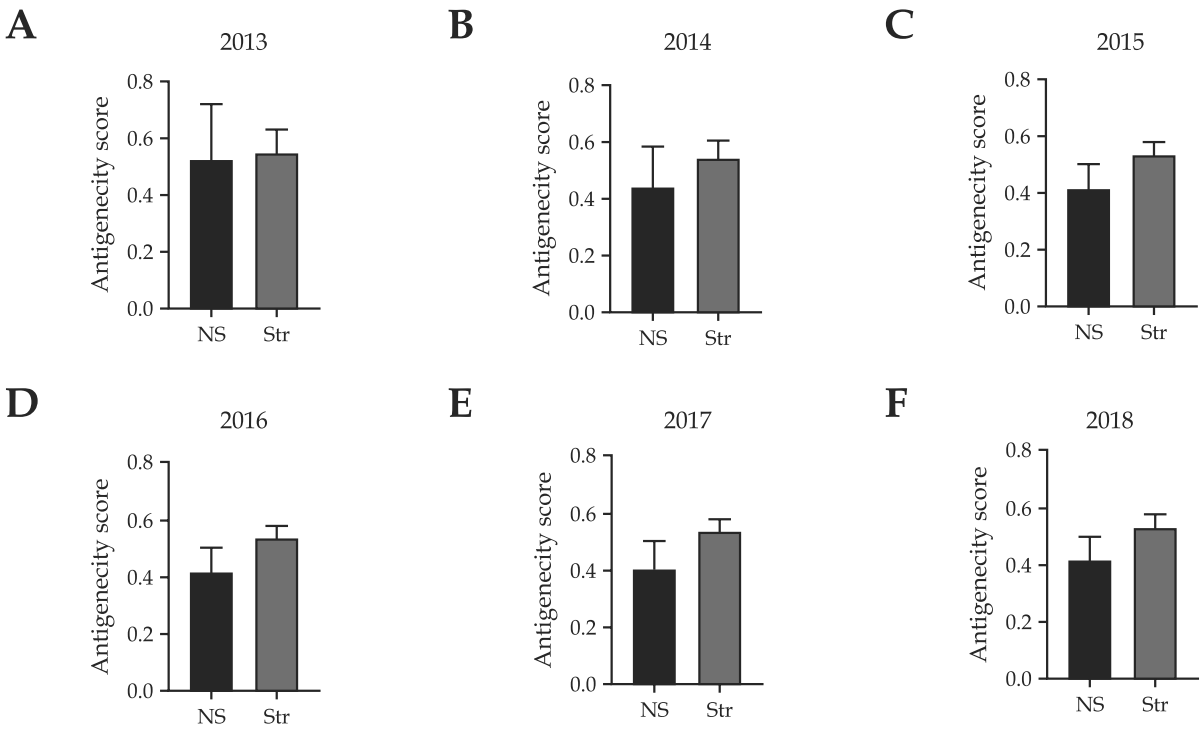

Fig 3. MERS-CoV nonstructural (NS) and structural (Str) proteins antigenicity scores. (A) Column bar graph showing the NS and Str proteins antigenicity scores during 2013 (B) 2014 (C) 2015 (D) 2016 (E) 2017 (F) 2018.

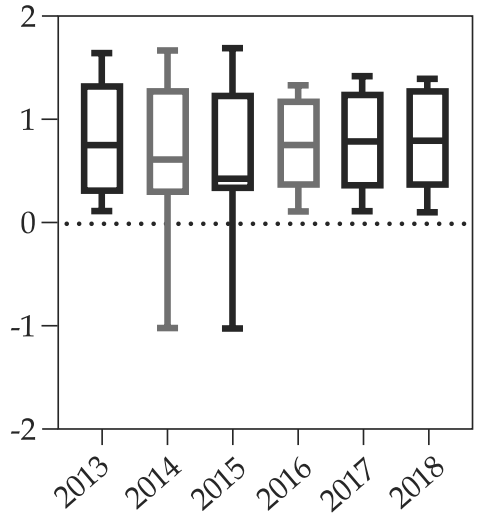

Fig 4. MERS-CoV pathogenicity scores. Box and whiskers plot showing the range of pathogenicity with cross-line at mean values.

In 2015, molecular diagnostic approaches and their global performances for the sensitive and specific detection of MERS-CoV RNA are well-illustrated in the first external quality evaluation MERS-CoV panel (Pas *et al,* 2015). MERS-CoV could be detected in all laboratories, but with varying degrees of sensitivity. The fact that 8% of labs reported false positives for MERS-CoV in a single assay demonstrates that there is potential for improvement and that confirmatory targets are important. This fact highlights the need for continued assessment of the accuracy of detection methods. In this work, we showed a decreasing antigenicity of MERS-CoV proteins. The impact of these changes on the accuracy of current diagnostics is to be elucidated.

Conflict of interest

The authors declare no conflict of interest.

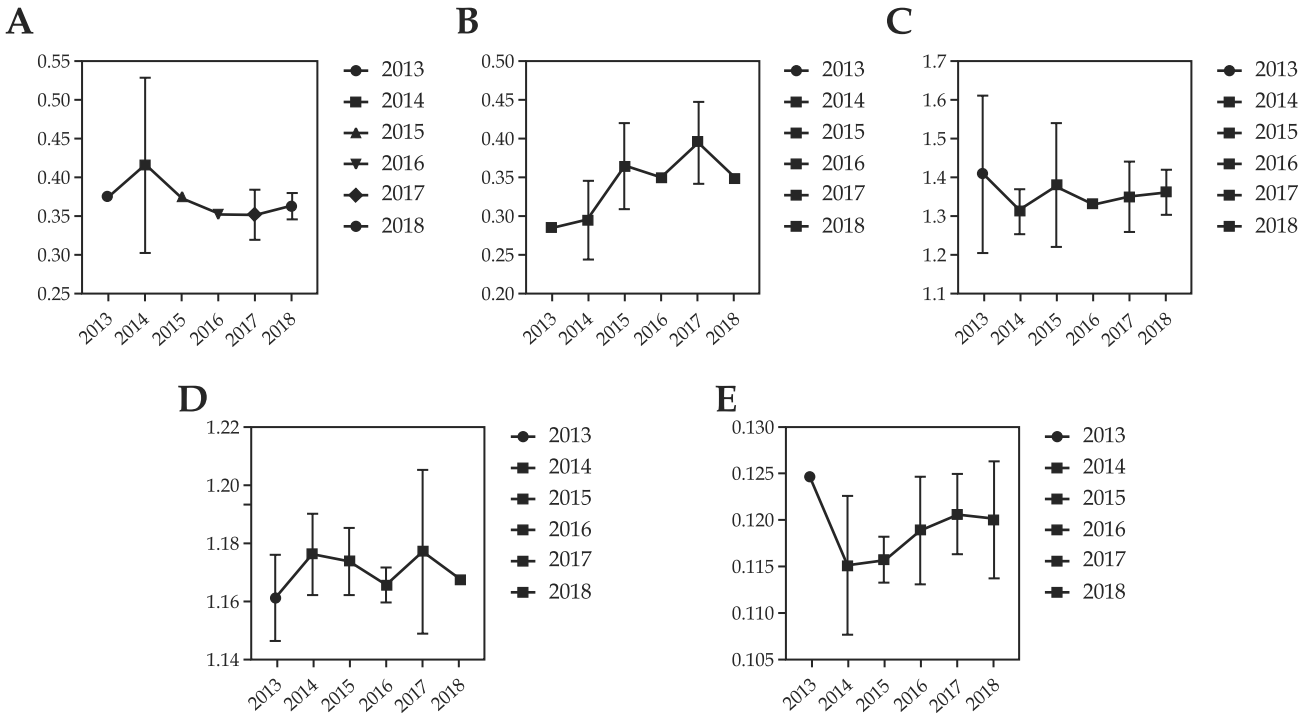

Fig 5. MERS-CoV proteins pathogenicity scores. (A) E protein (B) M protein (C) E protein (D) S protein (E) Polyab.

Acknowledgements

This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Project # 171002; Grant 271).

References

Alagaili AN, Briese T, Mishra N, Kapoor V, Sameroff SC, Burbelo PD, de Wit E, Munster VJ, Hensley LE, Zalmout IS, Kapoor A, Epstein JH, Karesh WB, Daszak P, Mohammed OB and Lipkin WI. Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. mBio. 2014; 5:884-814. DOI:10.1128/mBio.00884-14.

Anthony SJ, Gilardi K, Menachery VD, Goldstein T, Ssebide B, Mbabazi R, Navarrete-Macias I, Liang E, Wells H, Hicks A, Petrosov A, Byarugaba DK, Debbink K, Dinnon KH, Scobey T, Randell SH, Yount BL, Cranfield M, Johnson CK, Baric RS, Lipkin WI and Mazet JA. Further evidence for bats as the evolutionary source of Middle East respiratory syndrome coronavirus. mBio. 2017; 8(2). Doi:10.1128/mBio.00373-17.

Corman VM, Ithete NL, Richards LR, Schoeman MC, Preiser W, Drosten C and Drexler JF. Rooting the phylogenetic tree of middle east respiratory syndrome coronavirus by characterization of a conspecific virus from an African Bat. Journal of Virology. 2014; 88(19):11297–11303. Doi:10.1128/jvi.01498-14.

de Groot RJ, Baker SC, Baric RS, Brown CS, Drosten C, Enjuanes L, Fouchier RA, Galiano M, Gorbalenya AE, Memish ZA, Perlman S, Poon LL, Snijder EJ, Stephens GM, Woo PC, Zaki AM, Zambon M and Ziebuhr J. Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the coronavirus study group. Journal of Virology. 2013; 87(14):7790-7792. Doi:10.1128/jvi.01244-13.

Doytchinova IA and Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC bioinformatics. 2007; 8(1):1-7.

Gupta A, Kapil R, Dhakan DB and Sharma VK. MP3: a software tool for the prediction of pathogenic proteins in genomic and metagenomic data. PloS one. 2014; 9(4), e93907.

Hemida MG, Chu DK, Poon LL, Perera RA, Alhammadi MA, Ng HY, Siu LY, Guan Y, Alnaeem A, Peiris M. MERS coronavirus in dromedary camel herd, Saudi Arabia. Emerging Infectious Diseases. 2014; 20(7):1231-4. doi: 10.3201/eid2007.140571.

Müller MA, Corman VM, Jores J, Meyer B, Younan M, Liljander A, Bosch BJ, Lattwein E, Hilali M, Musa BE, Bornstein S and Drosten C. MERS coronavirus neutralizing antibodies in camels, Eastern Africa, 1983-1997. Emerging Infectious Diseases. 2014; 20:2093-2095. DOI:10.3201/eid2012.141026.

Pas SD, Patel P, Reusken C, Domingo C, Corman VM, Drosten C, Dijkman R, Thiel V, Nowotny N, Koopmans MP and Niedrig M. First international external quality assessment of molecular diagnostics for Mers-CoV. Journal of Clinical Virology. 2015; 69:81-85. Doi: 10.1016/j.jcv.2015.05.022.

van Boheemen S, de Graaf M, Lauber C, Bestebroer TM, Raj VS, Zaki AM, Osterhaus AD, Haagmans BL, Gorbalenya AE, Snijder EJ and Fouchier RA. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. mBio. 2012; 3(6):00473–12.

van Doremalen N, Hijazeen ZS, Holloway P, Al Omari B, McDowell C, Adney D, Talafha HA, Guitian J, Steel J, Amarin N, Tibbo M, Abu-Basha E, Al-Majali AM, Munster VJ and Richt JA. High Prevalence of Middle East Respiratory Coronavirus in Young Dromedary Camels in Jordan. Vector Borne Zoonotic Diseases. 2017; 17:155-159. DOI:10.1089/vbz.2016.2062.

Wang Q, Qi J, Yuan Y, Xuan Y, Han P, Wan Y, Ji W, Li Y, Wu Y, Wang J, Iwamoto A, Woo PC, Yuen KY, Yan J, Lu G and Gao GF. Bat origins of MERS-CoV supported by bat coronavirus HKU4 usage of human receptor CD26. Cell Host Microbe. 2014; 16(3):328-337. Doi:10.1016/j.chom.2014.08.009.

WHO. Middle East respiratory syndrome coronavirus (MERS-CoV) 2018 [cited 2019 Mar 26]. Available from: http://www.who.int/emergencies/mers-cov/en/

Woo PC, Lau SK, Li KS, Tsang AK and Yuen KY. Genetic relatedness of the novel human group C betacoronavirus to Tylonycteris bat coronavirus HKU4 and Pipistrellus bat coronavirus HKU5. Emerging Microbes and Infections. 2012; 1(11):e35. Doi: 10.1038/emi.2012.45.

Xu J, Jia W, Wang P, Zhang S, Shi X, Wang X and Zhang L. Antibodies and vaccines against Middle East respiratory syndrome coronavirus. Emerging Microbes and Infections. 2019; 8(1):841-856. Doi: 10.1080/22221751.2019.1624482.

Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD and Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. The New England Journal of Medicine. 2012; 367:1814-1820. DOI:10.1056/NEJMoa1211721.