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# Molecular detection of MERS-cor virus in the camels in different areas of Iraq

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## Abstract

Middle East Respiratory Syndrome (MERS) is a zoonotic viral disease that can be transmitted from dromedaries to human beings. Therefore this study was conducted to investigate the prevalence of MERS-cor virus in the camels during the period from October 2017 to December 2018. A total of 70 nasal swap samples were collected randomly from three different areas in Iraq, (male and female) with age ranged from 14 days to 2 years. Nasal swap samples were divided into identical triazole and transport media for viral RNA extraction and cultivation of the suspected cases for Mers-co virus isolation in embryonic tissue culture cells. The extracted RNA was tested by Nano drop to check RNA concentration and estimation of RNA purity. Cultivation of the samples in embrocated tissue culture cells showed visible CPE after 48 hr. Detection of the Mers-cor virus by real-time PCR showed that two males and 2 females from Najaf and Waist governorate were infected with the virus. In conclusion; Detection of Mers-co virus indicates the importance of the controlling its spread. The propagation and adaptation of the virus to grow and isolated in chicken embryo fibroblast cell culture as the first time work in Iraq is recommended instead of propagation of the virus cell line tissue culture which is not available and not applicable in Iraq.

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## Introduction

Middle East Respiratory Syndrome (MERS) is a zoonotic viral disease that can be transmitted from dromedaries to human beings. The causative virus was widely and quickly expanded to include more than 28 countries of Arabic Peninsula, Mediterranean region, North and West Africa, Asia including Korea and USA (Gardner *et al.,* 2016; Yaseen *et al.*, 2017). The majority of reported cases had been linked to exposure in KSA (WHO, 2018).

The virus was reported for the first time in 2012 after viral genome sequencing from sputum sample from a patient who die in Jeddah-KSA in 2012 (Zaki *et al.*, 2012). However, till the end of October 2018, the World Health Organization had announced that a total of 2266 cases of laboratory-confirmed MERS including 804 related deaths (fatality rate 35.5%) were reported globally.

It was well documented that MERS-CoV was widely prevalent in dromedary camels in the Middle East and some parts of Africa. Thus zoonotic transmission was likely originated from this animal species and is expected to continue for a long period of time in these regions supporting that KSA dromedary camel had significantly higher MERS-CoV carrier rates indicated an endemicity of the virus in the Arabian Peninsula predates the 2012 human MERS index case (Younan *et al.,* 2016; Wernery *et al.*, 2017).

The transmission of MERS-CoV between dromedary camels and humans mainly through respiratory droplets has been documented in several countries since MERS-CoV was found to be extensively replicated and shaded through upper respiratory tract (Adney *et al.*, 2014). It has been found that MERS-CoV isolated from dromedaries is genetically and phenotypically similar to that from humans (Omrani *et al.,* 2015; Mohd *et al.*, 2016; Hemida *et al.*, 2017). Other possible sources and vehicles of infection include food-borne transmission through consumption of unpasteurized camel milk and raw meat, medicinal use of camel urine (Gossner *et al.*, 2016). Although MERS has been associated with high fatality rate in human, MERS coronavirus (MERS-CoV) infection in dromedaries is usually asymptomatic and rarely cased mild respiratory signs (Okba *et al.*, 2017). The common entry receptor of MERCoV is dipeptidyl peptidase 4 (DPP4).

The emergence of MERS-CoV in humans from dromedary camels, and potentially earlier in time from bats, was facilitated human-to-human spread which was reported in hospital outbreaks and travelers returning from the Middle East and their close contacts particularly during Hajj and Umrah (Gossner *et al.,* 2016; Gautret *et al.*, 2016; Almaghrabi and Omrani, 2017; Hasan *et al.*, 2017 ).

As a result of the importance of the virus along with the few studies deal with this subject, this study was performed to investigate the prevalence of MERS-cor virus in the camels in different areas of Iraq.

## Materials and methods

Seventy nasal swabs were collected randomly from camels in different areas in Iraq.

The tip of the swab were placed in tube containing 3 ml viral transport media(VTM) After squeezing the tip of the swap and vortexing, the samples were transferred as soon as to the laboratory by cooled box & 300µl of the solution was taken for RNA extraction & stored the rest of the sample at –80◦C.

Two types of samples were done, one for viral isolation and another for RNA extraction. Three camels' aggregation areas were unchanged to check the surveillance study of the MERs COR virus.

*Preparation of Primary chick embryo fibroblast cell culture*

Cells were grown using the technique described for chicken embryo liver cells in tissue culture flasks by Villegas (1989).

*Viral RNA Extraction*

The viral RNA was extracted from all frozen swab (nasal swab) by using.

Dirct-Zol™ Total RNA extraction kit (Zymo Research, USA).

*The extracted RNA estimation*

The extracted RNA was checked by using Nan drop spectrophotometer that check RNA concentration and estimation of RNA purity through reading the absorbance in at (260 /280 nm).

*MERS-CoV dtec-RT-qPCR Test*

The MERS-CoV dtec-RT-qPCR test comprises a series of specific targeted reagents designed for the Middle East respiratory syndrome coronavirus detection by using qPCR.

*PCR set-up protocol*

Reverse transcription and qPCR reaction could be achieved in a single tube by using LyoMix RT-qPCR (BLUE CAP). One-Step protocol is recommended to prevent contamination, reduce errors, and save significant materials and time (Table 1).

**Results and discussion**

The result of detection by PCR is shown in the Fig (1). Table 2 revealed that out of 70 camels tested for the presence of MERS-CoV, only 4 (5.8%) were positive by using PCR technique and the remaining 66 (94.2%) were negative for the MERS-CoV RNA.

**Table 1.** Reaction pre-mix contents.

| Reagent | Volume |
|---|---|
| LyoMix RT-qPCR (BLUE CAP) | 4 µl |
| TargetSpecies dtec-RT-qPCR-mix (AMBER TUBE) | 1 µl |
| (DNase/RNase free water (GREEN CAP) | 10 µl |
| Reaction pre-mix volume | 15 µl |

**Table 2.** MERS CoV RNA positivity rate by PCR.

| Categories | Frequency | Percentage | 95% confidence interval |
|---|---|---|---|
| Negative | 66 | 94.2 | 89.2 - 100 |
| Positive | 4 | 5.8 | 0- 10.8 |
| Total | 70 | | |

The results of MERS CoV RNA detection (Table 3) by PCR found that only one camel was positive within 1 year age group (1.4%), while 3 camels were positive in the 13-24 months age group (4.3%). Therefore, the difference was significantly higher in the older camels ($\chi$ = 8.021, P= 0.005).

*Positivity rate by gender*

Regarding the gender, table (4) showed that the PCR results revealed that 2 (2.9%) of each of male and female camels were positive, and thus there was no significant difference in the positivity rate concerning the gender (P= 1.0).

**Table 3.** MERS-CoV RNA PCR results by camel age.

| Agr groups (ms) | PCR result | | Pearson Chi-Square value | *P value* |
|---|---|---|---|---|
| | Negative (%) | Positive (%) | | |
| < 1-12 | 55 (78.6%) | 1 (1.4%) | 8.021 | 0.005 [S] |
| 13-24 | 11 (15.7%) | 3 (4.3%) | | |
| Total | 66 (94.3%) | 4 (5.7%) | | |

*Positivity rate by locality*

The PCR results in the table (5) showed that 2 (2.9%) camels were positive in each of Gammas (Al-Najaf Al-Ashref) and Baddra and Jassan- (Wasit), while none of these was positive in Hor Al-Damledge area. The difference among these localities was statistically not significant (P= 0.8).

**Discussion**

In the present study only 4 out of 70 tested camels (5.7%) were positive for MERS-CoV RNA by RT-qPCR technique. These findings are surely less than the 28% reported by Al-Qadisiya study which based on the detection of MERS-CoV antigen in camel's nasal swabs using the rapid immunochromatographic MERS-CoV Camel Strips (Albusalih and Alrodhan, 2016). Undoubtedly, these laboratory procedures are

generally used for preliminary rapid field surveys as low-cost, simple-to-use, rapid tests for screening of infectious diseases since their results are obtained within 10-15 minutes (Zhou *et al.*, 2012). Furthermore, these assays are characterized by relative sensitivity and specificity making them as a useful tool for the rapid diagnosis and epidemiological surveillance of MERS-CoV infection in dromedary camels (Song *et al.*, 2015). On the other side, in Al-Qadisiya study only 15% of the enrolled camels were positive for MERS-CoV RNA by real-time quantitative PCR (Albusalih and Alrodhan, 2016). Again this result is higher than the 5.7% PCR positivity rate obtained in the current study.

**Table 4.** MERS-CoV RNA PCR results by camel gender**.**

| Gender | PCR result | | Pearson Chi-Square value | *P value* |
|---|---|---|---|---|
| | Negative (%) | Positive (%) | | |
| Male | 32 (45.7%) | 2 (2.9%) | 0.003 | 1.0[NS] |
| Female | 34 (48.6%) | 2 (2.9%) | | |
| Total | 66 (94.3%) | 4 (5.8%) | | |

**Table 5.** MERS-CoV RNA PCR results by locality**.**

| Locality | PCR result | | Pearson Chi-Square value | *P value* |
|---|---|---|---|---|
| | Negative (%) | Positive (%) | | |
| Hor Al-Damledge | 11 (15.7%) | 0 | 0.86 | 0.8[NS] |
| Ghamas/ Al-Najaf Al-Ashref | 24 (34.3%) | 2 (2.9%) | | |
| Baddra & Jassan/ Wasit | 31(44.3%) | 2 (2.9%) | | |
| Total | 66 (94.3%) | 4 (5.8%) | | |

The increasing use of RT-qPCR techniques in microbiology surely due to its ability for detection of nucleic acid instead of protein, which is much more sensitive method compared to conventional PCR method, with shorter analytical time and lower detection limit.

Its high specificity and sensitivity, give it a huge potential to serve as a powerful detection tool in various industries such as medical, veterinary, and agricultural industries (Sue *et al.*, 2014). However, different studies used these techniques had yielded variable results, similar to the situation here between Al-Qadisiya study and "Baghdad study", and these may be related to multiple reasons including proper samples, good primer design, accuracy of the technique, source of these materials (Zhang *et al.*, 2016; Kuypers and Jerome, 2017).

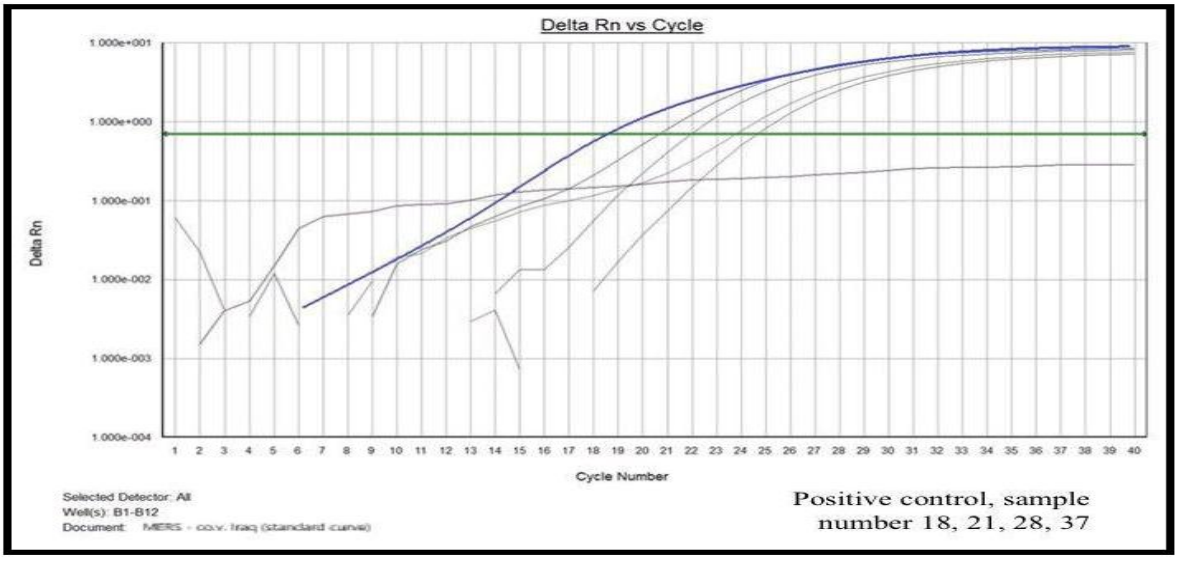


**Fig. 1.** Real-Time PCR.

**Conclusion**

Detection of Mers-co virus indicates the importance of the controlling its spread. The using of real-time PCR for diagnoses of Mers-cor virus and further surveillance studies of the disease was recommended as the rapid and accurate method for Mers-cor virus. The propagation and adaptation of the virus to grow and isolated in chicken embryo fibroblast cell culture as the first time work in Iraq is recommended instead of propagation of the virus cell line tissue culture which is not available and not applicable in Iraq.

**References**

**Adney DR, Van Doremalen N, Brown VR, Bushmaker T, Scott D, de Wit E, Bowen RA, Munster VJ.** 2014. Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels. Emerging Infectious Diseases Journal **20(12),** 1999-2005. http://dx.doi.org/10.3201/eid2012.141280.

**Albusalih SF, Alrodhan MA.** 2016. Screening and Molecular Characterization of Middle East Respiratory Syndrome-Coronavirus in Human and Camel. M.Sc. Thesis. College of Veterinary Medicine-University of Al-Qadisiya.

**Almaghrabi RS, Omrani AS.** 2017. Middle East respiratory syndrome coronavirus (MERS-CoV) infection. British Journal of Hospital Medicine **78(1),** 23-26. http://dx.doi.org/10.12968/hmed.2017.78.1.23.

**Gardner LM, Chughtai AA, MacIntyre CR.** 2016. Risk of global spread of Middle East respiratory syndrome coronavirus (MERS-CoV) via the air transport network. Journal of Travel Medicine **23(6),** 1-8. http://dx.doi.org/10.1093/jtm/taw063.

**Gautret P, Benkouiten S, Al-Tawfiq JA, Memish ZA.** 2016. Hajj-associated viral respiratory infections: A systematic review. Travel Medicine and Infectious Disease **14(2)**, 92-109. http://dx.doi.org/10.1016/j.tmaid.2015.12.008.

**Gossner C, Danielson N, Gervelmeyer A, Berthe F, Faye B, Kaasik AK, Adlhoch C, Zeller H, Penttinen P, Coulombier D.** 2016. Human-Dromedary Camel Interactions and the Risk of Acquiring Zoonotic Middle East Respiratory Syndrome Coronavirus Infection. Zoonoses Public Health **63(1),** 1-9.

**Hasan ASH, Karim SA, Usama AA.** 2017. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) seropositivity rate among human in Diyala province-Iraq. Iraqi Journal of Community Medicine **30(3),** 123-7.

**Hemida MG, Elmoslemany A, Al-Hizab F, Alnaeem A, Almathen F, Faye B, Chu DK, Perera RA, Peiris M.** 2017. Dromedary Camels and the Transmission of Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Transbound. Emerging Disease **64(2),** 344-353.

**Kuypers J, Jerom KR.** 2017 Application of digital PCR for clinical microbiology Journal of Clinical Microbiology **55(6),** 1621-1628. http://dx.doi.org/10.1128/JCM.00211-17

**Mohd HA, Al-Tawfiq JA, Memish ZA.** 2016. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) origin and animal reservoir. Virology Journal **13,** 87-96.

**Okba NM, Raj VS, Haagmans BL.** 2017. Middle East respiratory syndrome coronavirus vaccines: current status and novel approaches. Current Opinion in Virology **23,** 49-58.

**Omrani AS, Al-Tawfiq JA, Memish ZA.** 2015. Middle East respiratory syndrome coronavirus (MERS-CoV): animal to human interaction. Pathogens and Global Health **109(8),** 354-62. http://dx.doi.org/10.1080/20477724.2015.1122852.

**Song D, Ha G, Serhan W, Eltahir Y, Yusof M, Hashem F, Elsayed E, Marzoug B, Abdelazim A, Al Muhairi S.** 2015. Development and validation

of a rapid immunochromatographic assay for detection of Middle East respiratory syndrome coronavirus antigen in dromedary camels. Journal of Clinical Microbiology **53(4),** 1178-82. http://dx.doi.org/10.1128/JCM.03096-14.

**Sue MJ, Yeap SK, Omar AR, Tan SW.** 2014. Application of PCR-ELISA in molecular diagnosis. Biomed. Research International **2014,** 653014. http://dx.doi.org/10.1155/2014/653014.

**Wernery U, Lau SK, Woo PC.** 2017. Middle East respiratory syndrome (MERS) coronavirus and dromedaries. Veterinary Journal **220,** 75-79. http://dx.doi.org/10.1016/j.tvjl.2016.12.020

**Arabi YM, Balkhy HH, Hayden FG, Bouchama A, Luke T, Baillie JK, Al-Omari A, Hajeer AH, Senga M, Denison MR, Nguyen-Van-Tam JS, Shindo N, Bermingham A, Chappell JD, Van Kerkhove MD, Fowler RA.** 2017. Middle East Respiratory Syndrome. The New England Journal of Medicine **376(6),** 584-94. http://dx.doi.org/10.1056/NEJMsr1408795.

**Younan M, Bornstein S, Gluecks IV.** 2016. MERS and the dromedary camel trade between Africa and the Middle East. Tropical Animal Health and Production **48(6),** 1277-82. http://dx.doi.org/10.1007/s11250-016-1089-3.

**Zaki AM, Van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA.** 2012. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. The New England Journal of Medicine **367(19),** 1814-1820. http://dx.doi.org/10.1056/NEJMoa1211721

**Zhang K, Lin G, Li J.** 2016. Quantitative nucleic acid amplification by digital PCR for clinical viral diagnostics. Clinical Chemistry and Laboratory Medicine **54(9),** 1427-33. http://dx.doi.org/10.1515/cclm-2015-1101

**Zhou G, Mao X, Juncker D.** 2012. Immunochromatographic assay on thread. Analytical Chemistry **84(18),** 7736-43. http://dx.doi.org/10.1021/ac301082d

**Zumla A, Hui DS, Perlman S.** 2015. Middle East respiratory syndrome. Lancet **386(9997),** 995-1007. http://dx.doi.org/10.1016/S0140-6736(15)60454-8.