

Seropositivity rate of Middle East Respiratory Syndrome Coronavirus among Iraqi dromedary Arabic camels

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Received: 19/6/2017

Accepted: 29/8/2017

Summary

This study was conducted to determine the Middle East Respiratory Syndrome Coronavirus infection rate among Iraqi dromedary camels and to explore its risk factor. A total of 167 blood samples were collected, ninety of them were selected randomly and included in the study from 50 males (55.6%) and 40 females (44.4%). The age range was 1-15 years. Samples were collected from Al Najaf-slaughter house. Sera were separated and tested for the presence of anti-MERS-CoV IgG using the recombinant Camel anti-MERS-CoV spike protein S1 domain (MERS-S1) IgG ELISA kit. The results revealed that 81(90.0%) of camels included in this study were positive for anti-MERS-CoV IgG, with 95% confidence interval for the prevalence rate (82.5-94.9). Additionally, the Inter-quartile range of anti-MERS-CoV IgG titer was (5-19.7) and a mean rank of 99.8 U/ml. The highest positivity rate was among camels 10-15 years old with statistically insignificant difference ($P= 0.88$). Similarly, the anti-MERS-CoV IgG Ab titer was insignificantly higher in the same age group ($P= 0.79$). The anti- MERS-CoV IgG positivity rate was equally distributed among female and male camels (90.0%), so the difference was statistically insignificant ($P=1$). While the mean, median and Inter-quartile range of anti-MERSCoV-IgG titer was insignificantly higher among males compared to females ($P=0.57$). In conclusion, the majority of Iraqi camels are infected by MERS-CoV. Further studies are urgently needed to explore the ability of Iraqi camels to transmit the virus to human population.

Keywords: Middle East Respiratory Syndrome, Coronaviruses, Hajj-associated viral infection.

Introduction

In 2012, a novel coronavirus associated with severe respiratory disease in humans emerged in the Middle East. Epidemiologic investigations identified dromedary camels as the likely source of zoonotic transmission of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (1 and 2). Though it is not confirmed yet, multiple surveillance and phylogenetic studies suggested that the virus was originated in bats (3). Furthermore, phylogenetic and sequencing data strongly affirmed that the MERS-CoV originated from bat ancestors underwent a recombination event in the spike protein, possibly in dromedary camels in Africa, before its exportation to the Arabian Peninsula along the camel trading routes (4). Additionally, although bats can serve as potential reservoirs for MERS-CoV, dromedary camels seemed to be the only animal host responsible for the spill over human infections (2 and 3). Further epidemiological data confirmed the inter-transmission from camels to humans, though

inter-human spread within health care settings is responsible for the majority of reported MERS-CoV cases (5 and 6). Of note, it was well documented that the seroprevalence of MERS-CoV antibodies is very high in dromedary camels in Eastern Africa and the Arabian Peninsula (7-9). Recombinant spike protein-specific immunofluorescence and virus neutralization assays were applied on dromedary camel serum samples from the UAE revealed that 632/651(97.1%) camels had antibodies against MERS-CoV (7). In Pakistan, anti- MERS-CoV neutralizing antibodies were detected in 39.5% of dromedaries, documenting significant expansion of the enzootic range of to Asia (10). High MERS-CoV RNA rate was detected in camels from Ethiopia (upto 15.7%), Burkina Faso (upto 12.2%), and Morocco (upto 7.6%). The RNA detection rate was higher in camels bred for milk or meat than in camels for transport, as well as in younger camels. Concurrently, high seropositivity rates (up to

100%) were found in Morocco and Ethiopia, followed by Burkina Faso (upto 84.6%) (9).

Experimentally, detection of infectious virus in nasal secretions continues for 7 days postinoculation, and viral RNA upto 35 days postinoculation. The pattern of shedding and propensity for the upper respiratory tract infections in dromedary camels may help explain the lack of systemic illness among naturally infected camels and the means of efficient camel-to-camel and camel-to-human transmission (11). In another study, it was found that the maximum duration of viral shedding from infected camels was 2 weeks after the first positive test result as detected in nasal swabs and in rectal swabs obtained from infected calves (2). So, this study aimed to detect the MERS-CoV infection rate among Iraqi dromedary camels.

Materials and Methods

This cross- sectional study was conducted in Diyala province -Iraq for the period from October 2016 to February 2017 to figure out the infection rate of MERS-CoV in dormitory camels through the detection of serum anti-MERS-CoV IgG. A total of 167 blood samples were collected, ninety of them were selected randomly and included in the study, 50 (55.6

%) were males and 40 (44.4%) were females. The age range was 1-15 years. Samples were collected from Al Najaf- slaughter house. Sera were separated and tested for the presence of anti-MERS-CoV IgG, using the recombinant Camel anti-MERS-CoV spike protein S1 domain (MERS-S1) IgG ELISA kit (Alpha Diagnostic International, USA). Ethically, oral consent of the owners was taken before collecting blood samples. Statistical analysis was done using Statistical Package of Social Science (IBM-SPSS) Version 23, and P values less than 0.05 were considered significant.

Results and Discussion

Dromedary camels are the most likely source for the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) that causes Middle East respiratory syndrome (MERS) in humans. Serological assays have provided evidences for the circulation of MERS-CoV IgG among dromedary camels in many countries. Results in (Table, 1) revealed that 81(90.0 %) of camels included in this study were positive for anti-MERSCoV IgG, with 95% confidence interval for the prevalence rate (82.5 - 94.9). Additionally, the Inter-quartile range of anti-MERSCoV IgG titer was 5-19.7 and a mean rank of 99.8 U/ml.

Table, 1: The positivity rate and titer of anti-MERS-CoV IgG among camels.

Total No.	IgG positive No. (%)	95% CI for prevalence rate	Serum anti-MERSCoV IgG titer (U/ml)			
			Range	Median	Inter-quartile range	Mean rank
90	81(90.0)	(82.5 - 94.9)	(0.6 - 55.1)	12.6	(5 - 19.7)	99.8

For the best of our knowledge this is the first Iraqi study in this field, and actually it is a part of larger study including determination of MERSCoV infection rate among Iraqi population. Undoubtedly, the most important result of this study is the high infection rate of MERSCoV among Iraqi camels, plus these camels harbor high anti-MERSCoV IgG titer which certainly reflects the presence of high viral load. Similar results had been obtained from camels in the Middle East, particularly Arabian Peninsula, Africa, and Asia (1, 2, 7, 9 and 10). Additionally, 84.5% of serologically-tested camels from Egypt and 91.4% camels from Sudan had MERS-CoV neutralizing antibodies (12). The importance of these

results is streaming from the regional and global scientific consent that dromedary camels are the only animal species for which there were convincing evidences, that it is a host species for MERS-CoV and hence it is considered a potential source of human infections (6, 8 and 13). It is well known that Iraq has along common land borders with Kigdom of Saudi Arabia (KSA), the country where the MERS-CoV was first recognized in 2012, and the single country which reported more than 80% of the MERS-CoV cases worldwide (14). These borders are mostly deserts in which the camels forming the main vehicle cross the borders for trading. Therefore, Iraqi and Saudi camels are usually

come in contact with each other where transmission of the virus may occur. Earlier epidemiological surveys had affirmed that the MERS-CoV was endemic in Saudi's camels and that Saudi Arabian dromedary camels show significantly higher MERS-CoV carrier rates than dromedary camels imported from Africa (4). This high prevalence of infection with MERS-CoV, actually were responsible for large human MERS-CoV outbreaks in KSA (8, 15 and 16).

The presence of high anti-MERS-CoV Ab titer among Iraqi's camels probably reflects the presence of high viral load and consequently high shedding of the virus with respiratory secretions and thus high infectivity of these camels. Several studies had documented that

camels with high viral load were able to shed the MERS-CoV particularly with respiratory secretions for long duration (2 and 11). Therefore, it is crucial to conduct further studies on Iraqi camels for molecular detection of MERS-coronaviral RNA, and to investigate the infectiousness of these camels. The distribution of the MERS-CoV IgG positivity rate according to age groups was shown in (Table, 2). It is clearly obvious that the highest positivity rate was among camels 10-15 years old compared to other age groups. However, the difference failed to reach the levels of statistical significance (P= 0.88). Similarly, the anti-MERS-CoV IgG Ab titer was insignificantly higher in the 10-15 years age group (P= 0.79).

Table, 2: The anti-MERS-CoV IgG positivity rate and titer according to age groups.

Age groups (No.)	IgG positive No. (%)	95% CI for prevalence rate	Serum anti-MERSCoV IgG titer (U/ml)			
			Range	Median	Inter-quartile range	Mean rank
< 5 (18)	16 (88.9)	(68.9 - 97.6)	(0.7 - 49.3)	11.2	(4.5 - 17.6)	41.9
5-9 (35)	31 (88.6)	(75.1 - 96)	(0.8 - 37.5)	12.2	(5 - 20.7)	45.8
10-15 (37)	34 (91.9)	(79.9 - 97.7)	(0.6 - 55.1)	13.1	(5.6 - 20.2)	47

P= 0.88 [NS], P= 0.79 [NS]

The results also found that there was insignificantly higher positivity rate among camels 10-15 years old plus insignificantly higher anti-MERS-CoV IgG Ab titer among the same age group. These findings in older camels is inconsistent with the finding of (9) who reported that the MERS-CoV RNA detection rate was higher in camels bred for milk or meat than in camels for transport as well as in younger camels. On the other hand, this result is consistent with (17), who reported that the serologic assays showed significantly higher infection rate in adult camels compared to juvenile camels. Generally it has been found that MERS-CoV seroprevalence increased with age of camels (18). Accordingly, the results of this study seem reasonable and acceptable since older camels are usually used for trading across the Iraqi- KSA borders, and hence, more chance to catch the MERSCoV infection from carrier camels (3 and 14).

Or through importation of camels from endemic areas, and in this regard, it was found that Saudi Arabian dromedary camels had significantly higher MERS-CoV carrier rates than dromedary camels elsewhere (4). Additionally, younger camels may still have protective levels of maternal anti-MERS-Co Ab., therefore, further studies on Iraqi dormitory camels required to address these queries, especially when IgM antibodies were targeted to point out the recently infected animals. Such antibodies appeared in blood circulation in recently infected animals and shifted to IgG later. The anti- MERS-CoV IgG positivity rate was equally distributed in female and male camels (90.0%), so the difference was statistically insignificant (P= 1). While the mean, median and Inter-quartile range of anti-MERSCoV- IgG titer was insignificantly higher among male compared to female (P=0.57), (Table, 3).

Table, 3: The anti-MERS-CoV IgG positivity rate and titer according to gender.

Gender (No.)	IgG positive No. (%)		Serum anti-MERSCoV IgG titer (U/ml)			
	Range	Median	95% CI for prevalence rate	Mean rank		
Female (40)	36 (90%)	(78 - 96.5)	(0.8 - 55.1)	11.7	(4.5 - 19.2)	43.8
Male (50)	45 (90%)	(79.5 - 96.1)	(0.6 - 49.3)	13.6	(5.1 - 20.7)	46.9

P= 1 [NS]

P= 0.57 [NS]

The above-mentioned findings indicated clearly that insignificant differences associated with sex of animals but males carried high titer of IgG when compared to female camels. Similar findings were mentioned by (10) when they reported that no significant results appeared in comparing the seropositivity of male camels to female, but male camels carried high titer of antibodies. In contrast (9) reported high seroprevalence rate to MERS-CoV in female camels raised for milk in comparison to males raised for meat or used for transport or travelling. They attributed their findings to the fact that female camels always grouped to gather with young camels which were highly susceptible to MERS-CoV. Male camels for meat or travelling were always separated from females and only mixed during reproductive activities.

Conclusion: Depending on anti-MERS-Cov antibody detection, the majority of Iraqi camels are infected by MERS-CoV. Further studies are urgently needed to explore the ability of Iraqi camels to transmit the virus to human population, and for the construction of national prevention and control strategy.

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معدل الإصابة المصلية الموجبة لمتلازمة الشرق الاوسط التنفسية في الجمال العربية العراقية

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الخلاصة

أجريت هذه الدراسة لمعرفة نسبة الإصابة بمتلازمة الشرق الاوسط التنفسية في الجمال العربية العراقية ذات السنم الواحد وإظهار عوامل الخطورة منها. حيث جُمع ١٦٧ نموذج دم من الجمال وأختبر ٩٠ نموذجاً منها عشوائياً اشتملت على ٥٠ نموذج من الذكور (٥٥,٦%) و ٤٠ نموذج من الإناث (٤٤,٤%). أما الأعمار لهذه الحيوانات فقد تراوحت ما بين ١-١٥ سنة. جمعت النماذج من مجازر الجمال في محافظة النجف الأشرف وفصلت الأمصال وفحصت لوجود الأضداد نوع IgG باستعمال إختبار الأليزا الحاوية على المستضد S1 الموجود في الامتدادات البروتينية لفيروس متلازمة الشرق الاوسط التنفسية من الجمال. أظهرت النتائج ان ٨١ (٩٠,٠%) من الجمال المفحوصة موجبة لوجود الأضداد نوع IgG المضادة لهذا الفيروس مع فاصل ثقة بمقدار ٩٥% مع معدل انتشار (٨٢,٥-٩٤,٩) ومعدل معيار (١,٦-٩٤,٣). كما أظهرت الدراسة ان الأعمار ما بين ١٠-١٥ سنة هي الأكثر بمعدل الإصابة مع فارق احصائي غير معنوي (P=0.88) وكان معيار الأضداد عالياً في المستوى العمري الواحد وغير معنوي إحصائياً (P=0.79) أما نسبة الإصابة ما بين الذكور والإناث فظهرت متساوية (٩٠%) وكانت إحصائياً غير معنوية (P=1) وكان معيار الأضداد عالياً في الذكور مقارنة بالإناث. تستنتج الدراسة وجود الإصابة بمتلازمة الشرق الاوسط التنفسية في الجمال العربية العراقية مما يستوجب إجراء دراسات واسعة حول عزل الفيروس ودراسة احتمالية انتقاله الى الإنسان.

الكلمات المفتاحية: متلازمة الشرق الاوسط التنفسية، فيروس كورونا، إصابات الحج الفيروسي.