Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Seropositivity Rate among Human in Diyala Province-Iraq

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Background: Middle East Respiratory Syndrome-Coronavirus (MERS-CoV) is a novel coronavirus discovered in 2012 and is responsible for acute respiratory syndrome in humans. The disease is heavily endemic in dromedary camel populations of East Africa and the Middle East.
Objectives: The aims of this cross-sectional study are to figure out the MERS-CoV infection rate and to explore its risk factors.
Subjects and method: This study was conducted in Diyala province -Iraq for the period from August 2016 to February 2017 to figure out the infection rate of MERS-CoV infection through the detection of serum anti-MERS-CoV IgG. A total of 90 participants were enrolled and subdivided into 3 groups; 40 were close contact with camels, 20 were normal healthy individuals and 30 individuals who visit Saudi Arabia for Hajj pilgrims or Umrah. 34 (37.8%) were female and 56 (62.2%) were male. The age range was 22-72 years. Serum samples from participants were collected and tested for the presence of anti-MERS-CoV IgG using the recombivirus human anti-MERS-CoV spike protein S1 domain (MERS-S1) IgG ELISA kit (Alpha Diagnostic International, USA). Human privacy was respected by taken participant's consent. However, 8 individuals were refused participation. Statistical analysis was done using Statistical Package of Social Science (SPSS), Version 18, and P values less than 0.05 were considered significant.
Results: The results revealed that 46(51.1%) of human subjects were positive for anti-MERSCoV IgG, with 95% confidence interval for the prevalence rate (40.9-61.3) with a mean titer of anti-MERSCoV IgG Ab of 99.8. U/ml. the highest positivity rate was found among those 40-49 years. However, the difference was failed to reach the levels of statistically significant (**P= 0.08**). Similarly, the anti-MERS-CoV IgG Ab titer was insignificantly higher in the 40-49 years age group (**P= 0.11**). The anti- MERS-CoV IgG positivity rate was insignificantly higher among females compared to males (61.8% *vs* 44.6%, **P = 0.12**). While the mean, median and Inter-quartile range of anti-MERSCoV- IgG titer was significantly higher among female compared to male (**P< 0.004**). The results also found that the anti-MERS-CoV IgG positivity rate and Ab titer were significantly higher among those people who visited KSA as Hajj pilgrims or Umrah (**P < 0.001** and P< **0.001)** respectively. The flu-like syndrome is the most significant symptom reported (P= 0.001).
Conclusion: Based on anti-MERS-Cov Ab detection, considerable infection rate by MERS-Cov was found particularly among those who had previous visit to KAS. Further confirmatory and surveillance studies are required for future setup of control measures.
Keywords: MERS-CoV, anti-MERS-CoV Ab, Hajj-associated viral infection.

Introduction:

The Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel enzootic betacoronavirus that was first described in September 2012. Though not confirmed yet, multiple surveillance and phylogenetic studies suggest that the virus was originated in bats [1]. Furthermore, phylogenetic and sequencing data strongly affirmed that MERS-CoV that originated from bat ancestors was 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 [2]. Additionally, although bats and alpacas can serve as potential reservoirs for MERS-CoV, dromedary camels were seemed to be the only animal host responsible for the spill over human infections [1,3]. 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 [4,5]. 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 [6-8].

The clinical spectrum of MERS-CoV infection in humans ranges from an asymptomatic or mild respiratory illness to severe pneumonia and multi-organ failure; overall mortality is around 35.7% that was found to be significantly increased by age[9,10] . Most confirmed cases so far were part of MERS-CoV clusters in hospital settings, affecting mainly middle-aged men and patients with a pre-existing chronic morbidities or immunosuppressed status and the male to female ratio was 1.7:1 [11-13]. Clinically, it has been reported that the predominant comorbidities included hypertension, diabetes, respiratory, and renal disease, and the fever was the most common complaint [14]. The median incubation period was 5.2 days (1.9 to 14.7), and the serial interval was 7.6 days (2.5 to 23.1) [14]. In Saudi Arabia, it has been found that the seroprevalence of MERS-CoV antibodies was significantly higher in camel-exposed individuals,

who might be the source of infection for patients with confirmed MERS who had no previous exposure to camels [15]. Further data from the same country found that the serological evidence of zoonotic transmission of MERS-CoV was not common among animal workers during 2012 [16].

Subjects and methods:

This cross- sectional study was conducted in Diyala province -Iraq for the period from August 2016 to February 2017 to figure out the infection rate of MERS-CoV infection through the detection of serum anti-MERS-CoV IgG. A total of 90 participants were enrolled. They were subdivided into 3 groups; 40 were close contact with camels (camel's owners and slaughter house workers), 20 were normal healthy individuals and 30 individuals who visit Saudi Arabia for pilgrimage and Umrah. 34 (37.8%) were female and 56 (62.2%) were male. The age range was 22-72 years. Serum samples from participants were collected and tested for the presence of anti-MERS-CoV IgG using the recombivirus human anti-MERS-CoV spike protein S1 domain (MERS-S1) IgG enzyme linked immunosobant assay (ELISA) kit (Alpha Diagnostic International, USA). Human privacy was respected by taken participant's consent. However, 8 individuals were refused participation. Statistical analysis was done using Statistical Package of Social Science (SPSS), Version 18, and P values less than 0.05 were considered significant.

Results:

Results in table (1) revealed that 46(51.1%) of human subjects included in this study were positive for anti-MERSCoV IgG, with 95% confidence interval for the prevalence rate (40.9-61.3). 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 study subjects.

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	**46 (51.1)**	**(40.9 - 61.3)**	**(0.6 - 55.1)**	**12.6**	**(5 - 19.7)**	**99.8**

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 those 40-49 years old compared to other age groups. However, the difference was failed to reach the levels of statistically significant (**P= 0.08**). Similarly, the anti-MERS-CoV IgG Ab titer was insignificantly higher in the 40-49 years age group (**P= 0.11**).

Table (2): The anti-MERS-CoV IgG positivity rate and titer by age groups.

Agr groups (No.)	IgG positi No. (%)	95% CI prevalence	Serum anti-MERSCoV IgG titer (U/ml)			
			Range	Median	Inter-quartile range	Mean rank
<40 (30)	12 (40)	(24 - 57.8)	(1.7 - 83)	2.7	(1.8 - 3.9)	38.2
40-49 (26)	18 (69.2)	(50.2 - 84.2)	(1.6 - 94.1)	4.5	(2.2 - 72.6)	52.6
50-59 (34)	16 (47.1)	(31.1 - 63.5)	(1.7 - 94.3)	2.6	(2 - 75.6)	46.5
	P= 0.08 [NS]			***P= 0.11 [NS]***		

Although the anti- MERS-CoV IgG positivity rate was higher among females (61.8%) compared to that in males (44.6%), the difference was statistically insignificant (**P = 0.12**). While the mean, median and **Inter-quartile range** of anti-MERSCoV- IgG titer was significantly higher among female compared to male (**P< 0.004**), table (3).

Table (3): The anti-MERS-CoV IgG positivity rate and titer by gender.

Gender (No.)	IgG positive No. (%)	95% CI for prevalence rate	Serum anti-MERSCoV IgG titer (U/ml)			
			Range	Median	Inter-quartile range	Mean rank
Female (34)	21 (61.8)	(45 - 76.6)	(1.7 - 94.1)	21.6	(2.4 - 73.8)	55.6
Male (56)	25 (44.6)	(32.2 - 57.7)	(1.6 - 94.3)	2.4	(1.8 - 4.45)	39.4
	P= 0.12 [NS]			***P< 0.004 [S]***		

The results in table (4) showed that the highest anti-MERS-CoV IgG positivity rate was among those people who visit KSA for pilgrimage or Umrah 93.3% compared to other participant's groups. The difference was statistically significant (**P < 0.001**).

Table (4): Anti-MERS-CoV IgG positivity rate according to participant groups.

Participant's group	Total No.	serum IgG positive	95% CI for prevalence rate
		No. (%)	
Camels close contacts	40	11 (27.5)	(15.6 - 42.5)
Normal healthy individuals	20	7 (35)	(17.2 - 56.8)
Hajj pilgrims or Umrah	30	28 (93.3)	(80.3 - 98.6)

P < 0.001

Likewise, table (5) showed that the anti-MERS-CoV IgG Ab titer was significantly higher among those people visited KSA for pilgrimage and Umrah compared to other participant's groups (**P< 0.001**).

Table (5): Anti-MERS-CoV IgG titer (U/ml) by participant's groups.

Participant's group	Total No.	Range	Median	Serum IgG titer (U/ml)	Mean rank
				Inter-quartile range	
Camels close contacts	40	(1.6 - 83)	2.0	(1.8 - 3.8)	30.2
Normal healthy individuals	20	(1.7 - 3.9)	2.6	(2.1 - 3.8)	36.4
Human pilgrimage and Umrah	30	(2.2 - 94.3)	73.2	(53.1 - 84.9)	72

P <0.001

Regarding the clinical symptoms, the results showed that participants without symptoms had significantly higher anti-MERS-CoV Ab titer compared to those with one or more symptoms (**P =0.003**). On the other hand, among those participants with symptoms, the flu-like syndrome was found to be the only symptoms significantly associated with high anti-MERS-CoV Ab titer (**P =0.001**),while other symptoms including, dry cough, reproductive cough, sore throat were insignificantly associated with anti-MERS-CoV Ab titer, table (6).

Table (6): anti-MERS-CoV IgG titer (U/ml) by clinical symptoms.

Any symptoms	Total No.	Range	Median	Serum IgG titer (U/ml)	Mean rank	P value
				Inter-quartile range		
Negative	37	(1.6 - 94.1)	5.0	(2.5 - 75.6)	55.2	**P =0.003**
Positive	53	(1.7 - 94.3)	2.3	(1.8 - 3.9)	38.8	
Flu-like symptoms						
Negative	68	(1.6 - 94.3)	2.3	2.3	37.9	**P =0.001**
Positive	22	(2 - 94.1)	69.2	69.2	69	

Discussion: First of all, it is worthy to mention two things; firstly, for the best of our knowledge this is the first Iraqi study on MERS-CoV infection in humans, and secondly, it is a part of larger study included exploration of infection rate by MERS-CoV in local Iraqi dormitory camels.

In present study the recombivirus human anti-MERS-CoV spike protein S1 domain (MERS-S1) IgG ELISA assay was used which was proofed to be correlated well compared with other serological assays in human [17]. The present results found that 46(51.1%) of Iraqi population were seropositive for anti-MERSCoV IgG, with a mean of 99.8 U/ml of anti-MERSCoV IgG titer. The study thought that it is really high infection rate. Unfortunately, similar studies were scarce in the literature. However, in KSA it has been found that the anti-MERS-CoV antibodies were confirmed in 15 (0·15%; 95% CI 0·09-0·24) of 10,009 people tested in six of the 13 provinces [15]. Actually that is necessitates an urgent local or country-wide surveillance studies to affirm these data and for setting up an effective national control precautions.

Moreover, a significantly higher infection rate was recorded among those people who had previous travel to KSA as Hajj pilgrims or Umrah ((93.3%). Additionally, the anti-MERS-CoV IgG Ab titer was significantly higher among those people (mean serum IgG titer was 72 U/ml). Upon reviewing the literature, several countries had denied any infection by MERS-CoV in their citizens after their return form KSA [18-20]. Apart from MERS-CoV cases occurred in KSA which were mostly secondary to large outbreaks in healthcare settings, it has been reported that travel-associated MERS infection remains low and the virus exhibited a clear tendency to cause large outbreaks outside the Arabian Peninsula as exemplified by the outbreak in the Republic of Korea [21]. More comprehensively, a systematic review and meta-analyses including 31 studies found that SARS coronavirus and MERS coronavirus were never isolated in Hajj pilgrims,

and that the most commonly isolated viruses from symptomatic patients during the Hajj by PCR were rhinovirus (5.9-48.8%), followed by influenza virus (4.5-13.9%) and non-MERS coronaviruses (2.7-13.2%) [22]. Similarly, respiratory tract infections in travelers /pilgrims returning to the UK from the Middle East were mainly due to rhinoviruses, influenza A, and influenza B, whilst MERS-CoV was not detected among patients studied [23]. Therefore, further studies are needed to verify the present results.

On the other side, 5 Iranian patients with laboratory-confirmed MERS-CoV, none had a history of travel, contact with animals, or consumption of camel milk products, but all of them had contact with a person who had been in Saudi-Arabia and experienced respiratory infection [24]. Probably this is forming the most acceptable explanation for the 35% MERS-CoV infection rate among normal healthy population in our study. It is important to know that person-to-person transmission of MERS-CoV is still mostly limited to health care settings [4,25].

The results also revealed that 27.5% of camel's contacts (camel's owners and slaughter house workers) were positive for anti-MERS-CoV IgG with a mean IgG titer of 30.2 U/ml. These results are not unusual as many previous studies had ascertained the central role of camels for transmission of MERS-CoV to human contacts [1,7,9]. An epidemiological study was reported that MERS-CoV human cases without documented contact with another human MERS-CoV case make up 61% (517/853) of all reported cases, and dromedary camels are the only animal species for which there is convincing evidence that it is a host species for MERS-CoV and hence a potential source of human infections [5] . It was also reported 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, provided further evidence of the zoonotic potential of MERS-CoV infection and strongly suggested that camels may have a role in the transmission of the virus to humans [3]. On the other side, Memish *et al.* (2015) [16] has reported that serological evidence of zoonotic transmission of MERS-CoV was not common among animal workers in Southern Saudi Arabia during July 2012.

The present study also found that the anti-MERS-CoV IgG positivity rate and the anti-MERS-CoV IgG Ab titer were insignificantly higher among those 40-49 years old. These results are in agreement with those reported by an epidemiological study in KSA which found that the majority of the affected patients were aged ≥ 40 years [10]. Among 39 patients with laboratory-confirmed MERS-CoV cases identified in KSA from March to May 2014, 54% were male, aged 40 ± 19 years and included 8% pediatric patients (<18-years old) [14]. Generally, cases of MERS-CoV infection in Jordan and KSA were rare among children [21].

Regarding the sex differences, anti- MERS-CoV IgG positivity rate was insignificantly higher among females versus males (61.8% *vs* 44.6%), However, the anti-MERSCoV- IgG titer was significantly higher in female compared to male. It seems that the results in this respect are controversial [14, 24, 26]. It was reported that most confirmed cases so far were part of MERS-CoV clusters in hospital settings, affecting mainly middle-aged men and patients with a chronic disease or immunosuppressed status [12]. Clinically, among all symptoms investigated including, dry cough, reproductive cough, sore throat, and shortness of breath, only flu-like symptom was significantly associated with high anti-MERS-CoV Ab titer. Studies in this regard had yielded variable results. Among patients with confirmed MERS-CoV infection in KSA, 81% were symptomatic at presentation and fever being the most common complaint (Alraddadi *et al*., 2016). Similar results had been reported by other studies [10, 20, 24] . However, the predominance of comorbidities included hypertension, diabetes, respiratory and renal disease seems universal in all these studies. It is important to mention that asymptomatic or mild MERS-CoV infection was recognized in UAE and during the Korean outbreak but transmission from these cases was not reported [9,27].

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