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Serological identification of MERS-CoV in camels of Wasit province, Iraq

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Abstract

Background: Since the first human case of Middle East Respiratory Syndrome (MERS) caused by Coronavirus (MERS-CoV) in 2012, several evidence bases have shown one-humped camels as the main reservoir host, from which infection is transmitted to humans.

Aim: Serological investigation of MERS in dromedary camels in Wasit province (Iraq), detection severity of infection, and association to some risk factors.

Methods: A total of 455 dromedary camels were selected randomly from two main districts in Wasit province, Iraq, during January and April (2023). Sera of all study camels were examined by enzyme-linked immunosorbent assay (ELISA), and titers of positive study animals were categorized according to their severity.

Results: Serological testing yielded 37.58% positive animals for MERS infection. According to the severity of positive ODs (titer), a total of 53.22%, 30.99%, 12.28%, and 3.51% showed mild, moderate, strong, and very strong infections, respectively. Regarding risk factors, significant elevation in seropositivity was seen in camels of >3–6 and >6 years old and reduced in camels of ≤3 years old with an elevated risk of MERS with increased age. Regionally, seropositivity and relative risk were increased in the camels of Shaykh Sa'd when compared with Al-Numaniyah. Regarding sex, no significant variation was detected between seropositive females and males; however, male camels appeared at higher risk than females. Association between the severity of MERS infection and risk factors revealed that there was a significant increase in mild and moderate infections in female camels of >6 years old; whereas strong and very strong infections were seen in male camels of 3–6 years old. Mild and very strong infections were recorded in Shaykh Sa'd; while moderate and strong infections in Al-Numaniyah.

Conclusion: The study indicated a longstanding existence of MERS-CoV in camels of Wasit province; therefore, recent infections or active viral excretion are required for confirmation by molecular approaches.

Keywords: MERS-CoV, *Camelus dromedarius*, Coronavirus, Serological test, ELISA.

Introduction

Middle East respiratory syndrome (MERS) is one of the most prevalent viral diseases in the last decade. It was first described in Saudi Arabia in 2012 and then reported in many countries in Africa, Asia, Europe, and America continents (Şenel and Topal, 2021; Islam *et al.*, 2023). MERS-CoV belongs to *Coronaviridae* Family in *Nidovirales* Order of *Orthornavirae* Kingdom, which is an enveloped virus of single-stranded RNA genome and a nucleocapsid of helical symmetry with the club-shaped spikes that project from their surface (Li *et al.*, 2019; Yan *et al.*, 2020). Although, the origin of the virus is not fully understood, it is believed that it originated in wild animals, such as bats, and then spread to domestic animals, such as camel, cattle, goats, and

sheep (Reusken *et al.*, 2013a; Malik, 2020; Islam *et al.*, 2023). However, the dromedary camels have been the only species so far to harbor specific antibodies against MERS-CoV, identifying them as the main potential reservoir that transmits the virus to humans (Hemida and Alnaeem, 2019).

Notably, most MERS infections in camels appeared asymptomatic, but minor clinical signs could be seen after some field or experimental infections, which include fever, anorexia, respiratory discharge, sneezing, and coughing (Sharun *et al.*, 2020). Traditional isolation of the virus is a great of challenge since the shedding of the virus occurs during 1–7 days after infection, and the virus can be detected in tissues of the upper respiratory system associated with its regional lymph nodes (Adney

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et al., 2014). Molecular studies of nasal swabs showed that the findings could remain positive after two weeks, and the next sampling after 1 or 2 months post-infection revealed negative results for viral RNA (Meyer et al., 2016; Muhairi et al., 2016; Ali et al., 2017a; Hemida et al., 2017). Different serological techniques were modified to identify MERS, such as neutralization test, protein microarray, western blot, immunofluorescence assay, and enzyme-linked immunosorbent assay (ELISA). Of these methods, ELISA is suitable for first-line screening as it is a simple operating procedure with short processing time and large throughput (Yan et al., 2020).

Global epidemiological researchers have well documented that the virus circulates extensively in dromedary populations with a high seroprevalence varied between 80% and 100% in some areas, especially, in the Arabian Peninsula (Dighe et al., 2019; Pestil et al., 2022). In Iraq, there were only two serological reports that were carried out in Najaf, Muthana, and Basra (Thwiny et al., 2018) and Diyala (Hasan et al., 2022) provinces; therefore, this study represents a unique survey to investigate seroprevalence of MERS in one-humped camels of Wasit province (Iraq) by ELISA with detection the association of infection to some risk factors.

Materials and Methods

Study samples

A total of 455 camels (*Camelus dromedarius*) of variable ages and sexes were chosen from two main districts in Wasit province (Shaykh Sa'd and Al-Numaniyah) during January and April (2023). Each study animal was subjected to jugular venous blood sampling (5 ml), under aseptic conditions, into a labeled free-anticoagulant gel glass tube that was then centrifuged at 1000 g for 5 minutes. Sera were kept in Eppendorf tubes at 4°C.

Serological testing

Following the manufacturers' instructions of Qualitative MERS-COV ELISA Kit (MyBioSource, USA), the sera and ELISA kit reagents were prepared, processed and the reaction was read at an optical density (OD) 450 nm using the Automated Microplate Photometer (MicroTech, USA) after adding of Stop Solution. The averages of positive and negative controls were determined at 1.23 and 0.11, respectively; the Cut-Off value was calculated following the provided equal (Average of negative control + 0.15), and the positive samples were determined at ≥ 0.26 . According to the level of positive ODs (titer), the severity of infection was categorized into 4 groups: mild, moderate, strong, and very strong infections.

Study limitation

The study investigates the seroprevalence of MERS in camels in two regions only, as these regions exhibited a high number of camels compared to other regions. Furthermore, the limited number of camels in the other

regions associated with far distances and the difficulty of obtaining the consent of owners to take blood samples are the main reasons that the study depends on only two regions that represent the province.

Statistical analysis

The *t*-test, one- and two-way ANOVA, as well as Odd-Ratio in the GraphPad Prism Software (GraphPad Inc, USA), was used to estimate differences between values of the study at $p < 0.05$. Values were represented as either percentage (%) or as Mean \pm Standard deviation (AL-Shaeli et al., 2022).

Ethical approval

The study was licensed by the Scientific Committee of the College of Veterinary Medicine (University of Wasit, Wasit, Iraq). In contrast, the collection of blood samples and data was carried out after the oral agreement of the camels' owners.

Results

Serological results

Serological testing of 455 camels by indirect ELISA yielded 171 (37.58%) positive animals for MERS infection and 284 (62.42%) negative animals (Fig. 1). According to their positive OD levels, a total of 53.22% (91/171), 30.99% (53/171), 12.28% (21/171), and 3.51% (6/171) showed mild, moderate, strong, and very strong infections, respectively (Fig. 2). Subsequently, the OD values (M \pm SD) were 0.355 ± 0.074 , 0.594 ± 0.051 , 0.799 ± 0.067 , and 0.968 ± 0.036 , respectively for mild, moderate, strong and very strong infections (Fig. 3).

Risk factors

In this study, values of positivity, odd ratio, and relative risk showed a significant variation among different groups of age (Table 1), region (Table 2), and sex (Table 3). For age, significant elevation in seropositivity was seen in camels of >3–6 (38.24%) and > 6 (41.67%) years old and reduced in those of ≤ 3 years old (27.96%). Also, the findings of Odd ratio and relative

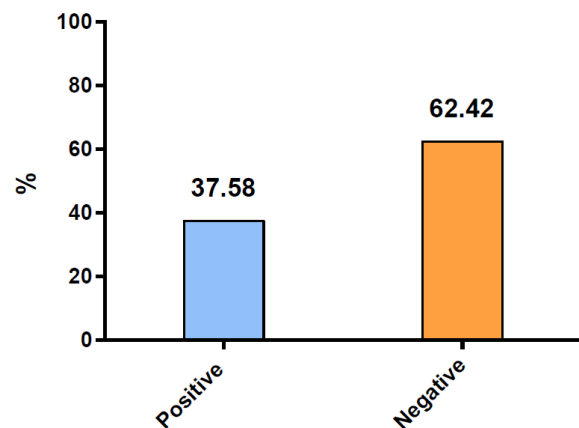


Fig. 1. Serological result for testing camel's sera by indirect ELISA.

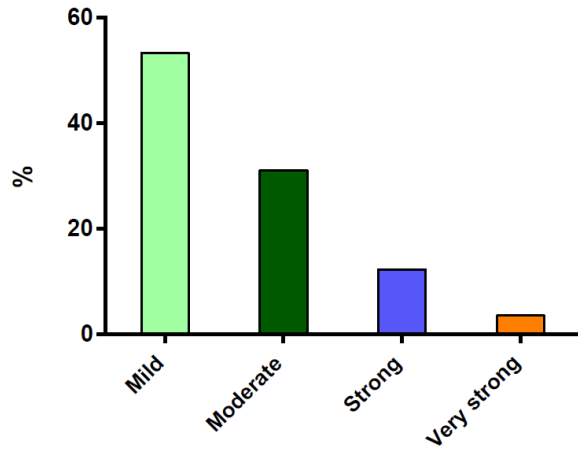


Fig. 2. Prevalence rate of positives according to severity of infection.

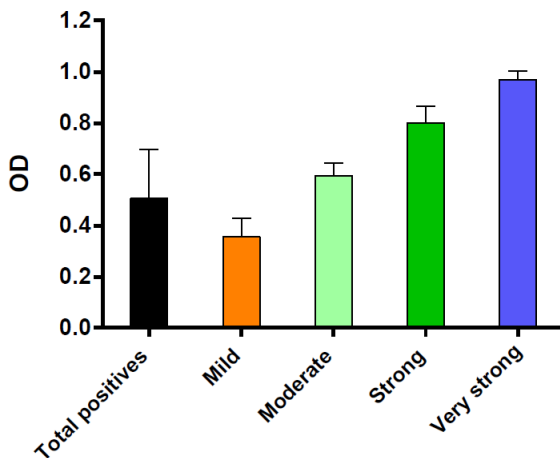


Fig. 3. Values of ODs of positives according to severity of infection.

Table 1. Association between positivity, Odd ratio and relative risk with age groups.

Age	Total no.	Seropositive		Odd ratio	Relative risk
		No.	%		
£ 3 years	93	26	27.96	0.5808	0.7640
> 3–6	170	65	38.24 *	1.0454	1.0203
> 6	192	80	41.67 *	1.3501 *	1.1441 *
<i>p</i> -value			0.0374	0.0013	0.0025

Significant increase (*).

risk were increased significantly ($p < 0.05$) with age, in particular in camels of >6 years old. Concerning study regions, significant increases ($p < 0.05$) in seropositivity were recorded in the camels of Shaykh Sa'd (42.16%) when compared to those of Al-Numaniyah (34.44%). In addition, values of Odd ratio

and relative risk were in camels of Shaykh Sa'd than Al-Numaniyah.

Regarding sex, no significant variation ($p < 0.0579$) was detected between seropositive females and males; however, male camels have appeared at a significantly greater MERS risk than females ($p < 0.05$).

Association of severity of MERS infection to risk factors
 Although the mild and moderate MERS infection increased significantly ($p < 0.05$) in camels of >6 years old (51.65% and 52.83%, respectively), strong and very strong infections were seen significantly ($p < 0.05$) in camels of 3–6 years old (76.19% and 50%, respectively), (Table 4).

Among both study regions, the findings of mild and very strong MERS-CoV infections were increased significantly ($p < 0.05$) in Shaykh Sa'd (53.85% and 83.33%, respectively), whereas moderate and strong infections were significantly higher in Al-Numaniyah (71.7% and 57.14%, respectively), (Table 5).

Regarding sex, mild and moderate MERS-CoV infections appeared significantly ($p < 0.05$) in females (69.23% and 64.15%, respectively), while the strong and very strong seropositive infections were reported in males (76.19% and 83.33%, respectively), (Table 6).

Discussion

The seroprevalence of MERS infection in study camels (37.58%) was less than the reported prevalence in Najaf (85%), Muthana (84.7%), and Basra (85.7%) (Thwiny *et al.*, 2018) and Diyala (90%) (Hasan *et al.*, 2022) provinces. In comparison with seroprevalence of MERS in camels of other countries, there was 0.24% in Kazakhstan (Orynbayev *et al.*, 2022), 4.12% in Canary Island (Gutiérrez *et al.*, 2015), 19.44% in Nigeria (Gaddafi *et al.*, 2020), 39.5% in Pakistan (Saqib *et al.*, 2017), 51.05% in Jordan (Ababneh *et al.*, 2021), and 52.7% in Turkey (Pestil *et al.*, 2022). Furthermore, the prevalence is 59.8%–97.1% in United Arab Emirates (Meyer *et al.*, 2014), 61.8% in Israel (David *et al.*, 2018), 68.9% in Egypt (Kandeil *et al.*, 2019), 70.81% in Saudi Arabia (Aljasim *et al.*, 2020), 80.4% in Tunisia (Eckstein *et al.*, 2021), 93% in Ethiopia (Fekadu *et al.*, 2017), and 100% in Oman (Reusken *et al.*, 2013b). The source of antigen is the main issue in different serological results; however, animal's age and sex, geographic location, and sampling population characteristics, as the imported camels have a significantly higher seropositivity than local breed counterparts, and diagnostic assay serving in sero-survey are other factors.

This study showed that mild infection was prevalent significantly among seropositive camels. Globally, few information is available about the longevity of antibody titers post-MERS-CoV infections. Meyer *et al.* (2016) found that neutralizing antibodies remain consistent during a year in camels following infection on a closed farm, whereas other researchers recognized that the titer of antibodies declines rapidly throughout 14 days

Table 2. Association between positivity, odd ratio and relative risk with region groups.

Region	Total no.	Seropositive		Odd- ratio	Relative risk
		No.	%		
Shaykh Sa'd	185	78	42.16*	1.3874*	1.1576*
Al-Numaniyah	270	93	34.44	0.7208	0.8638
<i>p</i> -value			0.0454	0.0015	0.0021

Significant increase (*).

Table 3. Association between positivity, odd ratio and relative risk with sex groups.

Sex	Total no.	Seropositive		Odd-ratio	Relative risk
		No.	%		
Female	285	103	36.14	0.8489	0.9291
Male	170	68	40	1.1780 *	1.0763 *
<i>p</i> -value			0.0579	0.0013	0.0016

Significant increase (*)

Table 4. Association of severity of MERS infection to age.

Severity	Total no.	≤3 years		>3–6		>6		<i>p</i> -value
		No.	%	No.	%	No.	%	
Mild	91	15	16.48	29	31.87	47	51.65*	0.0245
Moderate	53	8	15.09	17	32.08	28	52.83*	0.0239
Strong	21	1	4.76	16	76.19*	4	19.05	0.0334
Very strong	6	2	33.33	3	50*	1	16.67	0.0427
Total	171	26	15.21	65	38.01	80	46.78	0.0358
<i>p</i> -value			0.0454		0.0485		0.0394	-

Significant increase (*)

Table 5. Association of severity of MERS infection to region.

Severity	Total no.	Shaykh Sa'd		Al-Numaniyah		<i>p</i> -value
		No.	%	No.	%	
Mild	91	49	53.85*	42	46.15	0.0499
Moderate	53	15	28.3	38	71.7*	0.0315
Strong	21	9	42.86	12	57.14*	0.0418
Very strong	6	5	83.33*	1	16.67	0.0189
Total	171	78	45.61	93	54.39	0.0441
<i>p</i> -value			0.0445		0.0485	-

Significant increase (*)

(Ali *et al.*, 2017b; Hemida *et al.*, 2017). However, many of these studies that were conducted variously to dedicate the seroprevalence of MERS had taken the samples opportunistically from stored samples/sera of dromedary camels when there was an investigation of human outbreaks or from tested samples for other means. In addition, not all studies used serological assays to determine or confirm seropositivity, and between those that did, cut-off positivity titers varied.

These findings are important since they supported the regional and global scientific consent as the one-humped camel is the only host species for MERS and, therefore, a potential source of infection in humans (Mirkena *et al.*, 2018; Al-Salihi and Khalaf, 2021).

Our results showed that seropositivity increased with age, as recorded by several studies (Ali *et al.*, 2017a; Miguel *et al.*, 2017; Kasem *et al.*, 2018; Ommeh *et al.*, 2018); however, in contrast to others (Deem *et al.*,

Table 6. Association of severity of MERS infection to sex.

Severity	Total no.	Female		Male		p-value
		No.	%	No.	%	
Mild	91	63	69.23*	28	30.77	0.0372
Moderate	53	34	64.15*	19	35.85	0.0434
Strong	21	5	23.81	16	76.19*	0.0298
Very strong	6	1	16.67	5	83.33*	0.022
Total	171	103	60.23	68	39.77	0.0479
p-value		0.0369		0.0465		-

Significant increase (*)

2015; Harrath and Abu Duhier, 2018; Thwiny *et al.*, 2018) that showed no effect of age in seropositivity results. Reusken *et al.* (2013a) mentioned that maternal immunity in dromedaries rapidly declined 14–35 days post-delivery, and a much higher titer of antibodies was generated against disease in adult dromedaries. Hence, alternative interpretations are that camels had just been infected and titers of antibodies were rising.

In this study, increasing in seroprevalence and risk of MERS infection was reported in the camels of Shaykh Sa'd when compared to Al-Numaniyah. These findings were in contrast with those seen in other Iraqi locations by Thwiny *et al.* (2018), but it was similar to those reported in different countries in Africa and the Middle East, such as Egypt, Senegal, Tunisia, and Saudi Arabian (Kandeil *et al.*, 2019). However, significant variation between study areas might be attributed to differences in camel management, herd size, exposure to wild animals, owners' ethnic background, and facility of sampling site. Frequent transporting of camels appears to be another cause for the increasing regional seropositivity because transportation generates stress and lowers infection resistance, increasing the chance for direct contact with infected camels (Islam *et al.*, 2023).

Although, no significant variation was detected between seropositive females and males, male camels appeared at greater MERS risk than females. Some individual reports indicate a significantly high prevalence in females (Ali *et al.*, 2017a; Miguel *et al.*, 2017), while others found the opposite (Kasem *et al.*, 2018) or do not record any significant difference (Thwiny *et al.*, 2018). Sikkema *et al.* (2019) mentioned that most female camels are sampled at farms, whereas the majority of males were looked at quarantine areas, live animal markets, and slaughterhouses.

Different studies indicated that positive rates of IgG antibodies increase gradually in dromedaries with age, whereas detection of MERS-CoV RNA decreases (Wernery *et al.*, 2015; Wang *et al.*, 2019; Loeffelholz and Tang, 2020). Much higher MERS seroprevalence could exist probably as a result of multiple MERS re-infections or long-lasting immune response toward

MERS infections. However, immunity is not sterilizing as MERS infection and shedding have also been detected in adult camels that have MERS antibodies (Haagmans *et al.*, 2016; Hemida *et al.*, 2017; van Doremalen *et al.*, 2017).

Conclusion

Our observations indicated that MERS might be circulated in dromedary camels of Wasit province, and the potential role of camels, as a reservoir, in the transmission of infection to humans is expected. Combining serological laboratory results of camels with sufficient background information (severity of infection, age, region, and sex) does result in valuable data. The sensitivity and specificity of ELISA, as well as comparative studying with other diagnostic techniques, are necessary in the detection of MERS infection. Mapping the movement of dromedaries is necessary to understand the underlying spatial transmission dynamics of MERS.

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Conflict of interest

All authors have no conflict of interest to disclose.

Authors contributions

HJG and AAS: Blood sampling and sera preparation. HSRA and HMAB: Serological testing of sera. HAJG: Statistical analysis of data. All authors read and approved the final copy of the manuscript.

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Data availability

All data supporting the findings of this study are available within the manuscript.

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