

Seroepidemiological study of Middle East Respiratory Syndrome (MERS) virus infection in Iraqi dromedary camels

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ABSTRACT

The main aim of this study was to investigate the presence of MERS-CoV antibodies in Iraqi camels, to assess the prevalence and interpret the results according to the epidemiological information. A total of 180 dromedary camel plasma samples from healthy animals of different sexes and ages were collected from the provinces Najaf, Muthanna and Basrah. All the camels appeared healthy on clinical examination. Blood plasma was analysed using the ELISA technique. A large proportion (153/180, 85%) of the dromedary camels sampled had antibodies to MERS-CoV. There was no significant difference in seropositivity to MERS-CoV according to the location and sex of the camels. The prevalence of antibodies was higher in camels less than 2 years old (39/44, 88.6%) than in camels aged 2-4 years; (47/58, 81%). In addition, the percentage of camels with antibodies was 85.9% (67 of 78) in camels older than four years. These results suggest that MERS-CoV is widespread in the camel populations throughout Iraq.

Key words: MERS-CoV; dromedary camels; ELISA; Iraq

Introduction

Coronaviruses, which belong to the enveloped RNA virus family Coronaviridae, are capable of causing disease in humans and animals. They consist of single-stranded positive-sense, nonsegmented genomes (CHAN et al., 2012). Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel coronavirus which emerged (ZAKI et al., 2012) and spread from the Arabian Peninsula to 25 countries in Middle East, North Africa, Europe, United States and Southeast Asia. All cases have had an epidemiological

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link to the Middle East, with confirmed cases in Iran, Jordan, Kuwait, Lebanon, Oman, Saudi Arabia, Qatar, United Arab Emirates and Yemen. Human-to-human transmission seems limited to family and healthcare settings, and is assumed to have contributed to the recent upsurge of cases (ECDC 2014). MERS-CoV is associated with severe respiratory symptoms and renal failure in infected persons (ZAKI et al., 2012; DROSTEN et al., 2013). Transmission of MERS-CoV has been reported via two routes, between infected and non-infected humans, and between dromedary camels and humans (COTTEN et al., 2014). The epidemiology of many human MERS-CoV infections could not be correlated to contact with diseased humans, and therefore it was suspected that these humans might have been infected from other sources. Since many infectious diseases have their origin in animals (WOOLHOUSE et al., 2012; WOOLHOUSE and GOWTAGE-SEQUERIA 2005), several livestock species, including dromedary camels, goats, sheep and cattle, have been investigated for the presence of MERS-CoV via serological methods (REUSKEN et al., 2013a; 2013b). The dromedary camel has been the only species so far to harbor neutralizing antibodies against MERS-CoV. Dromedary camels have been found to harbour specific antibodies against MERS-CoV, identifying them as the main potential reservoir (REUSKEN et al., 2013b). Comparison of genome sequences between viruses isolated from infected humans and camels supports the conclusion that transmission of MERS-CoV occurs from camels to humans (BRIESE et al., 2014; MEMISH et al., 2014). MERS-CoV infections in dromedary camels are often asymptomatic or associated with short periods of nasal discharge (rhinitis). Since antibody responses following coronavirus infection remain detectable for many years (CAO et al., 2007), the seroepidemiology of potential animal species for a MERS-CoV-specific antibody is a logical approach. Enzyme-linked immunosorbent assay (ELISA) is a good tool for seroepidemiological studies because other tests, such as immunofluorescence assays or virus neutralisation assays, require a longer time for analysis in professional laboratories, or even on live MERS-CoV within high biosecurity containment facilities. The detection of MERS-CoV has been described in the neighboring countries but so far no study has been published in Iraq. Thus, the prevalence and epidemiology of MERS-CoV in Iraqi camel populations remains largely unknown. The purpose of this study was to investigate the presence of MERS-CoV antibodies in camels, to evaluate the prevalence and the epidemiology of MERS-CoV in Iraqi camels.

Materials and methods

Dromedary camels and blood samples. A total of 180 dromedary camel (*Camelus dromedarius*) plasma samples were collected from Najaf province (n = 40), Muthanna province (n = 98) and Basrah province (n = 42). These provinces are found in the south and south-west of Iraq, where camels reside (Fig. 1). The blood samples were taken from camels of different sexes (98 males and 82 females) and of different ages: less than two years (n = 44), two to four years (n = 58) and older than four years (n = 78).



Fig. 1. Distribution of camels in Iraq (AL-REKABY, 2016)

Blood samples were obtained by jugular vein puncture, according to standard veterinary procedures. Samples were collected by drawing 10 mL using a sterile syringe for each animal. Blood was placed in labeled sterile tubes containing anti-coagulant (EDTA). All blood collection tubes were inverted gently five times for direct mixing after collection. All plasma samples were obtained during the period from 1st September 2014 to 31st August 2015, and were transported directly by cooled box to the laboratory and stored at -70 °C until further analysis.

A general clinical examination was performed on all camels. The examination included general condition, vital signs such as body temperature, heart rate and respiratory rate, and examination of the superficial lymph nodes and the mucus membrane. All camels appeared healthy and no abnormal signs were noticed.

Serological analysis. Blood plasma was analysed using the anti-MERS-CoV ELISA Camel (IgG) kit, manufactured by EUROIMMUN AG (Lübeck, Germany) to detect specific antibodies, following the manufacturer's instructions. Samples were classified as negative, borderline or positive according to the recommendations from the test kit manufacturer.

Statistical analysis. Statistical Package for Social Science (SPSS) version 15 software was used to analyze the data. Chi-square (X^2) test was used to assess the significance of differences between groups. P value <0.05 was considered to be significant.

Results

A large proportion (153/180, 85%) of dromedary camels sampled had antibodies to MERS-CoV. The location of the camels tested and their serostatus were shown in Table 1. There was no significant difference in seropositivity to MERS-CoV according to the location of the camels; the seropositivity was 85%, 84.7% and 85.7% in Najaf, Muthana and Basrah province respectively ($P>0.05$).

Table 1. Seropositivity to MERS-CoV according to location of camels

Province	Total tested	% Positive (n)	% Borderline (n)	% Negative (n)
Najaf	40	85 (34/40)	5 (2/40)	10 (4/40)
Muthana	98	84.7 (83/98)	4.1 (4/98)	11.2 (11/98)
Basra	42	85.7 (36/42)	2.4 (1/42)	11.9 (5/42)
Total	180	85 (153/180)	3.9 (7/180)	11.1 (20/180)

($P>0.05$)

The age-groups of the tested camels and their serostatus were shown in Table 2. The prevalence of antibodies was higher in camels less than 2 years old (39/44, 88.6%) than in camels 2-4 years old; (47/58, 81%). In addition, the percentage of camels with antibodies was 85.9% (67 of 78) in camels older than four years ($P>0.05$).

Table 2. Seropositivity to MERS-CoV according to camels' age

Age groups (years)	Total tested	% Positive (n)	% Borderline (n)	% Negative (n)
< 2	44	88.6 (39/44)	0 (0/44)	11.4 (5/44)
2 - 4	58	81 (47/58)	5.2 (3/58)	13.8 (8/58)
> 4	78	85.9 (67/78)	5.1 (4/78)	9 (7/78)
Total	180	85 (153/180)	3.9 (7/180)	11.1 (20/180)

($P>0.05$)

The prevalence of antibodies according to sex is shown in Table 3. There was no significant difference in seropositivity to MERS-CoV according to the sex of the camels; the seropositivity of males was 84.7% while in females it was 85.4% ($P>0.05$).

Table 3. Seropositivity to MERS-CoV according to the camels' sex

Sex	Total tested	% Positive (n)	% Borderline (n)	% Negative (n)
Male	98	84.7 (83/98)	2 (2/98)	13.3 (13/98)
Female	82	85.4 (70/82)	6.1 (5/82)	8.5 (7/82)
Total	180	85 (153/180)	3.9 (7/180)	11.1 (20/180)

($P>0.05$)

Discussion

The MERS-CoV serological methods were applied in a previous study (PERERA et al., 2013). The efficacy of the serological test used in this survey shows it to be a good tool and it is recommended for future use. The manufacturer's information indicates that sensitivity and specificity are almost 100%. The high sensitivity and specificity of the kit detect specific anti-MERSCoV antibodies against S1 antigen and it has been used successfully by other authors evaluating MERS-CoV in camels (MÜLLER et al., 2014).

This study is the first comprehensive countrywide survey of dromedary camels in Iraq. Early serological investigations to reveal antibodies to MERS-CoV in dromedary camel victims have been reported in Jordan (REUSKEN et al., 2013a), Egypt (PERERA et al., 2013) and Saudia Arabia (HEMIDA et al., 2013). The results of this study have shown that camels in Iraq have MERS-CoV antibodies at high rates (85%). Dromedary camels in the Arabian peninsula and in North and East Africa also have a very high antibody titer to MERS-CoV ($>90\%$) (PERERA et al., 2013; REUSKEN et al., 2014a; CORMAN et al., 2014) in comparison with those in the Canary Islands (antibody titer is only 14%) and in northern Europe (no seropositive results have been conducted) (GUTIERREZ et al., 2015). These differences may be due to the exposure of dromedary camels to active infection with MERS-CoV, which may be more widespread in some areas.

In Iraq, camels reside in only four provinces: Anbar, Najaf, Muthanna and Basrah. In this study, samples were collected from only three provinces. Anbar was excluded due to security restrictions. There was no significance difference in seropositivity to MERS-CoV between the provinces. This may be due to the continuous movements of the camels during the year: in winter the camels move to Basrah province, while in summer they move to Muthana and Najaf provinces searching for grass. A study carried out in Saudi Arabia revealed regional differences in seropositivity between Tabuk in the northwest, Taif in the west, and Gizan in the southwest (ALAGAILI et al., 2014); however dromedary

camels from the central province of Riyadh and the eastern province, Al Ahsa, showed no significant difference in seropositivity to MERS-CoV (HEMIDA et al., 2013).

In this study, the highest percentage of seropositivity (88.6%) was in the <2 years age group (young camels), suggesting that animals might be infected as juveniles since maternal antibodies in dromedary camels reportedly decline rapidly two to five weeks after birth (WERNERY 2001). On the other hand, seropositivity decreased to 81% in the 2-4 years age group then increased to 85.9% in the >4 years age group suggesting that animals might be re-infected in adulthood.

This was the first study conducted to evaluate seropositivity to MERS-CoV in dromedary camels in Iraq. It found that dromedary camels have evidence of antibody prevalence to MERS-CoV suggesting that the virus is widespread in camel populations in Iraq. Almost all the camels have a ratio >2 which according Euroimmun means the camels have IgM antibodies so there is an acute infection. Although none of them showed any clinical signs related to MERS infection, it is known that infected camels do not usually show any signs of infection (CHU et al. 2014). For further confirmation, it is highly recommended to undertake gene sequencing which should be closely related to human-specific MERS-CoV sequences. We were unable to attempt virus isolation and molecular characterization due to the lack of appropriate facilities. Human infections resulting from contact with acutely infected camels have been shown (MEMISH et al., 2014), and such cases may be a source of limited human-to-human transmissions. The nasal and/or fecal shedding of MERS-CoV by animals with high levels of neutralising antibodies suggests that the presence of antibodies does not confer sterilising immunity (REUSKEN et al., 2014b). In view of this, cross-sectional investigations of existing antibodies in human populations are of interest (CAUCHEMEZ et al., 2013). Detection of antibodies in large parts of the population would suggest widespread and long-standing circulation of MERS-CoV. In contrast, the absence of antibodies would suggest that large portions of the population are susceptible to infection, increasing the risk of an epidemic. Finally, comparisons of antibody prevalence among humans who are exposed to livestock versus those who are not exposed, could provide clues to potential sources of zoonotic infection.

However, although the animal reservoir has been identified, the route of infection and types of exposures remain largely unknown. Moreover, infections with MERS-CoV may go undetected in Iraq since routine surveillance systems are simply not in place due to the lack of diagnostic capacity and diagnostic laboratory networks. It is important to shed light on the circulation of the virus in Iraq to be prepared for potential outbreak scenarios, and to be able to mitigate the risk of MERS-CoV infections for the Iraqi population. Therefore it is necessary to build diagnostic capacities and to gain more insight into camels and their role as reservoirs for MERS-CoV. The Iraqi camel population needs to

be systematically screened in different provinces. Efforts should focus on humans that have a high risk of contracting infections due to their interaction with camels, such as herders and abattoir workers. MERS CoV diagnostic tests should be considered for all patients with unexplained severe pneumonia in Iraq. MERS-CoV from Iraqi camels needs to be characterized and compared to MERS-CoV in the Middle East.

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SAŽETAK

Istraživanje je poduzeto s ciljem da se iračke jednogrbe deve pretraže na prisutnost protutijela specifičnih za virus srednjoistočnog respiratornog sindroma (MERS-CoV) kako bi se na temelju rezultata mogla donijeti epidemiološka procjena o širenju infekcije. Ukupno je pretraženo 180 uzoraka krvne plazme klinički zdravih jednogrbih deva različita spola i dobi na područjima Najaf, Muthanna i Basrah. Za pretragu plazme rabljen je komercijalni ELISA komplet. U većine pretraženih deva (153/180, 85 %) dokazana su protutijela za MERS-CoV. U serološki pozitivnih deva nije ustanovljena značajna razlika s obzirom na lokaciju i spol. Prevalencija je bila veća u deva starosti do dvije godine (39/44, 88,6 %) nego u onih u dobi od 2 do 4 godine (47/58, 81 %). U deva starijih od četiri godine postotak pozitivnih iznosio je 85,9 % (67 od 78). Rezultati pokazuju da je MERS-CoV proširen u populaciji deva diljem Iraka.

Cljučne riječi: MERS-CoV; jednogrbe deve; ELISA; Irak
