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Research Article

Sero-detection of Foot and Mouth Disease Virus Serotypes A and O in One-humped Camels *(Camelus dromedarius)* in the Middle of Iraq

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Abstract

Foot and mouth disease (FMD) is a severe trans-boundary animal disease caused by a foot and mouth disease virus (FMDV) that spreads through Asia and Arabic countries. The current study aimed to investigate the FMDV antibodies in Iraqi dromedary's camels (Camelus dromedaries). A total of 520 serum samples were collected from clinically healthy camels (265 females and 255males) from different areas in the three Provinces of Iraq (Al-Najaf, Al-Muthanna, and Al-Qadisiyah) from February to July 2019 and divided into three groups based on the age of the camels. All sera samples were screened for antibodies against the non-structural protein (NSP) of FMDV using ELISA and further the NSP positive serum samples were screened for antibodies against structural proteins of FMDV serotype O A and C by liquid phase blocking ELISA (LPB-ELISA).The Result indicated that 10% of the sera were positive for NSP antibodies and FMDV serotype A found to be predominant. It was also observed that NSP positive was more in samples collected from female camels (11.30%) than male camels (8.60%), NSP FMDV antibodies were detected in camels of all ages. In summary, our study showed, for the first time in Iraq, that camels are more susceptible to the A and O FMD serotypes.

Keywords: *FMD, NSP, Camels, LPBE, Iraq*

Irak'ın Orta Bölgesinde Tek Hörgüçlü Develerde *(Camelus dromedarius)* Şap Hastalığı Virüsü Serotipleri A ve O'nun Sero-Tespiti

Öz

Şap hastalığı (FMD), Asya ve Arap ülkelerine yayılan ayak ve ağız hastalığı virüsünün (FMDV) neden olduğu ciddi bir sınır ötesi hayvan hastalığıdır. Bu çalışmada Irak tek hörgüçlü develerinde *(Camelus dromedaries)* FMDV antikorlarının araştırılması amaçlandı. Şubat-Temmuz 2019 tarihleri arasında Irak'ın üç vilayetinde (El-Necef, El-Muthanna ve El-Kadisiyah) farklı bölgelerden klinik olarak sağlıklı develerden (265 dişi ve 255 erkek) toplam 520 adet serum örneği toplandı ve develerin yaşına göre üç gruba ayrıldı. Tüm serum numuneleri, ELISA kullanılarak FMDV'nin yapısal olmayan proteinine (NSP) karşı antikor varlığı yönünden tarandı ve NSP pozitif serum örnekleri, sıvı fazı bloke eden ELISA (LPBE) ile FMDV serotipi O, A ve C'nin yapısal proteinlerine karşı antikor varlığının tespiti amacıyla tarandı. Sonuçlar, serumların %10'unun NSP antikorları için pozitif olduğunu ve FMDV serotip A'nın baskın olduğunu gösterdi. Ayrıca NSP pozitiflik oranının dişi develerden (%11.30) toplanan örneklerde erkek develerden (%8.60) daha fazla olduğu, NSP FMDV antikorlarının her yaştan devede bulunduğu belirlendi. Özetle, bu çalışma ile Irak'ta ilk kez develerin A ve O FMD serotiplerine daha duyarlı olduğu tespit edildi.

Anahtar sözcükler: *FMD, NSP, Deve, LPBE, Irak*

INTRODUCTION

Foot and Mouth Disease (FMD) is a highly contagious viral disease that can affect all species of cloven-hoofed animals and wildlife, including cattle, buffaloes, sheep, goat, pigs, elephant, camel, and deer, causing severe economic loss in the livestock industries worldwide [1]. FMD is caused by FMD virus (FMDV) belonging to the genus Aphthovirus, which has seven serotype es namely FMD serotype A, O, C, SAT 1, SAT 2, SAT 3 and Asia1[2]. Although the mortality rate for FMD is below 5%, the disease still leads to a significant loss in livestock productivity and trade. The virus can cause

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severe lesions in the myocardium of young animals, resulting in death with a high mortality rate among younger animals [3]. Camels are important domestic animals in many areas of the world, such as the Arabian countries and countries in middle and East Asia, North and East Africa, as well as in South America and the disease is endemic in these regions. Camelidae are susceptible to FMD, similar to cattle, sheep, goats, and pigs [4], with clearly distinguishable clinical signs.

Iraq is home to approximately 58000 dromedary camels of two breeds and there is significant utilization of camel milk, meat, leather, and wool. Most camels are pastured in close groups with herds of other large and small ruminants [5]. FMD is endemic in Iraq, and the first notable case of FMD was recorded in 1937, and in 1952 FMD outbreaks are regularly reported among the large and small ruminants in various regions of Iraq [1,5] and the predominant FMDV serotypes detected were O, A, and Asia1. The prevalence rate of FMD in Iraq was 68.7%, 46.6%, and 30% in cattle, buffalo, and small ruminants, respectively. However, there is no information on the prevalence and detection of FMD virus (FMDV) in one-humped camels in Iraq [6]. The viral isolation and ELISA are the gold standards in FMD diagnosis, and the most reliable indicator of FMD viral infection is the detection of antibodies in the serum against polyproteins 3ABC using liquid phase blocking ELISA (LPBE) [7], the Non-structural protein (NSP) enzyme linked immune sorbent assay (ELISA) test discriminates the animals that have been infected from those that have been vaccinated and the test would be able to detect continued viral circulation and would therefore be extremely useful for serological surveys. Given the absence of prior data on the sero-surveillance, in the present study we investigated the serological evidence of the natural exposure of camels, which were reared together with ruminants, to the FMD virus [8].

MATERIAL and METHODS

This study was carried out with the permission of Ethical Committee in the College of Veterinary Medicine, University of Al-Qadisiyah under Ref No. 543/2018.

Animals and Study Area

The study was conducted using 520 Arabian one-humped camels *(Camelus dromedarius)* sampled over a period of six months (February to July 2019) from areas distributed in three Provinces (Al-Najaf, Al-Muthanna, and Al-Qadisiyah Provinces) in the middle of Iraq, which include the central area stretching from the Euphrates to the western frontier, towards the border with Saudi Arabia. This is the region where the majority of Iraqi camels are grazed.

Sampling

In total, 520 blood samples were collected by jugular vein puncture using vacutainer tubes containing serum clot activators from apparently healthy Arabian camels (255 males and 265 females) depending on the clinical examination and case history as well as general conditions and their activities, appetite and close inspection for each lesions or any abnormal behaviors within the herds. Of these, 120 blood samples were collected from slaughtered camels (100 males and 20 females) in an abattoir in the Al-Najaf Province and 400 blood samples were collected from camels (155 males and 245 females) that were in daily contact with ruminants during rearing or grazing. The blood samples were immediately mixed and transported to laboratory on wet ice and refrigerated overnight. The separated serum was centrifuged at 3000 g for 10 min, aliquot, labeled and stored at -20°C until further use. The sera were grouped based on the age of the camels as; under one-year group (<1y), 125 samples, 60 males and 65 females, the1-3y group, (which included 253 samples, 148 males and 105 females) and the >3y group (87 samples, 47 males and 40 females).

Detection of Antibodies Against NSP of FMDV

The sera were screened using the FMDV-ELISA kit (PrioCHECK -prionics, Lelystad B.V., Netherlands) to detect antibodies against the non-structural protein (NSP) of the FMDV. This assay was performed according to the manufacturer's instructions. The optical density was read at 450 nm, and results were expressed as percentage inhibition (PI), which was calculated according to the formula below:

$$PI = 100 - (OD\ test\ sample/OD\ Neg) \times 100$$

Sera with PI >50% were scored as positive [8].

Liquid Phase Blocking Enzyme ELISA (LPBE)

The sera that were scored as positive for NSP by ELISA were further screened using the LPBE kit (FMD World Reference Laboratory, Pirbright Institute, UK) for the detection of antibodies against structural proteins of the three FMDV serotypes (O, A, and C) according to the protocol prescribed by the manufacturer. Optical densities (OD) were read using a microplate reader at 492 nm. A positive reaction was that in which the OD was reduced by more than 50% compared with the OD of the reference antigen controls, as described previously [9].

Statistical Analysis

The obtained data were statistically analyzed for the means and significances between the groups by ANOVA using the SPSS software (IBM SPSS Statistics, version7).

RESULTS

A total of 52 sera from the camels were positive for NSP of FMDV (10%; 52\520), and a higher incidence of FMD infection was observed in female camels (11.3%; 30\265) than in males (8.6%; 22\255) *(Table 1)*.

Table 1. *Sero-positive detection of antibodies against NSP of FMD virus in dromedary camels*			
Sex	**No. of Tested Samples**	**No. of Seropositive Samples**	**%**
Male	255	22	8.6[a]
Female	265	30	11.3[b]
Total	520	52	10
Different superscript letters refer to significant variations (P≤0.05)			

Table 2. *Sero-positive detection of antibodies against NSP of FMD in camels of different age groups*			
Age in Years	**No. of Tested Samples**	**No. of Seropositive Samples**	**%**
< 1	125	14	11.2[a]
1-3	253	22	8.6b
> 3	142	16	11.2[a]
Total	520	52	10
Different superscript letters refer to significant variations (P≤ 0.05)			

Table 3. *LPBE results of the serotype analysis in the NSP-positive sera*									
Sex	**Total**	**FMD Serotypes**							
		A+	**%**	**O+**	**%**	**C+**	**%**	**-ve**	**%**
Male	22	11	50[aA]	8	36.3[Ba]	-	-	3	13.6[aC]
Female	30	19	63.3[bA]	8	26.6[bB]	-	-	3	10[aC]
Total	52	30	57.6[A]	16	30.7[B]	-	-	6	11.5[aC]
Differences in superscript small letters refer to significant vertical variation and differences in superscript capital letters refer to significant horizontal variation (P≤0.05)									

A significantly higher percentage of seropositive samples was recorded in the <1 y and >3 y groups (11.2% in both) than in the 1-3y group (8.6%; 22\253) *(Table 2)*.

The result of LPBE revealed that the FMDV serotypes A and O were detected in the NSP positive serum samples. The predominant FMDV serotype in all NSP positive sera was the A serotype (57.6%; 30\52) and 16 out of 52 sera were positive for the O serotype (30.7%; 16\52).The C serotype was not detected in the screened sera, and 6 out of 52 sera were negative for all three FMD serotypes. Further, the A serotype was predominantly detected in female camels (63.3%), as compared to males (50%) *(Table 3)*.

Higher incidence of infection was observed in camels during April (55.7%; 29\52), then in March (23.07% ;12\52), so in May (17.3%;9\52) but incidence of infection was lower during February (3.8%; 2\52). No infection was detected during June and July.

DISCUSSION

This study was the first to detect antibodies against NSP of FMDV in the sera of dromedary camels in Iraq. The result revealed that 10% of the screened sera collected from Iraqi camels exhibited sero-evidence of NSP FMD indicating the exposure of dromedaries to FMDV infection and these results are consistent with the report of the ability of dromedaries to develop specific antibodies against FMDV [10]. Findings of the present study are in agreement with other published reports on the detection of FMD antibodies in one-humped camels in other countries such as Nigeria [11] , the Kingdom of Saudi Arabia [12] and Egypt [13] using the ELISA test. It was reported that FMD infection in dromedary camels in Egypt acts as the source of infection of other susceptible animals [14]. However, many studies failed to detect FMD antibodies in camels in Sudan [15], and in the United Arab Emirates [16]. The sero-negative FMD detection and failure to isolate FMD from camels have also been previously reported and that suggest these examined camels were not exposure to FMDV, or they developed very low titer of non detectable specific antibodies [17].

In our study, NSP-FMD antibodies were detected more in female camels than in males, and camels less than a year old and more than 3 years old exhibited a higher incidence of FMD infection. Although there are no reports on the effect of age and gender on FMD infection in camels, similar results have been reported in other ruminants, such as cattle [18,19]. The physiological and hormonal differences between both sexes, such as gestation, calving, and lactation in female cattle can act as stressors that influence the immune status and decrease resistance to many microbial infections. The higher incidence FMD virus in female camels may be due to breeding practices in camel

rearing, which permit female camels to come into contact with other camels and other animals in the pasture. This along contact with infected animals in addition with handlers during milking, may underlie more exposure to FMDV caused the increased seropositivity for FMD infection in female camels.

Camel husbandry practices also permit younger camels to associate closely with other susceptible small ruminants, especially sheep (because young camels are smaller in size compared with older camels); this might increase exposure rates to the disease source. The higher incidence of seropositivity for FMD infection in aged camels could be attributed to excessive exposure times to FMD source that increased with age while younger camels are homestead and in less contact with other animals in pasture or in workplace [20].

In our study, the A and O serotypes were detected in serum samples collected from Iraqi camels with the A serotype being the predominant serotype. This is consistent with earlier published reports where FMDV serotypes A and O were detected in camels [11]. The occurrence of two FMDV serotypes A and O in ruminant population was officially registered in Iraq, Iran, Turkey, Saudi Arabia, and Jordan and the O serotype was reported in Kuwait and it was shown that the camels were affected with endemic FMDV serotypes when they are contact with the susceptible ruminants [10]. The LPBE results revealed that six sera that were positive for NSP FMD were negative for the A, O, and C serotypes, suggesting that infected camels are sero-converted but with very low titer of antibodies depending on route of infection or these samples were positive for other FMD virus serotypes that were not checked by the kit, such as SAT1 and Asia, as previously reported in Iraq [21]. On other hand, the antibodies against FMD virus serotype C was not detected in camels sera but A and O serotypes were detectable, our finding is in agreement with the study about FMD infection in ruminants in middle of Iraq [22] which indicates that the A and O FMDV serotypes are predominant and most FMD outbreak was caused by A serotype but not C serotype [23].

Interestingly, among the examined camel sera, we detected FMD virus antibodies in a higher number of samples collected in April and higher seropositive sera in the spring season. This might be because this season provides the ideal micro-environment for the viability and transmission of the FMDV, leading to increased infection among animals.

Moreover, in the spring season, the animals begin grazing on new green pastures in mixed groups and spend long periods of time in close contact with new animal herds among pastures, after the dry winter season, which might lead to higher exposure to FMD infection. The grazing system of camel herds that allows daily contact with other ruminants in the same herd and the process of grazing, buying, selling, and seasonal migration of camels throughout Iraq and into neighboring countries across borders facilitate the transmission of FMDV consequently raising the level of infection in the studied camel herds.

By analyzing the sera of the dromedary camels in three Iraqi provinces, we found that camels are susceptible to FMD infection, particularly to the A and O serotypes, and thus, could play important roles in the transmission of FMD among other domestic animals and in the epidemiology of the disease in this region.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

SHA and QHK designed the study and interpreted results. QHK and AJ were involved in sampling. SHA and QHK carried out sero-detection examination. All authors read and approved the final manuscript.

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