AENSI Journals
Advances in Environmental Biology
ISSN:1995-0756 EISSN: 1998-1066
Journal home page: http://www.aensiweb.com/aeb.html

Prevalence of Pasteurella multocida and Mycoplasma Arginini and in dromedary camel (Camelus dromedarius) in Iran: the effect of season on P. multocida prevalence

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ARTICLE INFO

Article history:
Received 15 April 2014
Received in revised form 22 May 2014
Accepted 25 May 2014
Available online 15 June 2014

Keywords:
Dromedary camel; Pneumonia; P. multocida; M. arginini; Season

ABSTRACT

Pasteurella multocida and Mycoplasma Arginini have been isolated from pneumonic lesions in camel. The aims of the present study were: 1) to determine the prevalence of P. multocida and M. Arginini, and 2) to investigate the effect of season on the prevalence of P. multocida and M. Arginini in dromedary camels. Lung samples (n = 971) were collected from pulmonary lesions of camels slaughtered in local abattoir over a 1-year period. DNA was extracted from the samples and PCR was implemented using proper primers for identification of P. multocida and M. Arginini. In total, the prevalence of P. multocida and M. Arginini was 10.7% (104/971) and 3.3% (32/971), respectively. The prevalence of P. multocida was affected by season (P = 0.016), and it was higher in the winter (43/296: 14.5%) as compared with the spring (17/224: 7.6%; adjusted odds ratio = 2.068; P = 0.017) and summer (16/226: 7.1%; adjusted odds ratio = 2.262; P = 0.008). However, the prevalence of M. Arginini was not influenced by season (P > 0.05). In addition, there was an association between the presence of P. multocida and M. arginini (Adjusted odds ratio = 2.958; P = 0.011). In conclusion, the present study substantiated the presence of P. multocida and M. arginini in pulmonary lesions in camel. Moreover, the results showed the effect of season on the prevalence of P. multocida. Additionally, an association between presence of P. multocida and M. arginini in pulmonary lesions was observed in the present study.

To Cite This Article: Ali Chitgar, Iraj Sohrabi Haghdost, Mahmood Jamshidian, Saeed Hesaraki, Prevalence of Pasteurella multocida and Mycoplasma Arginini and in dromedary camel (Camelus dromedarius) in Iran: the effect of season on P. multocida prevalence, ***Adv. Environ. Biol., 8(9),*** *792-796, 2014*

INTRODUCTION

Dromedary camel (Camelus dromedarius) is native to the unfavorable conditions of rough mountains as well as dry and desert areas. They have unique capability of adaptation to hot and arid environments [3]. As dromedary camels are able to survive under harsh conditions and still provide milk, meat and draught power [16,20], they are considered as economically important domestic animals in arid and semi-arid regions [23], including some regions of Iran [13].

Pneumonia, which could demolish the productivity of the animal [17,4], might be caused by different pathogens including viruses, bacteria and fungi [2,4]. In this context, Pasteurella multocida has been isolated from pulmonary lesions in camel [1]. In addition, Mycoplasma Arginini has been observed to be associated with pneumonia in camel. Nevertheless, little information is available on the epidemiology of either the aforementioned bacteria or other pathogens contributing to development of pneumonia in camel [4,1,21]. Therefore, the present study was conducted to determine the prevalence of P. multocida and M. Arginini as well as the effect of season on the prevalence of each microorganism in camels slaughtered at an abattoir in Tehran province, Iran.

MATERIAL AND METHODS

2.1. Sample collection:

Lung samples (n = 971) were collected from pulmonary lesions of dromedary camels slaughtered at an abattoir located in Shahr Rey, Tehran, Iran over a 1-year period from October 2012 to September 2013. Samples were immediately transported to laboratory after collection and were maintained at -80 ºC until further analysis.

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2.2. DNA extraction:

The sample was harvested in 50 µl of STES buffer (Tris-Hcl 0.2M, NaCl 0.5M, SDS 0.1% and EDTA 0.01M) and homogenized by vortexing. A 20 µl TE buffer and 60 µl of Phenol- chloroform (1:1) were added and vortexed vigorously for one min followed by centrifuging for 5 min at 13000 rpm. The upper aqueous phase was removed and mixed thoroughly with 40 µl of sodium acetate (3M) and 100 µl of absolute ethanol and left at -20 °C for 30 min. The mixture was centrifuged and the pellet was washed with 1ml of 70% ethanol and air dried. DNA was resuspended in 50 µl TE buffer (pH 8.3) and stored at -20 °C.

2.3. PCR assay:

Amplification of P. multocida putative transcriptional regulator genes and M. arginini 16S-23S rRNA intergenic spacer regions was performed using appropriate primers (Kong *et al*. 2001; Liu *et al*. 2004; Table 1).

Table 1. Sequences of oligonucleotide primers for genes used to identify P. multocida and M. arginini.

Microorganism	Target and primer name	Primer sequence (5'–3')
P. multocida	Pm0762 (forward)	TTGTGCAGTTCCGCAAATAA
	Pm0762 (reverse)	TTCACCTGCAACAGCAAGAC
M. arginini	MAS	CCGTAAACGATGATCATTAGTCGGTGGAGAGTTC
	MAA	CGATATTCAGTTTTCAAAGAACAAATTGAGAGATAGGTC

PCR was performed in a total volume of 25µL containing 10 ng of extracted DNAs, 2.5 µL of 10X PCR buffer (Cinagen, Tehran, Iran), 1.25 mmol / L (1.5 mmol / L for M. arginini) of $MgCl_2$, 100µmol/L of each d NTP (Cinagen), 1 U of Taq DNA polymerase (Cinagen), and 0.5l mol/L of each primer. Amplification steps for P. multocida included: an initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 40 sec, annealing at 55 °C for 20 sec, 72 °C for 45 sec and the final extension at 72 °C for 10 min. the PCR program for M.arginini consisted of an initial denaturation at 96 °C for 2 min, followed by 33 cycles of 96 °C for 30 sec, annealing at 58 °C for 1min, 74 °C for 1 min and the final extension at 72 °C for 10 min. PCR products were visualized on agarose gel (1%).

2.4. Statistical analysis:

The effects of season on the prevalence of P. multocida and M. Arginini were analyzed using GLIMMIX procedure including function link logit in the model, in which season and identification of P. multocida (in the analysis of M. Arginini prevalence) and M. Arginini (in the analysis of P. multocida prevalence) were considered as fixed effects and month of year was incorporated as random effect. LSMEANS statement was used to perform multiple comparisons in case there was any effect of season. Multivariable logistic regression analyses produced and adjusted odds ratios (AORs) as the estimates of strength of difference between values of prevalence. All analyses were conducted in SAS (SAS, 2008). Differences at $P \leq 0.05$ were considered statistically significant, and $0.05 < P \leq 0.10$ were designated as a tendency to differ.

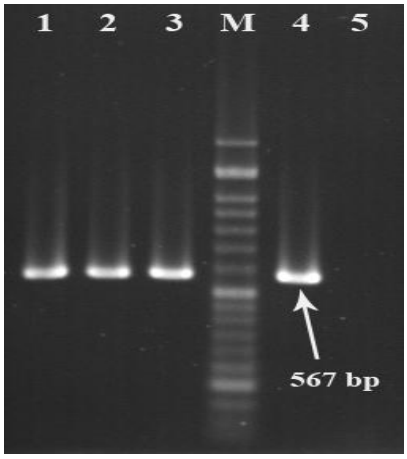
3. Results:

P. multocida was identified in 10.7% (104/971) of the lung samples (Fig. 1). Season influenced the prevalence of P. multocida (P = 0.016; Table 2).

Table 2: Estimated adjusted odds ratios (AORs) and their 95% confidence intervals (CI) for the effects of season and M. arginini the prevalence of P. multocida. Numbers in parentheses are actual numbers.

Effect	Class	Prevalence of P. multocida (%)	AOR	95% CI	P-value
Season	Spring	7.6 (17/224)	2.068	1.142-3.742	0.016
	Summer	7.1 (16/226)	2.262	1.234-4.144	—
	Fall	12.4 (28/225)	1.215	0.727-2.033	—
	Winter	14.5 (43/296)	—	—	—
M. arginini	Positive	25.0 (8/32)	2.958	1.280-6.838	0.011
	Negative	10.2 (96/939)	—	—	—

The prevalence of P. multocida in the winter (14.5%) was higher than that in the spring (7.6%; AOR = 2.068; P = 0.017) and summer (7.1%; AOR = 2.262; P = 0.008; Table 2). The prevalence of P. multocida in the fall (12.4%) was tended to be higher than that in the summer (AOR = 1.861; 95% confidence = 0.975-3.553; P = 0.060; Table 2). However, other differences in the prevalence of P. multocida between seasons were not significant (P > 0.05). In addition, the prevalence of P. multocida was associated with M. arginini being identified (AOR = 2.958; P = 0.011; Table 2).



The prevalence of M. arginini was 3.3% (32/971) in camel lung samples (Fig. 2). The prevalence of M. arginini did not differ among spring (2.7%), summer (3.5%), fall (4.0%) and winter (3.0%; P > 0.05; Table 3).

Table 3: Estimated adjusted odds ratios (AORs) and their 95% confidence intervals (CI) for the effects of season and P. multocida the prevalence of M. arginini. Numbers in parentheses are actual numbers.

Effect	Class	Prevalence of M. arginini (%)	AOR	95% CI	P-value
Season	Spring	2.7 (6/224)	0.978	0.339-2.817	0.866
	Summer	3.5 (8/226)	1.318	0.494-3.509	—
	Fall	4.0 (9/225)	1.376	0.533-3.546	—
	Winter	3.0 (9/296)	—	—	—
P. multocida	Positive	7.7 (8/104)	2.958	1.280-6.838	0.011
	Negative	2.8 (24/867)	—	—	—

But the prevalence of M. arginini was associated with P. multocida being identified (AOR = 2.958; P = 0.011; Table 3).

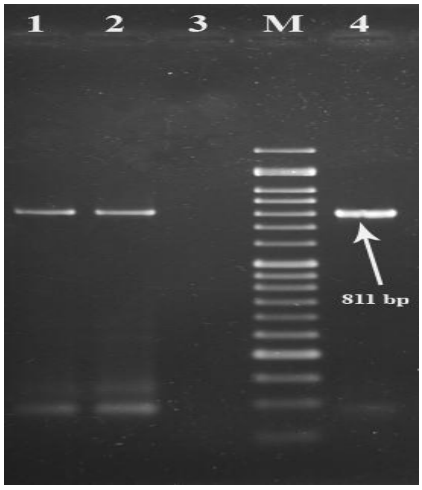


Fig. 1: Results of PCR amplification using primers of gene Pm0762 used for identification of P. multocida.

Discussion:

The present study revealed the presence of P. multocida in 10.7% of camel lung samples. Abubakar *et al*. [1] isolated P. multocida from 4.4% of pulmonary lesions of dromedary camels in Nigeria. In contrast, investigating bacterial agents associated with pneumonia in camels in Jordan, Al-Tarazi, [2] detected P. multocida in none of the specimens examined. P. multocida has also been observed to be associated with pneumonia in other ruminants including cattle [22,15], sheep [11] and goat [9] as well. Odugbo *et al*. [11] reported the prevalence of 9.4% for P. multocida in pneumonic samples in sheep.

The risk for detection of P. multocida was higher in the winter in the present study. It has been observed that high relative humidity could increase the survival of P. multocida in the environment [19]. Given that the relative humidity is highest during the winter in the area, where samples were collected [24], it could be speculated that high relative humidity in the winter might have contributed to the higher prevalence of P.

multocida during the winter in the present study. Alternatively, the higher prevalence of P. multocida could be attributed to the accommodation system of camels during the cold season. Considering the cold weather and poor pastures during the cold season, camels are handfed and maintained in drylot during the most of the fall and the entire winter, which would increase the accommodation density of camels. High-density accommodation has been indicated to result in accumulation of gaseous ammonia [12]. Exposure to ammonia causes irritation of respiratory tract, thereby predisposing animals to pulmonary infections [12]. Moreover, exposure to gaseous ammonia has been found to increase the growth and survival of P. multocida in the respiratory tracts of pigs [6]. Accordingly, the higher prevalence of P. multocida in the winter and to some extent in the fall could be attributed to the potential higher exposure to ammonia.

The prevalence of M. arginini was 3.3% in the present study. Elfaki *et al*.[4] isolated M. arginini from 8.8% of pulmonary lesions in camels. Presence of M. arginini in lung is not limited to camels and has been reported in other ruminant species including cattle [15], sheep [7,18] and goat [5] as well. Tabatabayi *et al*. [18] isolated M. arginini from 79% of ovine pneumonic lung in Iran. The present study revealed an association between presence of P. multocida and M. arginini in pulmonary lesions of dromedary camels. Jones *et al*. [7] found no association between the frequency of M. arginini isolation and the degree of pulmonary pathological change in sheep. Further, endobronchially inoculating goats with M. arginini, Goltz *et al*. [5] observed that M. arginini challenge failed to cause pulmonary lesions but resulted in temporary elevation in monocytes, neutrophils and fibrinogen, and concluded that M. arginini might not be a primary pathogen in terms of caprine pneumonia. Elfaki *et al*. [4], as similar to the present study, only investigated lung samples with pulmonary lesions for detection of M. arginini. To our knowledge, no study has assessed normal healthy lung specimens for detection of M. arginini. It seems that the pathogenicity of M. arginini with regard to pneumonia in camel requires to be elucidated by further controlled studies in which the presence of M. arginini to be investigated in both pneumonic and normal health lung tissues. On the other hand, intratracheal inoculation of P. multocida has been indicated to result in typical pneumonia in sheep [11]. In addition, Abubakar *et al*. [1] detected P. multocida merely in specimens associated with pulmonary lesions and no P. multocida was found in normal lung tissue in camel, implying that P. multocida could play a role as a pathogen with regard to camel pneumonia. Nevertheless, the association of P. multocida and M. arginini in camel remains to be investigated by further studies.

In conclusion, the prevalence of P. multocida and M. arginini was 10.7% and 3.3%, respectively. The prevalence of P. multocida was higher during the cold season, which could be attributed to the cold weather and/or housing system during the cold season, both of which should be considered by farmers in order to decrease the prevalence of pneumonia in camels, thus improving the productivity of animals. Moreover, there was association between the presence of P. multocida and M. arginini in pulmonary lesions, which needs further studies to be elucidated.

ACKNOWLEDGEMENT

The authors wish to thank Dr. P. Mortazavi and Mr. M. Maleki for his kind assistance in the present study.

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