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MILK AMYLOID A – A POTENTIAL BIOMARKER TO DETECT SUBCLINICAL MASTITIS IN LACTATING DROMEDARY CAMEL

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ABSTRACT

The objective of the present study was to investigate the possible use of Milk Amyloid A (MAA) as a sensitive biomarker to detect subclinical mastitis in the lactating dromedary camel. Quarter milk samples (n=120), from 65 milking dromedary camels, were collected to evaluate somatic cell count (SCC) and MAA. At the area under the curve of 0.859 for MAA (P<0.001) and cut off points of 306000 cells/ml and 1040 ng/ml for SCC and MAA, respectively. The sensitivity and specificity of the MAA test to detect subclinical mastitis were 100 and 43.9%, respectively. In conclusion, it might be possible to use MAA measurement, as screening test, for early detection of sub-clinical mastitis in dromedary camel.

Key words: Camel, milk amyloid A, somatic cell count, subclinical mastitis

Prevalence of mastitis in camel was assumed to be low, due to the thin streak canal, covering udder to restrict suckling (Manefield and Tinson, 1996; Wernery and Kadden, 2002), the least contact of udder to contaminated bed throughout rest period (personal observation), the low density of population scattered throughout the pasture and finally the common practice of hand milking rather than machine milking. Although, machine milking has been adopted for camel in very few countries (Nagy *et al,* 2013; Yagil, 1982), dairy camel industry still depends on hand milking in most countries worldwide. It is anticipated that with the development of machine milking, problems associated with mastitis could rise in dairy camel similar to other milk-producing animals. Regardless of anatomical and environmental factors which may help to reduce subclinical mastitis in camel, the prevalence of subclinical mastitis, based on quarter infection rates, varied from 15-67.4%; among different studies (Alamin *et al,* 2013; Abera *et al,* 2010, Bhatt *et al,* 2004; Seifu and Tafesse, 2010).

Somatic Cell Count (SCC) is still the gold standard method to detect mastitis. It has been shown that the correlation between the severity of mastitis and SCC may not be always good (Schepers *et al,* 1997). It is important to know that the sensitivity and specificity of SCC in detection of subclinical mastitis is not high enough and bacterial culture is a labour-intensive and time-consuming technique (Shirazi-Beheshtiha *et al,* 2012). Therefore, new biomarkers with higher diagnostic value and faster turn around times in detecting subclinical mastitis are needed (Akerstedt *et al,* 2007; Shirazi-Beheshtiha *et al,* 2012).

Acute phase proteins (APPs) are a group of proteins whose plasma concentrations increase (positive APPs) or decrease (negative APPs) during the systemic acute phase response which occurs following stress or local tissue inflammation such as infection, injury, trauma, or other tissue necrosis (Baumann and Gauldie, 1994; Gabay and Kushner, 1999; Murata *et al,* 2004). Serum amyloid A, a major

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bovine positive APP, is produced mainly by the liver. It has antimicrobial function through attaching on gram-negative bacteria and thereby facilitating phagocytosis (Larson *et al,* 2005). In the last several years, there has been considerable progress on milk APPs including milk amyloid A (MAA), milk albumin, α-lactoglobulin and immunoglobulin (Ig). Amyloid A is also produced by the mammary gland and therefore it is present in milk from dairy animals. It has been suggested that intra-mammary synthesis of MAA is increased during mastitis (McDonald *et al,* 2001). MAA has been suggested as a good biomarker for early identification of subclinical mastitis (Grönlund *et al,* 2003; Safi *et al,* 2009; Gerardi *et al,* 2009) and its diagnostic value was determined in bovine subclinical mastitis (Shirazi-Beheshtiha *et al,* 2012). Many efforts have been invested to find alternative biomarkers to replace or complement SCC, e.g. milk haptoglobin and whey proteins (Akerstedt *et al,* 2007; Shirazi-Beheshtiha *et al,* 2012). The objective of the present study was to investigate the diagnostic value of MAA for identifying subclinical mastitis in dromedary camel.

Materials and Methods

Experimental location and animals

This study was conducted during 2011-2012 between months May and June, in Golestan Province (longitude: 37°1′35" N; latitude: 54°13′17"E; altitude: 0 m) of I.R. Iran, as the main camel milk producing province within the country. Dromedary milking camels (n=65), 7-11 years of age, 2-4 months after calving, with the average daily milk production of 6 kg were used in this study. They were milked thrice daily (5:00, 16:00, 21:00 Hrs) and maintained on pasture throughout the day.

Experimental design

Camel milk samples were collected from individual quarters (n=120) of 65 apparently healthy milking camels without observable clinical signs of mastitis. Five minutes prior to milking, camel received oxytocin (20 I.U, IM), the teats were cleaned and the camel calf released to stimulate the dam. After discarding the first few squirts of milk, about 50 ml of milk were collected into sterile bottle for SCC estimation. Samples were kept on ice and transported to the laboratory. In the laboratory, samples assigned into 2 tubes. For SCC estimation, samples were examined freshly within 8 hours after milk collection. Samples for MAA measurement were frozen at -20°C until assay.

Somatic Cell Count

Milk samples for SCC were collected into the tube with potassium dichromate (Floka, Boches, Switzerland). Somatic cells were counted using Fossomatic machine (Fossomatic 5000, Fossomatic Company, Denmark). Prior to SCC, standard sample, consisting 389,000 cells, was used to calibrate the machine. Samples of 25 ml volume were assigned into special racks and allowed to be automatically homogenised and counted individually by the detector.

Assay for determination of MAA

MAA concentration was measured using a commercial ELISA kit (Mast ID RANGE Milk Amyloid A Assay, cat TP-807, Tridelta Development Ltd, Wicklow, Ireland) according to the manufacturer's instruction. The optical density of samples was measured using an automated plate reader (Model ELX 800; Bio-Tek Inc., VT, USA). Assay was validated for camel milk and the sensitivity of assay was 0.1 ng/ml.

Statistical analysis

All statistical analyses were performed using SPSS statistical software version 16 (SPSS, 2007). The SCC was considered as the gold standard test. To achieve high diagnostic sensitivity and specificity for the diagnosis of subclinical mastitis, different cutoff points were selected for SCC (51000, 108000 and 306000 cell/ml) using receiver operating characteristic (ROC) analysis, and the area under the ROC curve (AUC) was estimated for MAA. AUC of 0.5-0.7, 0.7-0.9 and >0.9 were considered as low, intermediate and high clinical accuracy to detect subclinical mastitis using MAA, respectively (Gardner and Greiner, 2006).

Results

Using SCC as a gold standard, several cutoff points for MAA with high sensitivity (approx. 90%), high specificity (approx. 90%) and moderate sensitivity (approx. 50%) and specificity (approx. 50%) were estimated. In this estimation, the SCC of 51,000 cells/mL, 108000 cells/ml and 306000 cells/ml, were considered as cutoff points for SCC. At the cutoff points of SCC with 51000 and 108000 cells/ml, the clinical accuracies for MAA were not significant (P>0.05, Table 1). At SCC of 306,000 cells/ml, the clinical accuracy of MAA was 0.859 (P=0.0001, Table 1). Accordingly, the respective sensitivity and specificity of MAA to detect subclinical mastitis in camel at the cutoff point of 1040 ng/ml (100 and

Table 1. Camel milk amyloid A (MAA) for diagnosis of subclinical mastitis based on somatic cell count (SCC) and bacterial culture results in camels.

Parameter	Cut off point (ng/ml)	Specificity(%)	Sensitivity (%)	False negative (%)	False positive (%)
CA (MAA):0.524 (P=0.644) Cut off for SCC: 51000 cell/ml	318	8.6	90.3	9.7	91.4
	1419	55.2	51.6	48.4	44.8
	4969.5	91.4	17.7	82.3	8.6
CA (MAA): 0.575 (P=0.176) Cut off for SCC: 108000 cell/ml	318	8.9	90.2	9.8	91.1
	1314.5	53.2	61	39	46.8
	5325	94.9	24.4	75.6	5.1
CA (MAA): 0.859 (P=0.0001) Cut off for SCC: 306000 cell/ml	1040	43.9	100	0	56.1
	3644.5	87.9	69.2	30.8	12.1
	13084.5	99.1	46.2	53.8	0.9

43.9%), 3644.5 ng/ml (69.2 and 87.9%) and 13084.5 ng/ml (46.2 and 99.1%) were estimated (Table 1).

Discussion

The objective of the present study was to investigate the possibility of using milk amyloid A protein (MAA) as an early biomarker to detect subclinical mastitis in dromedary camel considering somatic cell Counts (SCC) as a gold standard. With respect to the area under the curve (AUC) of 0.859 for MAA (P<0.0001) and the cut off points of 306000 cells/ml and 1040 ng/ml for SCC and MAA, respectively, the sensitivity and specificity of the MAA test to detect subclinical mastitis in camel were 100 and 43.9%, respectively. SCC is still the gold standard used for detection of subclinical mastitis and it is an important factor in milk quality (Akerstedt *et al,* 2007). Since the sensitivity and specificity of SCC is low and a suitable SCC cut off for detection of camel subclinical mastitis was not determined, new more sensitive and specific biomarkers are needed.

The acute phase proteins have not been studied in camel milk. Since they are rapidly leaked from blood into the milk during udder inflammation, it makes them even more interesting and reliable as early markers for mastitis, especially in the subclinical forms. There is no information about the relationship between MAA and SCC in camel. More recently, we have elaborated the cut-off point of SCC, as gold standard (306,000 cells/ml), to detect subclinical mastitis in dromedary camel (un-published data). MAA demonstrated a strong sensitivity (100%) to detect subclinical mastitis but moderate specificity (43.9%) to detect healthy udders. In order to achieve high diagnostic sensitivity and specificity, different cutoff points were tested for MAA using ROC analysis. The respective AUC of >0.9, 0.7-0.9 and 0.5-0.7 were considered as high, moderate and low accuracy in diagnostic tests (Gardner and Greiner, 2006). MAA at the cutoff point of >1640 ng/ml with clinical accuracy of 95%, had sensitivity of 90.6% and specificity of 98.3% in diagnosing subclinical mastitis in cattle (Safi *et al,* 2009). MAA at the cut off point of >1600 ng/ml, had a high diagnostic sensitivity (92.3%) and specificity (92.1%) to detect sub-clinical mastitis in dairy cows (Shirazi-Beheshtiha *et al,* 2012). The difference between the clinical accuracy of MAA reported in cattle and what we reported in the present study could be attributed to the fact that SCC is not a suitable gold standard in camel. Although using SCC as the gold standard in cattle is under a question in many papers, but it seems that there is a higher correlation between SCC and MAA concentrations in cattle compared to camel.

Subclinical mastitis could be a major concern in camel. To our knowledge, the diagnostic value and cutoff points of MAA for subclinical mastitis in camel have never been reported. This is the first study describing MAA as a potential biomarker for detecting subclinical mastitis in camel. The results of the present study showed that the MAA test at the cutoff value of 1040 ng/ml has high diagnostic sensitivity (100%) and moderate diagnostic specificity for detection of subclinical mastitis in dairy camels and therefore, it could be used as a reliable alternative or complement test to the routine SCC in the early diagnosis of camel subclinical mastitis.

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