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Research Article

Practicability of somatic cell count and electrical conductivity as subclinical mastitis diagnostic tests in camels (*Camelus dromedarius*)

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ABSTRACT: Critical gaps exist in our understanding of the diagnostic reliability of subclinical mastitis tests in dromedary camels. Using a retrospective longitudinal cross-sectional approach, 191 lactating camels were randomly sampled from 47 camel herds to investigate at first the practicability of somatic cell count (SCC) and electrical conductivity (EC) tests as subclinical mastitis tests in camels through their validations by California mastitis test (CMT) score, and then through the subsequent employments of those objective means in assessing certain potential risk markers predisposing camels to this disease. Results indicate the reliability and validity of SCC test, in contrast to EC test, in distinguishing subclinical mastitic udders in camels, as demonstrated by the strong interrelationships (r = 0.83 *vs* 0.12; R^2 = 0.80 *vs* 0.02), excellent agreement beyond chance (kappa coefficient = 0.76 *vs* 0.09) between SCC test and CMT scores, as well as by the high sensitivity of SCC test [Area Under Curve (AUC) = 0.94 *vs* 0.48] in distinguishing mastitic udders compared to the EC test. Based on the SCC test, the calculated overall prevalence rate for subclinical mastitis was 35 %, and the breed, parity, and lactation period were the only risk markers predisposing camels to subclinical mastitis. Collectively, it can be concluded that the objective SCC test possesses considerable diagnostic merit for early detection of subclinical mastitis in camels, while the EC test was non-satisfactory and non-diagnostic. Accordingly, it seems logical to base herd management decisions on SCC readings using the cut-off Log_{10}SCC value of 5.67 (or SCC = 472.50 × 0^3 cells mL^{-1}).

Keywords: dairy, milk production, risk marker, human consumption, welfare

Introduction

Mastitis is a complex and multi-etiologic public health disease that is usually associated with suppressed milk production, altered milk composition, impaired preservation and processing, and increased veterinary costs and culling rates, as well as impacted product hygiene and security (Abdelgadir, 2014; Nagy et al., 2013). Heavy biological and economical losses are inevitable unless early and effectual methods of detection are used. However, detecting and treating mastitis greatly depends on the accuracy and effectiveness of the diagnosis.

According to the pathological signs, the inflammatory reaction in the parenchymal tissue of the mammary gland is mainly divided into clinical and subclinical forms (Constable et al., 2016). Several cases of both forms have been reported in camels (Al-Juboori et al., 2013; Al-Salihi et al., 2017; Hawari and Hassawi, 2008; Tuteja et al., 2003). While the clinical form of mastitis is self-evident and easily diagnosed, the subclinical form requires an indirect means of diagnosis. The California mastitis test (CMT) is widely considered the most validated field test -based on microbiological testing- to determine the degree of mammary gland inflammation and microbiological infection in lactating camels (Al-Dughaym and Fadlelmula, 2015; Saleh and Faye, 2011), where important management decisions regarding the cost-effective prevention and control of mastitis are fundamentally based on this test (Abdelgadir, 2014; Salah et al., 2013). Nevertheless, owing to the subjectivity of the CMT, there has been a great deal of research investigating the employment of more objective means of detecting subclinical mastitis and infection status in lactating camels, such as somatic cell count (SCC) and electrical conductivity (EC) tests (Nagy et al., 2013; Samara et al., 2014). There is a dearth of information on the basal levels and reliability of those objective tests in camels, which suggests a non-satisfactory, unrefined, questionable practicability, and emphasizes the need for further studies to clarify these issues. Therefore, the question of whether SCC and/or EC tests may have any reliable agreement with the CMT in diagnosing subclinical mastitic cases will be approached and examined herein.

Moreover, compared to other ruminants, few published reports have examined the conditions or evaluated the risk markers for subclinical mastitis in lactating camels (Aljumaah et al., 2011; Saleh et al., 2013). Employing objective means like SCC and EC tests to understand such marker–disease association, after been validated by the CMT score, may subsequently give us more insight into the potential predisposing markers for developing positive mastitis cases in camels. The outcomes are expected to enhance our planning of mastitis control programs with implications for the security of dairy industry.

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Materials and Methods

Study area description

This study was conducted in Riyadh province, the second largest of the 13 provinces of Saudi Arabia in terms of area. Riyadh is located in the center of the country at an altitude of 612 m above sea level between latitude 24°41′15″ N and longitude 46°43′18″ E, and contains the national capital, the city of Riyadh.

According to the recent results of the agricultural census published by the Saudi General Authority for Statistics, the total number of dromedary camels in Saudi Arabia is about 1.4 million, of which 33 % (about 500,000) are located in Riyadh Province followed by the Eastern Province (18 %) and Makkah Province (14 %). Furthermore, lactating camels (i.e., four years old and above) represented 46 % of camel females, and indigenous breeds were the highest among the exotic and hybrid breeds with 98 % of the total number (SGAS, 2015).

Study design

This observational study was based on cross-sectional surveys conducted as part of a larger project started back in 2007, aiming to understand the complex interplay of markers associated with the prevalence of subclinical mastitis in lactating dromedary camels in Saudi Arabia. The necessary camel size for the study was determined by using the sample-size formula recommended by Thrusfield (2005), where a Z value of 1.44, confidence level of 85 %, absolute precision of 5 %, and expected prevalence rate of subclinical mastitis of 33 % (Aljumaah et al., 2011) were all used. In total, 191 lactating camels were randomly sampled by employing a simple random sampling method from 47 camel herds fundamentally selected based on the accessibility and willingness of owners, where we believe that the sampled population was representative of the available herds in the region. Herd sizes ranged from 10-267 camels, while the daily milk production ranged between 4-15 kg.

The study drew on the superiority, legitimacy, and validity of the CMT in detecting subclinical mastitis in lactating camels, whereby agreements between the CMT, SCC, and EC tests for diagnosing subclinical mastitis were first determined. The practicability of the SCC and EC tests was then investigated in more detail by looking at multiple variables. For this, camel attributes that may be associated with developing subclinical mastitic cases included the type of husbandry system, breed, parity number, and lactation period for each sampled animal.

It is worthwhile to mention that 1) the records were obtained using a questionnaire designed specifically for the present study; 2) written consents were obtained from all owners; 3) interviewer and courtesy biases were reduced by allowing the owners to give their free opinions to questions needed to collect the required parameters, in addition to other questions; 4) data from all questionnaires were verified, rechecked, and filtered by two individuals; and 5) the whole project was carried out in accordance with the current laws on animal welfare and research in Saudi Arabia, and were approved by the internal Research Ethics Committee at the College of Food and Agriculture Sciences of King Saud University (N°. 28-49005791).

Sample analyses

Before sampling, all camels were subjected to physical examinations (visualization/palpation), rectal temperature measurements, and blood sample testing to identify any cases of overt clinical mastitis or systemic infections. Any animal with a clinical problem was excluded from the study. Therefore, only clinically healthy lactating camels were sampled.

Immediately post-clinical examination, milk samples (100 mL) were collected from each quarter in sterilized universal bottles after disinfecting the teats and discarding the first milk jets (about 10 mL). Collected samples were placed inside an ice box and immediately transferred to the laboratory to be analyzed. Within approximately 12 h after collection, milk samples were processed for CMT (Bovi-Vet, Kruuse, Germany), SCC (Fossomatic™, Minor, Foss, DK-3400 Hillerod, Denmark), and EC (Direct-ION, Dover, Kent, UK) by two trained technicians. To reduce any bias, these technicians were supervised to make sure that they adhered to the manufacturer's instructions in carrying out these tests.

Data preparation and statistical analyses

In total, 758 quarter-milk examinations were performed. Samples with negative or trace CMT scores (-ve; *t*) were, at first, deemed to be healthy quarters (i.e., CMT score = 0), while those with positive CMT scores (i.e., +1, +2, and +3) were considered subclinically affected quarters (Constable et al., 2016). The obtained data were analyzed using SAS (Statistical Analysis System version 9.1), where the PROC MEANS procedure was used to obtain the descriptive statistics of the CMT, SCC, and EC test results, in addition to the SCC and EC test results by the CMT score. Notably, the obtained distribution of SCC was positively skewed (i.e., to the right, Table 1); and therefore, logarithmic transformation (base 10) was used to reduce the skewness and improve the normality of the SCC values. In fact, $\log_{10}$SCC values were used in all analyses.

Differences in the overall means of $\log_{10}$SCC and EC tests by the CMT score (0, +1, +2, and +3) were determined by using the PROC GLM procedure, while differences in these means as influenced by different risk markers were determined using the PROC MIXED procedure. Mean differences, in both cases, were elaborated using the PDIFF option. The interrelationships among the CMT, $\log_{10}$SCC, and EC test results were attained using the PROC CORR and PROC REG procedures.

Table 1 – Descriptive analysis of somatic cell count (SCC) and electrical conductivity (EC) test results as classified by the California mastitis test (CMT) in lactating dromedary camels.

Test[1]	Descriptive analysis							
	Mean	Minimum	Maximum	Standard Deviation	Standard Error	Coefficient of Variation	Skewness	Kurtosis
CMT 0 (n = 510)								
SCC, $\times 10^3$cells mL^{-1}	233.46	10.00	2870.00	253.64	11.23	108.64	5.26	46.42
Log_{10}SCC	5.20	4.00	6.46	0.40	0.02	7.72	-0.34	0.14
EC, mS cm^{-1}	7.34	3.63	11.60	1.59	0.07	21.64	-0.03	-0.29
CMT +1 (n = 141)								
SCC, $\times 10^3$cells mL^{-1}	685.48	90.00	1858.00	356.10	29.99	51.95	0.90	0.69
Log_{10}SCC	5.77	4.95	6.27	0.24	0.02	4.21	-0.49	0.18
EC, mS cm^{-1}	6.88	4.11	12.33	1.61	0.14	23.44	0.60	0.12
CMT +2 (n = 67)								
SCC, $\times 10^3$cells mL^{-1}	1468.21	523.00	5013.00	770.08	94.08	52.45	1.98	5.92
Log_{10}SCC	6.12	5.72	6.70	0.20	0.02	3.23	0.45	0.08
EC, mS cm^{-1}	7.68	3.72	13.48	1.99	0.24	25.85	0.22	-0.06
CMT +3 (n = 40)								
SCC, $\times 10^3$cells mL^{-1}	3855.58	2155.00	6835.00	1090.63	172.44	28.29	0.68	-0.05
Log_{10}SCC	6.57	6.33	6.83	0.12	0.02	1.83	0.14	-0.76
EC, mS cm^{-1}	8.45	4.34	12.72	1.83	0.29	21.69	-0.07	-0.44

[1]n = number of udder quarters tested.

The receiver operating characteristic (ROC) analysis was also performed, as attained by the CMT score, for the SCC and EC tests to compare their sensitivity, specificity, positive and negative likelihood ratios, and positive and negative predictive values for detecting subclinical mastitis in camels, using the SigmaPlot software (SigmaPlot v12.0).

Additionally, the kappa statistic was used to assess the agreement between these indicators beyond chance alone, where agreements between these tests were regarded as evidence of validity. The kappa coefficient (KC) ranges from 1 (complete agreement) to 0 (no agreement); a KC > 0.75 represented excellent agreement beyond chance, a KC < 0.40 represented poor agreement, while a KC in the range of 0.40-0.75 represents intermediate to good agreement (Landis and Koch, 1977). The following defined cut-off levels were used: CMT scores of 0 were assigned as negative (-ve), while those with positive CMT scores were assigned as positive (+ve); log_{10}SCC values of < 5.67 (as retrieved by ROC analysis) were assigned as negative (-ve), while those with log_{10}SCC values of ≥ 5.67 were assigned as positive (+ve); and EC values of < 8.44 mS cm^{-1} (as retrieved by ROC analysis) were assigned as negative (-ve), while those with EC values of ≥ 8.44 mS cm^{-1} were assigned as positive (+ve).

On the other hand, univariate and multivariate analyses of logistic regression were used to determine the relative risk of developing subclinical mastitis for different markers using the PROC LOGISTIC procedure. Odds ratio (OR) estimates were calculated according to the SCC and EC tests, using 5.67 and 8.44 mS cm^{-1} as the cut-off values, respectively. The probability value, which denotes statistical significance, was declared at $p < 0.05$ throughout the study.

Results

Concordance between the CMT, SCC, and EC tests for subclinical mastitis diagnosis

The results revealed that the mean values (± SE) for CMT, SCC (10^3 cells mL^{-1}), log_{10}SCC, and EC (mS cm^{-1}) were 0.55 ± 0.03, 624.01 ± 34.91, 5.46 ± 0.02, and 7.35 ± 0.06, respectively, while the median values were 0, 285.01, 5.46, and 7.47, respectively. There were no differences in overall means of the measured CMT ($p < 0.67$), SCC ($p < 0.63$) and EC ($p < 0.92$) scores as influenced by udder quarter, where the mean values (± SE) for the left-rear, right-rear, left-front, and right-front quarters were 0.60, 0.50, 0.58, 0.53 (± 0.07) for CMT score, 5.48, 5.42, 5.48, 5.47 (± 0.04) for log_{10}SCC, and 7.33, 7.31, 7.35, 7.43 (± 0.13) for EC (mS cm^{-1}) (data not shown). Moreover, simple descriptive analyses of the obtained SCC and EC data, as classified by the CMT score, showed that the SCC data were positively skewed, while EC data were normally distributed (Table 1). The logarithmic transformation improved the normality of the SCC values, as presented in Table 1. For quarters with CMT scores of negative (n = 510), +1 (n = 141), +2 (n = 67), and +3 (n = 40), the overall means of the log_{10}SCC and EC (mS cm^{-1}) values were 5.20, 5.77, 6.12, 6.57, and 7.34, 6.88, 7.68, 8.45, respectively (Table 1).

The results also demonstrated that the overall means of SCC and EC were generally increased ($p < 0.001$) as the CMT score increased (Table 2). Notably, however, subclinical mastitic samples (i.e., CMT scores of +1, +2, and +3) had an average log_{10}SCC mean value (± SE) of 6.01 ± 0.02, which was greater ($p < 0.001$) than that of healthy samples, while subclinical mastitic samples had an average EC mean value (±

SE) of 7.39 ± 0.11 mS cm^{-1}, which was not different ($p > 0.70$) than that of healthy samples (Table 2). Accordingly, a high positive correlation was obtained between the CMT score and $\log_{10}$SCC ($r = 0.83$; $p < 0.001$), while a weak positive relationship was observed between the CMT score and EC ($r = 0.12$; $p < 0.001$). The relationship between the $\log_{10}$SCC and EC was surprisingly found to be a weak negative correlation, but not significant ($r = -0.03$; $p < 0.44$). Furthermore, the obtained results of regression analyses showed that $\log_{10}$SCC was linearly increased as the CMT score increased [$\log_{10}$SCC = 5.22 + 0.47 × CMT score; $R^2 = 0.57$; $p < 0.001$], and the EC linearly increased as the CMT score increased, but only very slightly, according to the obtained coefficient of determination [EC = 7.22 + 0.23 × CMT score; $R^2 = 0.02$; $p < 0.001$]. However, the relationship between the SCC test and CMT scores was found to be exponential, where SCC was exponentially increased as the CMT score increased [SCC (10^3 cells mL^{-1}) = 246.81 × $\exp^{(0.91 \times \text{CMT score})}$] ($R^2 = 0.80$; $p < 0.001$).

The reliability of both the SCC and EC tests in detecting subclinical mastitis was further compared with the CMT score using ROC analysis (Table 3 and 4). When subclinical mastitis was defined according to CMT score as noted before, the area under the ROC curve was 0.94 (0.92-0.96, 95 % CI) *vs.* 0.48 (0.44-0.53), cut-off value was 5.67 (in $\log_{10}$) *vs.* 8.44 (in mS cm^{-1}), sensitivity was 0.86 (0.81-0.90) *vs.* 0.31 (0.26-0.38), specificity was 0.91 (0.88-0.94) *vs.* 0.76 (0.71-0.79), positive likelihood ratios was 9.74 *vs.* 1.28, negative likelihood ratios was 0.16 *vs.* 0.90, positive predictive values was 0.91 *vs.* 0.56, and negative predictive values was 0.87 *vs.* 0.52 for SCC *vs.* EC tests, respectively (Table 3 and 4, Figure 1A and B).

The kappa statistic was also used to assess the validity of these indicators. The KC estimated indicated an excellent agreement between the SCC test and CMT scores (KC = 0.76, $p < 0.001$), a poor agreement between the EC test and CMT scores (KC = 0.09, $p < 0.02$), and a poor but not significant agreement between the SCC and EC (KC = 0.07, $p < 0.06$) when the pre-defined cut-off levels were used for each test (Table 5).

Association between developing subclinical mastitis and predisposing markers

After examinations, the risk markers recorded in the current study were as follows: three types of hus-

Table 2 – Differences in the somatic cell count (SCC) and electrical conductivity (EC) test means (± standard errors) according to the California mastitis test (CMT) measured in lactating dromedary camels.

Test	CMT score[1]				*p* value
	0 (*n* = 510)	+1 (*n* = 141)	+2 (*n* = 67)	+3 (*n* = 40)	
SCC, ×10^{3}cells mL^{-1}	233.46 ± 19.13^d	685.48 ± 35.96^c	1468.21 ± 52.16^b	3855.58 ± 67.51^a	< 0.000
$\log_{10}$SCC	5.20 ± 0.02^d	5.77 ± 0.03^c	6.12 ± 0.04^b	6.57 ± 0.06^a	< 0.000
EC, mS cm^{-1}	7.34 ± 0.08^b	6.88 ± 0.14^c	7.68 ± 0.20^b	8.45 ± 0.24^a	< 0.000

[1]*n* = number of udder quarters tested; [a-d]Means within the same row bearing different letters are significantly different at $p < 0.05$.

Table 3 – Sensitivity and specificity reports of somatic cell count test (in $\log_{10}$), when udder quarters were categorized according to the California mastitis test, as influenced by multiple risk markers.

Markers		Parameters[1]											
		n	PNC	PPC	Mean ± SEM	AUC	COV	SENS	SPEC	LR +	LR –	PV +	PV –
Type of Husbandry	Nomadic	190	59.14	40.86	5.57 ± 0.04^a	0.93	5.68	0.89	0.90	8.56	0.12	0.90	0.89
	Semi-nomadic	464	67.76	32.24	5.44 ± 0.03^b	0.95	5.67	0.87	0.92	11.34	0.15	0.92	0.87
	Settled	104	64.42	35.58	5.52 ± 0.05^{ab}	0.89	5.61	0.81	0.87	6.34	0.22	0.86	0.82
Breed of the Animal	Majaheem	270	69.40	30.60	5.39 ± 0.03^c	0.96	5.61	0.90	0.92	10.64	0.11	0.91	0.91
	Maghateer	196	69.11	30.89	5.55 ± 0.05^b	0.96	5.70	0.91	0.94	15.54	0.10	0.94	0.91
	Shual	156	49.35	50.65	5.69 ± 0.04^a	0.85	5.65	0.80	0.78	3.55	0.26	0.78	0.79
	Sufur	136	69.17	30.83	5.41 ± 0.05^c	0.97	5.68	0.90	0.97	26.46	0.10	0.96	0.91
Number of Parities	One	167	66.06	33.94	5.46 ± 0.04^b	0.98	5.77	0.91	0.94	14.48	0.10	0.94	0.91
	Two	170	71.43	28.57	5.46 ± 0.04^b	0.91	5.68	0.73	0.95	15.51	0.28	0.94	0.78
	Three	212	69.23	30.77	5.48 ± 0.04^b	0.93	5.66	0.87	0.91	9.83	0.15	0.91	0.87
	Four and more	209	55.12	44.88	5.64 ± 0.04^a	0.97	5.67	0.90	0.91	9.97	0.11	0.91	0.90
Periods of Lactation[2]	First	195	56.02	43.98	5.58 ± 0.04^a	0.97	5.67	0.96	0.92	11.28	0.04	0.92	0.96
	Second	279	69.96	30.04	5.45 ± 0.04^b	0.91	5.64	0.77	0.89	6.76	0.26	0.87	0.80
	Third	284	66.67	33.33	5.50 ± 0.03^{ab}	0.95	5.66	0.86	0.92	11.36	0.15	0.92	0.87
Overall		758	65.15	34.85	5.46 ± 0.02	0.94	5.67	0.86	0.91	9.74	0.16	0.91	0.87

[a-c]Means within the same column bearing different letters are significantly different at $p < 0.05$. [1]*n* = number of udder quarters tested, where 191 camels from 47 herds were used leading to 758 quarters; PNC = percentage of negative counts; PPC = percentage of positive counts; AUC = area under curve; COV = cut-off value; SENS = sensitivity; SPEC = specificity; LR + = positive likelihood ratio; LR - = negative likelihood ratio; PV + = positive post-test probability value; and PV - = negative post-test probability value. [2]The first (extend from parturition to three months postpartum), second (extend from three to six months postpartum), and third periods of lactation (extend from six months postpartum to the end of lactation), respectively.

Table 4 – Sensitivity and specificity reports of electrical conductivity test (in mS cm^{-1}), when udder quarters were categorized according to the California mastitis test, as influenced by multiple risk markers.

Markers		Parameters[1]											
		n	PNC	PPC	Mean ± SEM	AUC	COV	SENS	SPEC	LR +	LR –	PV +	PV –
Type of Husbandry	Nomadic	190	81.94	18.06	6.88 ± 0.14^{b}	0.37	4.09	0.98	0.07	1.05	0.25	0.51	0.80
	Semi-nomadic	464	70.91	29.09	7.25 ± 0.08^{a}	0.56	8.35	0.44	0.75	1.78	0.74	0.64	0.57
	Settled	104	68.27	31.73	7.09 ± 0.16^{ab}	0.48	9.58	0.10	1.00	0.90	1.00	0.53	9.58
Breed of Animal	Majaheem	270	61.40	38.60	7.67 ± 0.12^{a}	0.53	6.12	0.86	0.27	1.18	0.51	0.54	0.66
	Maghateer	196	76.02	23.98	7.04 ± 0.13^{b}	0.47	8.34	0.35	0.79	1.66	0.82	0.62	0.55
	Shual	156	73.08	26.92	6.82 ± 0.13^{b}	0.51	8.46	0.35	0.84	2.19	0.78	0.69	0.56
	Sufur	136	87.12	12.88	6.76 ± 0.14^{b}	0.40	4.93	1.00	0.16	1.19	0.00	0.54	1.00
Number of Parities	One	167	81.41	18.59	6.59 ± 0.13^{c}	0.40	8.41	0.24	0.82	1.33	0.93	0.57	0.52
	Two	170	81.71	18.29	6.84 ± 0.12^{c}	0.43	8.47	0.21	0.85	1.42	0.93	0.59	0.52
	Three	212	72.50	27.50	7.16 ± 0.12^{b}	0.50	8.46	0.33	0.76	1.37	0.88	0.58	0.53
	Four and more	209	58.33	41.67	7.71 ± 0.12^{a}	0.47	9.57	0.15	0.95	2.95	0.90	0.75	0.53
Periods of Lactation[2]	First	195	88.37	11.63	6.34 ± 0.13^{b}	0.52	5.49	0.82	0.32	1.21	0.55	0.55	0.64
	Second	279	71.92	28.08	7.56 ± 0.12^{a}	0.57	8.41	0.44	0.79	2.15	0.70	0.68	0.59
	Third	284	63.93	36.07	7.33 ± 0.09^{a}	0.46	10.09	0.11	0.94	1.66	0.95	0.62	0.51
Overall		758	72.75	27.25	7.35 ± 0.06	0.48	8.44	0.31	0.76	1.28	0.90	0.56	0.52

[a-c]Means within the same column bearing different letters are significantly different at $p < 0.05$; [1]*n* = number of udder quarters tested, where 191 camels from 47 herds were used leading to 758 quarters; PNC = percentage of negative counts; PPC = percentage of positive counts; AUC = area under curve; COV = cut-off value; SENS = sensitivity; SPEC = specificity; LR + = positive likelihood ratio; LR - = negative likelihood ratio; PV + = positive post-test probability value; and PV - = negative post-test probability value. [2]The first (extend from parturition to three months postpartum), second (extend from three to six months postpartum), and third periods of lactation (extend from six months postpartum to the end of the lactation), respectively.

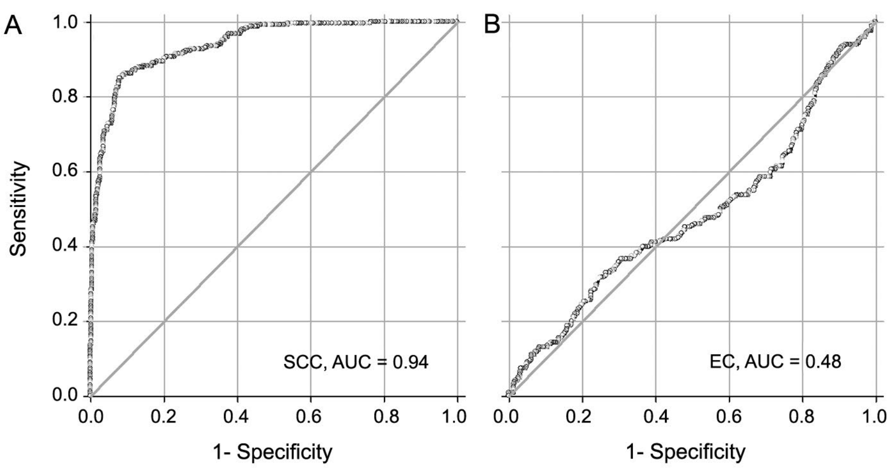

Figure 1 – The area under the receiver operating characteristics (ROC) curves of the somatic cell count (SCC) test (A) and the electrical conductivity (EC) test (B) when udders were categorized according to the California mastitis test (CMT) score (Negative or trace CMT scores being healthy [n = 510], positive CMT scores (+ 1, +2, or +3) having mastitic quarters [n = 248]) in lactating dromedary camels. The cut-off value and area under the ROC curve for the SCC test were 472.50×10^3 cells mL^{-1} ($\log_{10}$ SCC = 5.67) and 0.94 (95 % CI = 0.92-0.96), while for the EC test, they were 8.44 mS cm^{-1} and 0.48 (95 % CI = 0.44-0.53), respectively.

Table 5 – Agreements between the California mastitis test (CMT), somatic cell count (SCC) test, and electrical conductivity (EC) test for mastitis diagnosis in lactating dromedary camels.

Test	Parameters[1]				
	OA	EA	KC	95 %CI	*p* value
	——— % ———				
CMT *vs* Log_{10}SCC	89.01	55.08	0.76	0.71-0.81	< 0.000
CMT *vs* EC	60.39	56.52	0.09	0.02-0.16	0.015
Log_{10}SCC *vs* EC	59.57	56.55	0.07	-0.00-0.14	0.061

[1]OA = observed agreement (%); EA = expected agreement (%); KC = kappa coefficient; and 95 % CI, 95 % confidence interval.

bandry system, i.e., (i) the nomadic system (n = 190, quarter numbers), where camels were hand-milked once per day, suckled their calves throughout the day excluding 2 h before sunset, watered twice per day (morning and evening), and traveled long distances (> 100 km) in search of food; (ii) the semi-nomadic system (n = 464), where camels were hand-milked once per day, suckled their calves throughout the day excluding 2 h before sunset, watered twice per day, and were housed and fed during the summer until the next grazing season; and (iii) the settled system (n = 104), where camels were hand-milked and suckled their calves twice per day, subjected to anti-suckling devices, watered *ad libitum*, and housed in enclosures to be fed indoors throughout the year. Additionally, there were four indigenous camel breeds i.e., (i) *Majaheem* (n = 270), (ii) *Maghateer* (n = 196), (iii) *Shual* (n = 156), and (vi) *Sufur* (n = 136); four categories of parity i.e., (i) one (n = 167), (ii) two (n = 170), (iii) three (n = 212), (vi) four or more parities (n = 209); and three periods of lactation i.e., (i) the first period (n = 195), extending from parturition to three months postpartum; (ii) the second period (n = 279), extending from three months postpartum to six months postpartum; and (iii) the third period (n = 284), extending from six month postpartum to the end of the lactation period.

The effects of these attributes as revealed by the SCC and EC test results are summarized in Tables 3 and 4. Regarding the husbandry system, the nomadic system had the highest ($p < 0.05$) overall mean $\log_{10}$SCC (41 %, percentage of SCC positive counts using 5.67 as a cut-off), followed by the settled system (36 %), and then the semi-nomadic system (32 %), while both the semi-nomadic (29 %, percentage of EC positive counts using 8.44 mS cm^{-1} as a cut-off) and the settled (32 %) systems had the highest ($p < 0.05$) overall mean EC, followed by the nomadic system (18 %). Regarding camel breeds, the *Shual* breed had the highest ($p < 0.05$) overall mean $\log_{10}$SCC (51 %), followed by the *Maghateer*, *Majaheem*, and *Sufur* (31 %) breeds, while the *Majaheem* breed had the highest ($p < 0.05$) overall mean EC (39 %) compared to those of the other breeds. The effect of parity on the overall means of both the $\log_{10}$SCC and EC was evidently high ($p < 0.05$) in the fourth parity (45 and 42 %, respectively), where the results revealed gradual increases in these values as the parity number increased. On the other hand, the highest ($p < 0.05$) overall mean $\log_{10}$SCC was obtained during the first period of lactation (44 %), followed by a decrease ($p < 0.05$) during the second period (30 %), and then an increase ($p > 0.05$) during the final period of lactation (33 %). Meanwhile, the highest ($p < 0.05$) overall mean EC was recorded during the second (28 %) and third (36 %) periods, and the lowest was recorded during the first period of lactation (12 %), as shown in Tables 3 and 4.

The sensitivity and specificity reports of both the SCC and EC tests were likewise influenced by these markers. As a matter of fact, the area under the ROC curve for the SCC test was in the 0.85-0.98 range, and its highest points were attained in the semi-nomadic system (0.95), *Sufur* breed (0.97), first parity (0.98), and first period of lactation (0.97) (Table 3 and Figure 2A, B, C and D); for the EC test the area under the ROC curve ranged from 0.37-0.57, and its highest points were attained in the semi-nomadic system (0.56), *Majaheem* breed (0.53), third parity (0.50), and second period of lactation (0.57) (Table 4 and Figure 3A, B, C and D).

To quantify the strength of association between certain predisposing markers and the prevalence of subclinical mastitis in lactating camels using SCC and EC tests, multiple logistic models were used. Among these markers, three in the present study were considered ($p < 0.05$) to be potential risk markers for developing subclinical mastitis (Table 6). These were the breed, parity number, and lactation period. However, significant associations with subclinical mastitis were only recorded, according to the SCC test, in the *Majaheem* ($p < 0.04$) and *Shual* ($p < 0.001$) breeds, second ($p < 0.05$) and fourth ($p < 0.001$) parities, and first period of lactation ($p < 0.02$); according to the EC test, significant associations were only shown in the *Majaheem* ($p < 0.001$) and *Sufur* ($p < 0.01$) breeds, second ($p < 0.002$) and fourth ($p < 0.001$) parities, and all periods of lactation ($p < 0.001$, $p < 0.03$, and $p < 0.001$, respectively) (Table 6).

Subsequently, the calculated values of OR according to the SCC test showed that the *Majaheem* breed had a lower ($p < 0.04$) probability of developing subclinical mastitis by a factor of 0.81 and 0.42 than the *Maghateer* and *Shual* breeds, respectively, but had a slightly higher (1.04 times) probability than the *Sufur* breed, while the *Shual* breed had ($p < 0.001$) a higher probability of being affected by subclinical mastitis than other breeds by a factor of 2.38 compared to both the *Majaheem* and *Sufur* breeds, and by a factor of 1.92 compared to the *Maghateer* breed. In addition, camels with two parities had a lower ($p < 0.05$) probability of developing subclinical mastitis by a factor of 0.84, 0.78, and 0.48 compared to camels with one, three, and four or more parities, respectively. Meanwhile, the risk of camels with four parities and more increased ($p < 0.001$) by 1.72, 2.09, and 1.67 times compared to camels with one, two, and three parities, respectively. Moreover, the association between subclinical infection and first period of lactation exceeded ($p < 0.02$) that during the second and third periods of lactation by 1.65 and 1.52 times, respectively (Table 6).

In contrast, according to the EC test, the *Majaheem* breed had a higher ($p < 0.001$) probability of developing subclinical mastitis by a factor of 2.29, 1.87, and 3.13 compared to that of the *Maghateer*, *Shual*, and *Sufur* breeds, respectively, while the *Sufur* breed had the lowest ($p < 0.01$) probability of being affected by subclinical mastitis by a factor of 0.32, 0.69, and 0.54 compared to the *Majaheem*, *Maghateer*, and *Shual* breeds, respectively. Additionally, camels with two parities had the lowest ($p < 0.002$) probability of developing subclinical mastitis by a factor of 0.77, 0.55, and 0.26 compared to that of camels with one, three, and four or more parities, respectively, while the predisposition of camels with four or more parities was increased ($p < 0.001$) by 2.85, 3.88, and 2.05 times compared to that of camels with one, two, and three parities, respectively. Finally, the prevalence risk computed for the odds of finding subclinical mastitis positive cases over the total cases was considerably higher ($p < 0.001$) during the third period of lactation by 4.31 and 1.38 times compared to that during the first and second periods of lactation, respectively, while the second period of lactation had ($p < 0.03$) a lower probability by a factor of 0.28 and 0.72 than the

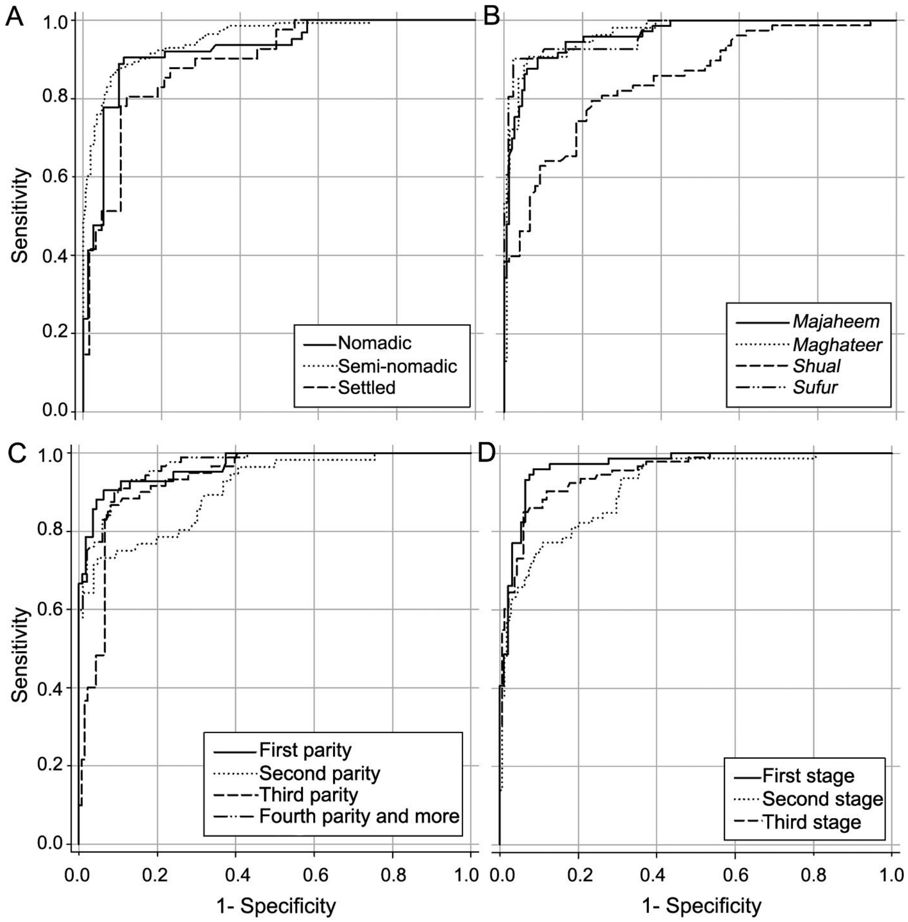

Figure 2 – The area under the receiver operating characteristics (ROC) curves of the somatic cell count test, when udders were categorized according to the California mastitis test score, as influenced by the husbandry system (A), breed of the animal (B), number of parities (C), and periods of lactation (D) in lactating dromedary camels (details are shown in Table 4).

first and third periods, respectively. Meanwhile, camels in the first period of lactation had a lower (p < 0.001) probability of developing subclinical mastitis by a factor of 0.23 compared to those in third period of lactation, and higher probability than those in second period by 3.57 times (Table 6).

Discussion

The camel is considered the fifth most important dairy animal in the world, following dairy cattle, water buffalo, goat, and sheep (Faye and Konuspayeva, 2012). Despite our best efforts in the last decade to engage in developing the camel dairy industry, including machine milking, animal milk ability, and milking manageability; few studies have since focused on udder health, one of the major and fundamental issues for dairy camel breeders. Indeed, studies concerning the epidemiology and pathogenicity of mastitis in camels have vital importance to dairy sub-sectors in developed and developing nations, where mastitis represents the most important factor affecting the production of camel milk and security of the industry (Gitao et al., 2017; Raziq et al., 2008).

In the present observational study, the practicability of SCC and/or EC tests as subclinical mastitis diagnostic tests in lactating dromedary camels was examined at first through their validations by the CMT score, and then through their subsequent employments in assessing certain potential markers predisposing camels to develop the disease. To our knowledge, this study is the first to investigate the reliability of using both of SCC and EC tests in diagnosing subclinical mastitis in camels, as well as the association between developing subclinical mastitis and certain potential risk markers using those objective means as diagnostic tests.

Validation of SCC and/or EC as subclinical mastitis diagnostic tests in camels

As previously noted, the value of the CMT as a screening test for early detection of subclinical mastitis in camels is widely validated based on microbiological testing (Abdelgadir et al., 2005; Abdelgadir, 2014),

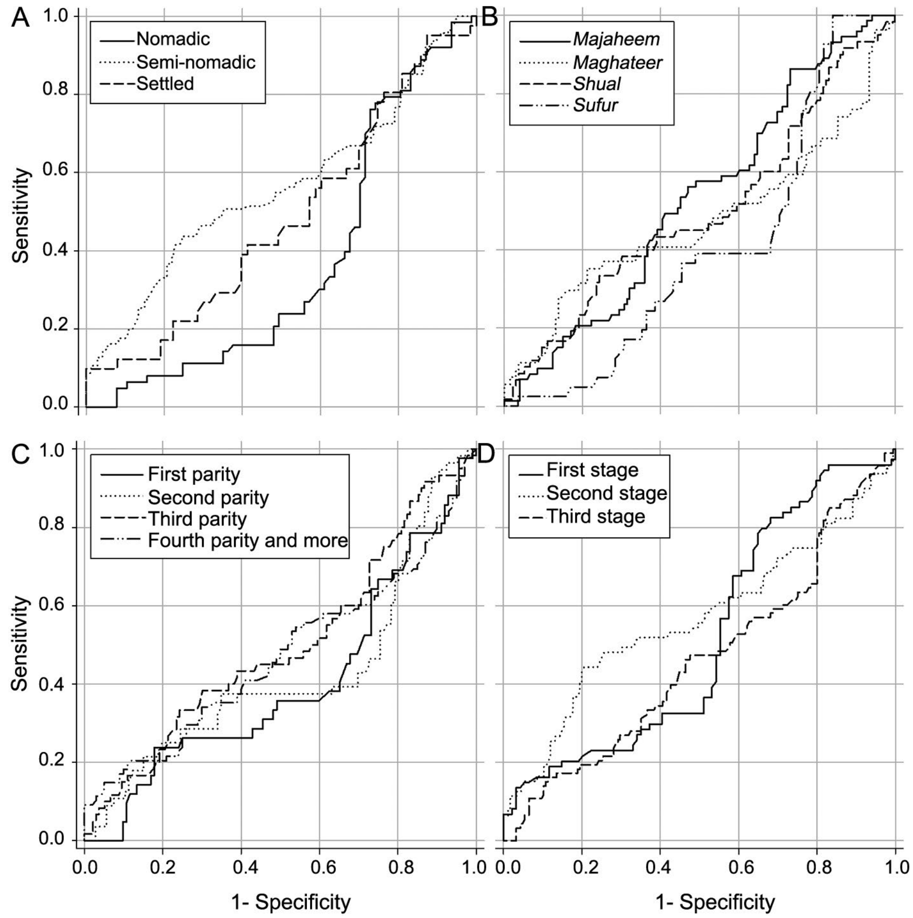

Figure 3 – The area under the receiver operating characteristics (ROC) curves of the electrical conductivity test, when udders were categorized according to the California mastitis test score, as influenced by the husbandry system (A), breed of the animal (B), number of parities (C), and period of lactation (D) in lactating dromedary camels (details are shown in Table 5).

which makes it the ideal test for important farm management decisions. Drawing on such superiority and legitimacy, the potential concordance between the CMT, SCC, and EC tests for subclinical mastitis diagnosis in camels was determined herein. The obtained results highlighted the reliability of the SCC test, in contrast to the EC test, in predicting subclinical mastitis in lactating camels. This conclusion was demonstrated by the attained findings that subclinical mastitic quarters had higher SCC than healthy quarters in comparison to the EC test (Table 2), which may therefore explain the strong positive correlation as well as the high coefficient of determination obtained between the SCC test and CMT scores. These observations are in accordance with other studies on camels (Nagy et al., 2013; Samara et al., 2014) and other species (Norberg et al., 2004; Sargeant et al., 2001). Actually, it was observed that the conductance of cattle milk decreases as the percentage of fat increases in mastitic milk samples, where fat globules hinder the conductance by occupying the volume of the conducting medium and impeding the mobility of the conducting ions (Mabrook and Petty, 2003; Lawton and Pethig, 1993); this may explain the obtained EC test results of the present study. Despite the fact that our data are in accordance with these observations in cattle, we previously observed -though there was a slight increase- that no difference ($p > 0.05$) in fat percentage was found between the milk samples collected from healthy and subclinically affected quarters (Aljumaah et al., 2011). Therefore, other patho-physiological avenues should be explored and discussed.

The reliability of the SCC test was also confirmed herein by further analyses of the ROC curves. In fact, our results implied that the SCC test was proven to determine mastitic udders with a higher sensitivity in camels compared to that of the EC test (Table 3 and 4; Figure 1A and B). In agreement, the calculated prevalence rate of subclinical mastitic cases herein was about 35 % based on the SCC test (Table 3), which is in accordance with our previous observations based on the CMT score (33 %, Aljumaah et al., 2011), but is in contrast to what was obtained herein according to the EC test (27 %, Table 4).

These deductions were additionally emphasized by the estimated excellent agreement beyond chance between the SCC test and CMT scores and the poor agree-

Table 6 – Associations between potential predisposing markers and the prevalence of subclinical mastitis in lactating dromedary camels, according to the SCC (cut-off of $log_{10}SCC = 5.67$) and EC tests (cut-off of EC = 8.44 mS cm^{-1}).

Markers	Comparisons[1]	Diagnostic tests			
		$Log_{10}SCC$		EC	
		Odds Ratio[2]	95 %CI	Odds Ratio[3]	95 %CI
Type of Husbandry	H1 *vs* H3	0.82	0.49-1.39	0.75	0.71-0.81
	H2 *vs* H3	1.56	0.71-1.87	0.70	0.41-1.21
	H2 *vs* H1	1.44	0.95-2.18	0.91	0.51-1.61
Breed of the Animal	B1 *vs* B4	0.96	0.61-1.54	0.32	0.18-0.57
	B2 *vs* B4	0.78	0.47-1.30	0.69	0.37-1.28
	B3 *vs* B4	0.42	0.25-0.69	0.54	0.30-1.00
	B2 *vs* B1	0.81	0.52-1.26	2.29	1.42-3.71
	B2 *vs* B3	1.92	1.20-3.09	1.23	0.72-2.10
	B3 *vs* B1	0.42	0.28-0.65	1.87	1.14-3.07
Number of Parities	P1 *vs* P4	1.72	1.01-2.68	2.85	1.68-4.82
	P2 *vs* P4	2.09	1.33-3.29	3.88	2.25-6.70
	P3 *vs* P4	1.67	1.10-2.52	2.05	1.30-3.24
	P2 *vs* P1	1.19	0.73-1.93	1.30	0.72-2.34
	P2 *vs* P3	1.28	0.81-2.03	1.82	1.07-3.09
	P3 *vs* P1	0.93	0.59-1.46	0.72	0.42-1.22
Periods of Lactation	L1 *vs* L3	0.66	0.44-0.98	4.31	2.52-7.36
	L2 *vs* L3	1.05	0.70-1.56	1.38	0.88-2.16
	L2 *vs* L1	1.65	1.08-2.51	0.28	0.16-0.50

[1]H1–H3 = nomadic, semi-nomadic, and settled husbandry systems, respectively; B1–B4 = *Majaheem*, *Maghateer*, *Shual*, and *Sufur* breeds, respectively; P1–P4 = one, two, three, and four or more parities, respectively; and L1–L3 = first (extend from parturition to three months postpartum), second (extend from three to six months postpartum), and third periods of lactation (extend from six months postpartum to the end of the lactation), respectively. [2]The probability values, which denote the statistical significance of the associations for each marker, according to the SCC test, are as follows: the nomadic ($p < 0.17$), semi-nomadic ($p < 0.18$), and settled systems ($p < 0.85$); the *Majaheem* ($p < 0.04$), *Maghateer* ($p < 0.73$), *Shual* ($p < 0.001$), and *Sufur* breeds ($p < 0.09$); one ($p < 0.47$), two ($p < 0.05$), three ($p < 0.69$), and four or more parities ($p < 0.001$); and the first ($p < 0.02$), second ($p < 0.14$), and third periods of lactation ($p < 0.26$). [3]The probability values, which denote statistical significance of the associations for each marker, according to the EC test, are as follows: the nomadic ($p < 0.68$), semi-nomadic ($p < 0.26$), and settled systems ($p < 0.16$); the *Majaheem* ($p < 0.001$), *Maghateer* ($p < 0.35$), *Shual* ($p < 0.71$), and *Sufur* breeds ($p < 0.01$); one ($p < 0.12$), two ($p < 0.002$), three ($p < 0.73$), and four or more parities ($p < 0.001$); and the first ($p < 0.001$), second ($p < 0.03$), and third periods of lactation ($p < 0.001$).

ment obtained between the EC test and CMT scores (Table 5). Remarkably, this is consistent with previous reports on cattle, where the ability of the EC test to denote subclinically mastitic samples from healthy ones was non-satisfactory (Mabrook and Petty, 2003; Norberg, et al., 2004). As a matter of fact, Younan et al. (2001) purely noted that EC readings were non-diagnostic in camels.

Thereby, these outcomes clearly bear substantial evidence that the objective SCC test, and not the EC test, have considerable diagnostic merit corresponding to the subjective CMT for the early detection of subclinical mastitis in lactating camels. As suggested by the obtained results herein, it consequently seems logical to base herd management decisions on the SCC test readings, using the cut-off $log_{10}SCC$ value of 5.67 (or SCC = 472.50×10^3 cells mL^{-1}, as retrieved by ROC analysis) to separate subclinically infected camels from healthy ones.

Association between developing subclinical mastitis and certain risk markers

On account of being invalidated by the CMT score, the results obtained regarding the potential predisposing markers associated with the prevalence of subclinical mastitis according to the EC test will therefore not be discussed.

With high sensitivity, the influence of camel attributes revealed that the nomadic system had the highest prevalence rate compared to other types of husbandry system according to the SCC test [AUC = 0.93, percentage of positive counts (PPC) = 41 %, Table 3]. This high rate was also observed in previously reported studies in camels, where the poor management of animals and inadequate hygiene during milking are common in nomadic camel herders, causing injury and predisposing the udder to bacterial infection, which can eventually lead to an increased SCC (Alshaikh and Salah, 1994; Ayadi et al., 2009). Improper handling such as this can cause a public health concern. In fact, washing hands and udders, dipping teats, treating teat lesions, unemploying anti-suckling devices, and applying basic hygienic measures were all demonstrated to reduce infections and microbial loads in husbandry systems that apply traditional hand milking methods (Abdelgadir et al., 2005; Abdelgadir, 2014; Gitao et al., 2017). Nevertheless, the prevalence risk computed herein using multivariable models for the odds of finding positive cases of subclinical mastitis over the total cases-using a $log_{10}SCC$ of 5.67 as the cut-off value-was found to be unaffected by the type of husbandry system (Table 6); this result thereby attests that this marker is definitely not associated with the prevalence of subclinical mastitis in camels.

Of the potential risk markers considered in this study according to the SCC test, the breed, parity, and period of lactation were the only significant risk markers that contributed to an increased prevalence of subclinical mastitis in camels (Table 6). Specifically, our results indicated that there is an association between developing subclinical mastitis in camels and the camels being *Majaheem* and *Shual* breeds, in their second and fourth parities, and in their first period of lactation.

Among the breeds, the *Shual* breed was more frequently affected, with a documented prevalence of 51 %, compared to 31 % in the *Majaheem* breed (Table 3), which may therefore explain the higher probability of the *Shual* breed being affected by subclinical mastitis than the *Majaheem* breed (by a factor of 2.38, Table 6). This confirms our previous observations based on the CMT score (Aljumaah et al., 2011). The reason for such differences between breeds is not clear, although the *Shual* breed is primarily a meat camel, while the *Majaheem* breed is preferentially selected by dairy farmers in Saudi Arabia. It might be possible that the higher probability of subclinical mastitis in the *Shual* breed could be ascribed to differences in the breed susceptibility to udder infection; the genetic make-up could explicate the differences in susceptibility, such as those manifested among other dairy animal breeds (Harmon, 1994; Leitner et al., 2004; Sharma et al., 2006). In fact, environmental selection (such as a larger quarter, narrower teat canal, and/or firmer teat sphincters) could have helped the *Majaheem* breed to become better adapted to a less hygienic environment, which is the main source of teat contamination and subclinical mastitis. Further studies critically evaluating the differences between breeds are required to understand these possibilities.

Moreover, the increased prevalence rate of subclinical mastitis from 29-45 % with an increasing parity number, from two to four or more, confirms several previous observations in camels (Abdelgadir et al., 2005; Aljumaah et al., 2011; Al-Salihi et al., 2017) and other animals (Kavitha et al., 2009; Syridion et al., 2012; Wilson et al., 1995). An increased number of parities implies that the age of the animal is also increased. As the age increases, the risk of developing subclinical mastitis increases in camels, as revealed herein by the calculated OR estimates (Table 6). This could be attributed to the fact that the teat canal in older lactating animals is more dilated and less elastic due to the years of cumulative stress of repeated milking, which encourages the introduction of environmental microorganisms into the teat canal, leading eventually to subclinical mastitis (Boscos et al., 1996; Dingwell et al., 2004). The cause of this increase could additionally be linked to a flabby udder suspensory system, lower immunity defense, and insufficient treatment efficacy (Ayadi et al., 2016).

On the other hand, the risk of subclinical mastitis infection in camels was highly recorded herein during the first period of lactation compared to other periods, where the association between subclinical infection and the first period of lactation exceeded that with other periods of lactation (Table 3 and 6). These findings indicate that an increased rate of mastitis in camels is likely to occur shortly after parturition, as in other species of dairy animals (Burvenich et al., 2007; Hussain et al., 2012). This corroborates our earlier results, which indicated that the risk of developing subclinical mastitis increased during the first period of lactation (Aljumaah et al., 2011). The higher prevalence risk of subclinical mastitis during the first period compared to that during other periods of lactation could be associated with decreased resistance of the mammary gland to infection as a result of immune system depression due to the hormonal changes that occur around the time of parturition and the onset of lactation (Sordillo, 2005; Burvenich et al., 2007). This prompts the question of whether this effect can be reversed. Obviously, additional studies are required to elucidate this; thus, further investigations are encouraged to continue in this line of research.

Limitations

This study is not without limitations. In fact, some shortcomings deserve to be noted. For example, more oriented studies (i.e. case controlled and/or cohort) are recommended to standardize the SCC test in camels, in order to confirm the obtained findings herein, by collecting a larger sample size of both healthy and affected animals as representative of the global camel population. In addition, information on the causative bacteria, history of infection, past antimicrobial use, and efficiency of potential control measures should all be assessed in future studies. There may also be a need to conduct broader studies to determine the effect of other quarter-, camel-, and herd-level potential risk markers not included in this study. Additionally, longitudinal studies are definitely imperative in order to establish the actual causal pathways between these risk markers and the development of subclinical mastitis.

Conclusions

The early detection of subclinical mastitis is crucial for efficient control of the condition. At the beginning of this study, it was not clear whether SCC and/or EC tests had strong agreements with the CMT in detecting subclinical mastitic cases, or if there was any association between developing subclinical mastitis and certain potential risk markers according to these tests. To our knowledge, this observational study is considered among the first to investigate such aspects of camel dairy production. In the present study, the validity of the objective SCC test, compared to the EC test, in having considerable diagnostic merit corresponding to the subjective CMT score for the early detection of subclinical mastitis in lactating camels was clearly substantiated. In fact, the reliability of the EC test was non-satisfactory and non-diagnostic in camels. Accordingly, it seems wise to base herd management decisions on SCC readings using the cut-off log_{10}SCC value of 5.67 (or SCC = 472.50 × 10^3 cells mL^{-1}), as suggested by the obtained results herein.

Despite the pre-mentioned shortcomings, this work should assist dairy camel practitioners in planning and adopting preventive and control measures by improving their awareness about the importance of proper herd health management and hygienic milking practices in to reduce the incidence of clinical mastitis and culling rate in dairy camels. Moreover, such research efforts may not only have implications for mastitis, but could be more broadly relevant to the productivity and welfare of camels. In fact, the outcomes of this work would create a new paradigm for future camel herd management through improving the production of camel milk and the security of the dairy camel industry, and offering as well considerable insights into making camel milk safer for human consumption.

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Authors' Contributions

Conceptualization: Aljumaah, R.S.; Almutairi, F. Data acquisition: Aljumaah, R.S.; Almutairi, F.; Ayadi, M. Data analysis: Aljumaah, R.S.; Al-Haidary, A.A.; Samara, E.M. Design of methodology: Aljumaah, R.S.; Almutairi, F.; Alshaikh, M.A. Writing and editing: Aljumaah, R.S.; Almutairi, F.; Ayadi, M.; Alshaikh, M.A.; Al-Haidary, A.A.; Samara, E.M.

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