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SUBCLINICAL MASTITIS IN DAIRY CAMELS IN ALGERIA: COMPARISON OF SCREENING TESTS

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Subclinical mastitis in dairy camels in Algeria: Comparison of screening tests

The aim of the present study was to determine a threshold values and to assess the effectiveness of four indirect tests for the diagnosis of subclinical mastitis in dairy camels comparing with bacteriological culture. One hundred fifty three milk samples from 17 lactating camels were subjected to bacteriological culture, where 84 milk samples were positive, 47 were negative and 22 samples were considered as contaminated. A total of 131 milk samples were screened by pH, electrical conductivity (EC), California mastitis test (CMT) and somatic cell count (SCC). The good combination of sensitivity and specificity were obtained with a threshold of 6.55, 7.2 mS/cm, score trace was considered as CMT (+) and 240000 cells/ml for the four tests, respectively. The sensitivity of the SCC, pH, EC and CMT was 72.61, 66.66, 47.61 and 39.28 %; the specificity 70.21, 38.02, 59.57 and 72.34 %; percentage accuracy 71.75, 51.14, 51.90 and 51.14 %; and positive predictive value 81.33, 47.61, 67.79 and 71.73 %, respectively. The SCC was significantly correlated with bacteriological culture ($r = 0.415$, $p < 0.05$). Kappa value of SCC was higher than that of other tests (SCC > CMT > EC > pH). In conclusion, the results suggest that the SCC was the most accurate, reliable, diagnostic method compared to other tests used in this study after cultural isolation for the detection of subclinical mastitis in dairy camel under field conditions.

Key words: camels; dromedaries; *Camelus dromedarius*; lactation; subclinical mastitis; screening tests; indirect tests; pH value; electrical conductivity; California Mastitis Test; somatic cell count; bacteriological test

Subklinični mastitis pri mlečnih kamelah v Alžiriji: primerjava presejalnih testov

Namen te študije je bil določiti mejne vrednosti in preveriti učinkovitost štirih posrednih testov za diagnozo subkliničnega mastitisa pri kamelah v primerjavi z bakteriološko kulturo. Sto triinpetdeset vzorcev mleka 17 kamel v laktaciji smo analizirali s kultivacijsko metodo, kjer je bilo 84 vzorcev mleka pozitivnih, 47 je bilo negativnih, 22 vzorcev pa je bilo okuženih. Na skupno 131 vzorcih mleka smo izmerili pH vrednost, električno prevodnost (EC) in število somatskih celic (SCC) ter opravili Kalifornijski test za mastitis (CMT). Dobro kombinacijo občutljivosti in specifičnosti smo dobili pri mejnih vrednostih: za pH 6,55, električno prevodnost 7,2 mS/cm, pozitivnim izidom CMT (+) in 240.000 somatskimi celicami na ml mleka. Občutljivost testa za SCC je bila 72,61, za pH 66,66, za EC 47,61 in za CMT 39,28 %. Specifičnost testov se je gibala med 38,02 in 72,34 %; natančnost med 51,14 in 71,75 % in pozitivna napovedna vrednost med 47,61 in 81,33 %. SCC je bilo statistično značilno povezano z bakteriološko kulturo ($r = 0{,}415$, $p < 0{,}05$). Kapa vrednost za SCC je bila višja kot pri drugih testih (SCC > CMT > ES > pH). Rezultati kažejo, da je v praktičnih pogojih reje SCC med primerjanimi metodami najnatančnejša in najbolj zanesljiva metoda za določanje subkliničnega mastitisa pri kamelah v primerjavi z bakteriološko metodo osamitve povzročiteljev mastitisa.

Ključne besede: enogrbe kamele; *Camelus dromedaries;* laktacija; subklinični mastitis; presejalni testi; posredni testi; pH vrednost; električna prevodnost; Kalifornijski test za mastitis; število somatskih celic; bakteriološki test

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1 INTRODUCTION

Many reports revealed that lactating camel can get mastitis easily (Abdel Gadir et al., 2006; Alamin et al., 2013; Al-Juboori et al., 2013), especially in its subclinical form (Hawari and Hassawi, 2008; Ahmed et al., 2011; Alamin et al., 2013; Husein et al 2013). Subclinical mastitis causes suffering of the animal, reduces milk yield, alters milk properties, impairs preservation and processing and represents a public health concern for consumers of camel milk (Tibary and Anouassi, 2000; Saleh and Faye, 2011).

Clinical mastitis in camel can be detected without special tests, by physical examination of the udder and milk (Abdurahman, 1995). However, sub-clinical mastitis refers to the existence of inflammation in the absence of gross signs (Bekele and Molla, 2001).

Detection of subclinical mastitis is difficult and depends upon various test procedures aimed at detecting the cause or the products of inflammation in milk. Cultural examination is considered the golden standard in order to establish reliable opinion about infection status of the udder (Tuteja et al., 2013).

Different methods have been suggested for detection of subclinical mastitis in camels, such as somatic cell count (SCC), California mastitis test (CMT) (Saleh and Faye 2011; Alamin et al., 2013; Ali et al., 2016), electrical conductivity (Eberlein, 2007; Ali et al., 2016) and pH estimation (Tuteja et al 2003; Ali et al., 2016).

These various tests are based upon detection of products of inflammation or changes in milk and have a well established role as screening test for predicting disease status of mammary gland in cattle, sheep and goat but their relevance for application to the camel is less known. Therefore, the aim of this study was to optimize and determine threshold values for pH, electrical conductivity, California mastitis test and somatic cell counts and also to evaluate the effectiveness of these methods for the diagnosis of subclinical mastitis in dairy camel in comparison with microbiological culture.

2 MATERIALS AND METHODS

2.1 SAMPLE COLLECTION

The study was carried out on 17 dairy camels (*Camelus dromedarius*) from the south-East of Algeria. Between November 2014 and September 2015, a total of 153 milk samples were collected during the routine morning milking.

Udders of the camels were examined visually and by palpating for the presence of any lesions, such as redness, pain, heat, and swelling. Moreover, milk samples from each animal were taken and checked for any change in color and consistency.

The teat end was then scrubbed with a cotton wool wetted with 70 % alcohol. After the teat-end had dried, the first strip was discarded and then approximately 50 ml of milk was collected into sterile bottles.

The samples were immediately transported on ice to the laboratory where they were examined using the pH, EC, SCC, CMT and the milk bacteriological culture.

2.2 MEASUREMENT OF pH

The pH of each milk sample was measured by digital pH-meter (Hanna HI 99161, Romania). The pH meter was calibrated in buffers of pH 4 and 7.

2. 3 ELECTRICAL CONDUCTIVITY (EC) TEST

The Electrical conductivity of milk samples (mS/cm) was detected by electrical conductivity meter (Hanna EC 215). The device was calibrated using standard buffer solutions.

2. 4 CALIFORNIA MASTITIS TEST (CMT)

Sub-clinical mastitis was diagnosed based on CMT results and the nature of coagulation and viscosity of the mixture (milk and CMT), which shows the presence and severity of the infection, respectively. Before sample collection for bacteriological examination, milk samples were examined for visible abnormalities, they were screened by CMT according to Quinn et al. (1999) from each animal, a squirt of milk sample was placed in each of the cups on CMT paddle and an equal amount of 3 % CMT reagent was added to each cup and mixed well.

2.5 SOMATIC CELL COUNT (SCC)

Somatic cell counts were determined according to Weisen (1974) using the microscopic count. The method involves diluting the sample of milk to 1/10 with Lazarus liquid. The suspension was homogenized and cells counted. In a drop between slide and cover slip, after eliminating the first 3 to 4 drops of the mixture. The cell concentration (cells/ml) was determined using a hemocytometer (Thoma cell) examined at a magnification 40x.

Three various threshold values for pH, electrical conductivity and somatic cell counts were used to

evaluate the effectiveness of these methods on screening of subclinical mastitis by evaluating its sensitivity (SE), specificity (SP), the positive predictive value (PPV) and negative predictive value (NPV) in comparison with bacterial culture. However, low threshold scores were used to assess the effectiveness of CMT test.

2.6 BACTERIOLOGICAL EXAMINATIONS

All milk samples, were randomly selected and used for bacteriological analysis. A loopful of each milk sample was streaked on defibrinated sheep (5 %) blood agar, nutrient agar, BCP (Bromcresol Purple Lactose) agar, and Chapman agar. Plates were incubated at 37 °C for 48 h. Grown colonies were subjected to the following tests as recommended by the National Mastitis Council (NMC, 1987): morphology, haemolysis pattern, Gram stain and biochemical classic tests. Samples with a growth of 5 or more identical colonies were considered positive for subclinical mastitis (1997; Pradieé et al., 2012). The growth of two or more morphological types (> 5 UFC per type) was considered as contamination and the result was excluded from the analysis (Gonzalo et al., 2005; Pradieé et al., 2012).

2.7 STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS software (Version 16). The results were considered significant if the associated p value was < 0.05.

Sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) and the accuracy of tests were calculated using standard two-by-two contingency tables.

The following formulas were used for diagnostic test evaluation:

Sensitivity is the proportion of truly diseased that had the test positive and it was calculated as:

Sensitivity = TP / TP + FN × 100.

Specificity is the proportion of truly non-diseased that had the test negative and it was calculated as:

Specificity = TN / FP + TN × 100.

Positive predictive value is the probability that a disease is present in animals with positive test results. It was calculated as:

Positive predictive values = TP / TP + FP × 100.

Negative predictive value is the probability that the disease is absent in animals with negative test results. It was calculated as:

Negative predictive values = TN / FN + TN x 100.

Where: TP = true positive, FP = false positive, TN = true negative, FN = false negative.

The Kappa and Chi-square statistical analysis were used to determine the level of agreement between the diagnostic tests and bacterial culture.

Kappa values above 0.7 indicated an excellent agreement, whereas values between 0.4 and 0.7 indicated a moderate agreement. Values under 0.4 indicated a poor agreement. The correlation was calculated by the Phi test. The correlation index was classified as high ($r > 0.7$), medium ($0.5 < r < 0.7$) and low ($r < 0.5$).

3 RESULTS

A total of 153 samples from 17 animals were examined with cultural examination, 84 samples were classified as positive (growth of 5 or more identical colonies), 47 samples were taken as negative (there was no growth) and 22 samples were considered as contamination (growth of two or more morphological types).

The sensitivity, specificity, predictive value and accuracy of various thresholds used for pH, EC, CMT and SCC tests, when cultural test was taken as standard are shown in Table 1, 2, 3 and 4 respectively.

The results of the study showed that the good combination of sensitivity, specificity, positive predictive value and negative predictive value of pH test was obtained while using a threshold of 6.55, with values of 66.66 %, 38.02 %, 47.61 % and 57.44 %, respectively (Table 1).

For electrical conductivity, the good combination of sensitivity, specificity, positive predictive value and negative predictive value were observed while using a threshold of 7.2 mS/cm, with values of 47.61 %, 59.57 %, 67.79 % and 38.88 %, respectively (Table 2).

Table 1: *Analysis of the pH values in comparison with bacterial culture*
Preglednica 1: *Analiza pH vrednosti v primerjavi z bakteriološko kulturo*

	pH threshold values		
Values observed	≥ 6.40	≥ 6.55	≥ 6.60
Sensitivity (%)	62.06	66.66	65.11
Specificity (%)	20.00	38.02	36.36
Positive predictive value (%)	85.71	47.61	33.33
Negative predictive value (%)	6.38	57.44	68.08
Accuracy (%)	57.25	51.14	45.80

Table 2: *Analysis of the electrical conductivity values in comparison with bacterial culture*
Preglednica 2: *Analiza električne prevodnosti v primerjavi z bakteriološko kulturo*

	Electrical conductivity threshold values (mS/cm)		
Values observed	≥ 6.5	≥ 7.2	≥ 7.5
Sensitivity (%)	59.52	47.61	31.32
Specificity (%)	36.17	59.57	78.72
Positive predictive value (%)	62.5	67.79	72.22
Negative predictive value (%)	33.33	38.88	39.36
Accuracy (%)	51.14	51.90	48.46

Table 4: *Analysis of the somatic cell count values in comparison with bacterial culture*
Preglednica 4: *Analiza števila somatskih celic v primerjavi z bakteriološkim testom*

	Somatic cell count threshold values (cells/ml)		
Values observed	≥ 200000	≥ 240000	≥ 300000
Sensitivity (%)	73.80	72.61	40.47
Specificity (%)	40.42	70.21	74.46
Positive predictive value (%)	68.88	81.33	73.91
Negative predictive value (%)	46.34	58.92	41.17
Accuracy (%)	61.83	71.75	52.67

The results indicated that the good combination of sensitivity, specificity, positive predictive value and negative predictive value of CMT test was obtained while considered score trace as CMT positive, with values of 39.28 %, 72.34 %, 71.73 % and 40 % respectively (Table 3).

Table 3: *Analysis of the California mastitis test values in comparison with bacterial culture*
Preglednica 3: *Analiza rezultatov kalifornijskega testa za mastitis v primerjavi z bakteriološko kulturo.*

	California mastitis test threshold values (cells/ml)	
Values observed	Score trace was considered as CMT (–)	Score trace was considered as CMT (+)
Sensitivity (%)	11.90	39.28
Specificity (%)	97.87	72.34
Positive predictive value (%)	90.90	71.73
Negative predictive value (%)	38.83	40
Accuracy (%)	42.74	51.14

The best combination of sensitivity, specificity, positive predictive value and negative predictive value of somatic cell count test was recorded while using a threshold of 240,000 cells/ml, with values of 72.61 %, 70.21 %, 81.33 % and 58.92 %, respectively (Table 4)

The comparison of considered thresholds characteristics, agreement and correlation values of various indirect tests in comparison with bacterial culture are given in Table 5.

The results showed that among the four tests, the SCC had the highest sensitivity (72.61 %), followed by pH (66.66 %), EC (47.61 %) and CMT (39.28 %). However, the specificity of the of CMT (72.34) were higher than those of SCC (70.21 %), EC (59.57 %) and pH (38.02 %). The accuracy of SCC, EC, pH and CMT was 71.75 %, 51.90 %, 51.14 % and 51.14 %, respectively.

The analysis of Kappa statistics revealed that SCC had a good agreement ($k = 0.411$) with the bacteriological culture examination. However, a poor concordances were observed between the bacteriological culture test and the pH, EC and CMT tests with values of ($k = 0.046$), ($k = 0.064$) and ($k = 0.099$), respectively.

Furthermore, based on Chi-square statistic test, only the SCC had an agreement ($p < 0.05$) with the results of culture examination. However, there was significant difference ($p > 0.05$) between the bacteriological culture test and the pH, EC and CMT tests.

The somatic cell count test showed the highest correlation ($r = 0.415$, $p < 0.05$) with bacterial culture test, followed by CMT ($r = 0.117$, $p > 0.05$), EC ($r = 0.069$, $p > 0.05$) and pH ($r = 0.049$, $p > 0.05$), respectively which showed a poor positive correlation with bacterial culture.

The comparison of various indirect diagnostic tests characteristics between the different studies is shown in Table 6. The observed differences with the results of the other studies indicate a wide range of possible tests properties.

4 DISCUSSION

The knowledge and effectiveness of different screening tests to detect subclinical form of mastitis have long been acknowledged as an imperative to the success of mastitis control and treatment in livestock animals. Many previous studies were reported for various species such as goat (Ghasemzadeh-Nava et al., 2008), sheep (Pradiee et al., 2012) and cattle (Langer et al 2014; Reddy et al.,

Table 5: *Comparison of considered thresholds characteristics, agreement and correlation values of various indirect tests in comparison with bacterial culture*
Preglednica 5: *Primerjava mejnih vrednosti, ujemanja in korelacij med različnimi posrednimi testi in bakteriološko kulturo*

	Threshold values			
	pH	Electrical conductivity (mS/cm)	California mastitis test	Somatic cell count (cells/ml)
Values observed	≥ 6.55	≥ 7.2	Score trace = CMT (+)	≥ 240000
Sensitivity (%)	66.66	47.61	39.28	72.61
Specificity (%)	38.02	59.57	72.34	70.21
Positive predictive value (%)	47.61	67.79	71.73	81.33
Negative predictive value (%)	57.44	38.88	40.00	58.92
Accuracy (%)	51.14	51.90	51.14	71.75
Kappa	0.046	0.064	0.099	0.411
Chi-square (*p* value)	0.577	0.427	0.181	0.000
R (Phi correlation)	0.049	0.069	0.117	0.415

Chi-square test with: $p < 0.05$ indicates an agreement between the results obtained for the diagnostic tests and the result of bacterial culture test, $p > 0.05$ indicates a disagreement between the results obtained for the diagnostic tests and the result of bacterial culture test.

2014). However, there is very little report about these aspects on sub-clinical mastitis in camels.

Bacterial culture is a standard method for examination of mastitis in camels (Tuteja et al., 2013). It may be carried out on individual quarter samples or on composite samples including milk from all quarters. In a mastitis control program, the cost of bacteriological culture in the laboratory can be greatly reduced by screening the camels with the indirect tests first and then culturing the positive reactors (Abdurahman et al., 1995; Almaw and Molla, 2000). These tests are indirect and detect only presence of inflammatory changes. Moreover, the intensity of the inflammation can be estimated qualitatively by CMT and quantitatively by SCC. The two tests have been used as diagnostic tools to detect subclinical mastitis in camel (Eberlein, 2007; Saleh and Faye, 2011). Similarly, technique that examines change in the milk conductivity (Ali et al., 2016) or its pH could be used to detect mastitis in camel (Tuteja et al., 2003).

Table 6: *Comparison of pH, EC, CMT and SCC characteristics*
Preglednica 6: *Primerjava značilnosti pH, EC, CMT in SSC metod*

Authors	Test	Sensitivity	Specificity	Basis
Current study	pH	66.66	38.02	Bacterial Culture
Langer et al (2014) (cows)		56.84	61.1	Bacterial Culture
Current study	EC	47.61	59.57	Bacterial Culture
Langer et al (2014) (cows)		25	87.1	Bacterial Culture
Reddy et al (2014) (cows)		56.52	84.84	Bacterial Culture
Current study	CMT	39.28	72.34	Bacterial Culture
Sharma et al. (2010) (cows)		86.07	59.70	Bacterial Culture
Pradiee et al (2012) (ewes)		28.67	87.06	Bacterial Culture
Langer et al (2014) (cows)		60.1	62.7	Bacterial Culture
Reddy et al (2014) (cows)		71.07	75.75	Bacterial Culture
Current study	SCC	72.61	70.21	Bacterial Culture
Sharma et al. (2010) (cows)		88.60	97.76	Bacterial Culture
Pradiee et al (2012) (ewes)		13.33	95.29	Bacterial Culture
Langer et al (2014) (cows)		39.8	84.8	Bacterial Culture
Reddy et al (2014) (cows)		65.21	78.78	Bacterial Culture

The results of present study indicate that the good combination of sensitivity and specificity of pH test was obtained while using a threshold of 6.55. However, our current findings are in disagreement with the findings of Tuteja et al. (2013) who reported that a threshold of 6.4 or more is indicative of sub-clinical mastitis in dairy camels. Sensitivity of pH test observed in present study was higher than that reported by Langer et al. (2014) for cows. Although, the specificity of this test was much lower than that reported by those authors, the low specificity observed for pH test suggests that pH of milk is not suitable method

for detection of subclinical mastitis in camels. The pH of milk may depend on the nature of fodder and water availability (Gorban and Izzeldin, 1997). Similarly, other factors may affect milk pH such as milk yield, lactation stage, milk composition and waiting time between sampling and testing. However, Ali et al. (2016) reported an increase in pH of camel milk samples in subclinical infection when compared to non-infected animals.

The current study indicated that a threshold of 7.2 mS/cm showed the good combination of sensitivity and specificity of electrical conductivity. This finding is comparable with that reported by Ali et al. (2016). In the present study, sensitivity and specificity of electrical conductivity was lower than that reported by Reddy et al.(2014). Likewise, based on this result, electrical conductivity cannot be used as an indirect test to detect subclinical mastitis in camel. This finding was in line with the previous report (Younan et al., 2001; Eberlein, 2007). Mastitis is not the only circumstance that causes changes in milk electrical conductivity and non-mastitis related variation in electrical conductivity is a major drawback. Non-mastitis factors influencing electrical conductivity include milk temperature, density, water, fat, protein percentage and breed (Elobeid et al., 2015).

The threshold values on SCC and CMT in camel milk were recent and not common. According to Merin et al., (2004), the SCC values in infected udder are lower in camel compared to the other ruminants. The present study showed that the good combination of sensitivity and specificity of these tests was obtained with a thresholds of 240000 cell/ml and score trace of CMT was considered as positive, respectively. This finding is supported by the report of Alamin et al (2013) who suggested that a threshold of SCC more than 2.5 × 105 cells/ml can be considered as indication of udder infection in camel. Sensitivity of CMT observed in our study was lower than that reported by Younan et al. (2001) which revealed a rate ranging from 68 %–77 % for major pathogen (*S. agalactiae* and *S. aureus*) in camel and that observed by Sharma et al. (2010) in cattle. The specificity of CMT concurs with the observations of Reddy et al. (2014) for cows but is lower than that reported by Younan et al. (2001) (91 %) for camel. The observed differences between the results of these studies indicate a wide range of possible CMT properties. This may be influenced by the subjectivity involved in interpreting the results of CMT. However, many researchers confirm that CMT can be used as a screening test to detect subclinical mastitis in camels (Saleh and Faye, 2011; Husein et al., 2013; Wanjohi et al., 2013).

In this study, SCC was found to be more accurate, sensitive, and specific than the CMT, pH and electrical conductivity tests compared to the bacteriological culture. Kathiriya and Shah (2009) compared the accuracy of SCC, CMT and pH for the detection of subclinical mastitis in dairy camels and stated that SCC was the most accurate test after examination using bacteriological culture. In addition, previous reports have confirmed that milk SCC can be used as a screening test to detect subclinical mastitis in camels (Bekele and Molla, 2001; Saleh and Faye, 2011). However, Alamin et al. (2013) reported that the SCC couldn't be used for detection of subclinical mastitis in camel.

The analysis of statistical results revealed that the pH, electrical conductivity and CMT test have a poor agreement (k = 0.046, 0.064 and 0.099, respectively) and poor correlation (r = 0.049, 0.069 and 0.117, respectively) with bacteriological examination. These results indicate that any single test may not be suitable in diagnosing sub-clinical mastitis. This finding is in agreement with previous studies (Younan et al., 2001, Bhatt et al., 2004 and Eberlein et al., 2007) who confirmed that electrical conductivity cannot be used for detection of subclinical mastitis in camels. However, many researchers (Abdel Gadir et al., 2006, Hawari and Hassawi, 2008 and Saleh and Faye 2011) reported that the positive correlation of CMT with the presence of mastitis pathogens in camel milk and stated that CMT is useful screening test in the detection of mastitis in camel and may serve to detect udder infected with major pathogens in a subclinical form. However, all of these studies subjected to cultural examination only the milk samples which had positive reaction on CMT.

In the present study only the SCC showed a good agreement ($k = 0.411$) and a good significant correlation ($r = 0.415$, $p < 0.05$) with the results of bacteriological culture. The value of the diagnostic test depends on its accuracy, sensitivity, specificity, predictive values, percentage agreement, and field applicability. In this study, SCC was found to be more accurate, sensitive, and specific than the other three tests (pH, EC and CMT) compared to the bacterial culture. Also, Guliye et al. (2002), Hawari and Hassawi (2008) reported that an increase in the number of somatic cells in camel milk is a good indication of the presence of pathogenic microorganisms in milk samples. Moreover, Kathiriya and Shah (2009) compared the accuracy of SCC, CMT and pH for the detection of subclinical mastitis in dairy camels and stated that SCC was the most accurate test after cultural examination. Similarly, Saleh and Faye (2011) confirm that SCC can be used as a screening test to detect subclinical mastitis in camels.

The observed discrepancy between the results of current study with those of other reports (Table 6) can be attributed to several possible factors such as animal species, subjective interpretation of the CMT and SCC results as well as the difference in the devices used for

measurement of pH and electrical conductivity of milk samples.

5 CONCLUSION

It is concluded that the SCC was the most accurate and reliable diagnostic method following the cultural isolation for the detection of sub-clinical mastitis in dairy camels under field conditions. Therefore, the combination between the SCC and CMT tests might be used effectively for diagnosis of sub-clinical mastitis in dairy camels. Moreover, the current study stated that the CMT, pH and EC were a poor predictor of udder infections. Therefore, the use of any single test may not be reliable in diagnosing sub-clinical mastitis in dairy camels.

6 AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author LH conducted the study. Author HA designed the study and served as principal supervisor. Author BH and MSM searched for the literatures and participated in manuscript writing. Authors AA wrote the protocols. All authors read and approved the final manuscript.

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