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Ability of Diagnostic Tests to Predict Subclinical Mastitis and Intramammary Infections in Quarters from Lactating Dairy Cows

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ABSTRACT

Subclinical mastitis (SCM) is the persistent inflammation of the mammary gland accompanied by subsequent damage to mammary gland tissue resulting in changes in composition of milk. This study was designed to evaluate the clinical utility of diagnostic tests like California mastitis test (CMT), pH, electrical conductivity (EC) for diagnosing SCM and intramammary infections. The profiles of CMT, pH, EC were studied in 135 composite milk samples which consisted of apparently healthy (n=25) and SCM (n=110) Holstein-Friesian dairy cows. Milk samples from Holstein- Friesian dairy cows were collected in different areas of Ganderbal district, Jammu & Kashmir from June 2017 to January 2019. The overall incidence of SCM among Holstein Friesian dairy cows was 81.48% by CMT. The rate of prevalence in +1, +2 and +3 CMT scores was 35.45%, 48.18% and 16.36% respectively. In this study, mean values of EC and pH in healthy animals was 4.01±0.09 mS/cm & 6.30±0.06, whereas in SCM it was 4.83±0.06 mS/cm and 6.90±0.05 respectively. The Receiver operating characteristics (ROC) curve analysis of EC and pH is at a cut-off values of 4.44 and 6.75. A significant increase ($p<0.0001$) in concentration and ROC curve analysis of EC and pH in milk were observed in dairy cows having sub-clinical mastitis as compared to the healthy animals.

***Key words*:** Subclinical mastitis, Diagnostic tests, EC, pH, ROC, Dairy cows

Subclinical mastitis (SCM) is the inflammation of the bovine mammary gland usually associated with milk alterations and changes in the pathology of the udder [1]. SCM is the predominant form of mastitis and is 5-40 times more prevalent than clinical mastitis [2]. The quantity and quality of milk in dairy cows with SCM is greatly altered which causes significant economic losses [3-4]. In SCM, no gross changes are observed which makes its detection imperative by laboratory diagnosis [5]. The extent of physical damage to the tissue of the udder is reflected by compositional changes in the milk. An important complete dietary food consumed by humans is milk that is enriched with numerous components [3]. Due to the overgrowing population the demand of milk and milk-based products has greatly increased [6]. In SCM, damage occurs to the blood-milk barrier that leads to the release of extracellular fluid components into the lumen of the alveolus, which decreases the rate of milk secretion. These components released from the inflamed quarters mix with secreted milk, which increases, pH and electrical conductivity. The severity of the inflammatory process is positively related to the magnitude of secretory components [7]. In SCM the quality and quantity of milk is greatly affected and the contaminated milk can serve as a source of zoonotic diseases.

Therefore, a simple, highly sensitive cow side test that is used for detecting SCM in bovines is California mastitis test (CMT) which gives a measure of somatic cells in milk. Other screening tests to detect SCM include the measurement of milk conductivity and pH. One of the earliest manifestations to detect intramammary infections (IMI) are the compositional changes in milk that increase its salinity. The elevated concentrations of anions and cations demonstrate the changes in electrical conductivity (EC). Milk pH is also considered as an indicator of subclinical

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mastitis because of increased membrane permeability which leaks blood components into the milk.

ROC is one of the best tools for evaluating the efficacy of diagnostic tests as it provides sensitive and specific results. Further, it allows in identifying the cut-off point that is considered as the best test value as it reduces the number of false-positive and negative values. The area under curve provides the accuracy of the diagnostic test and a value at cut-off point of 1.0 signifies complete insight between cases and non-cases. However, one of the greatest hurdles that prevent the use of these techniques is their low sensitivity and specificity. Therefore, this study was conducted with the purpose of assessing the effectiveness of various diagnostic tests for the identification of subclinical mastitis and intramammary infections in dairy cattle.

MATERIALS AND METHODS

Study area and population

A cross-sectional observational study was undertaken on 135 lactating cross-bred Holstein Friesian dairy cows reared in different areas of district Ganderbal of J&K from the period of June 2017 to January 2019.

Study methodology

California mastitis test (CMT)

California mastitis test (CMT) was performed according to the protocol of Hoque *et al.* [8].

Electrical conductivity (EC)

A digital electric conductivity meter (Eutech, Singapore) was used to measure the electric of selected milk samples.

Milk pH

pH of all milk samples was measured by a digital electric pH meter.

Statistical analysis

Descriptive statistical analysis of EC and pH in milk was performed through GraphPad prism (version 8) software. Receiver Operating Characteristics curve analysis was performed through Sigma plot 13 software in order to determine sensitivity and specificity.

RESULTS AND DISCUSSION

Prevalence of mastitis

Relation between the subclinical mastitis and CMT

Milk samples from 135 Holstein-Friesian dairy cows were collected which included apparently healthy controls (n= 25) and sub-clinical infected (n=110) animals. All the milk samples were subjected to CMT and the results were revealed as scores ranging from 0 to 3. The relation between CMT scores and SCM are shown in (Table 1, Fig 1). Out of 135 milk samples, 110 were positive for SCM with 39 (35.45%), 53 (48.18%) and 18 (16.36%) positive cases had a CMT score of +1, +2 and +3 respectively.

Relation between the subclinical mastitis and Electrical conductivity (EC)

Sub-clinically infected Holstein-Friesian dairy cows showed numerically higher variation in levels of electrical conductivity in milk (CV= 5.10% vs 7.72%) as compared to the healthy control group. Also, skewness statistics revealed a higher distribution of milk electrical conductivity in subclinical mastitis as compared to the healthy group, as scores clustered to the right. The mean concentration of electrical conductivity in the milk of healthy animals was 4.01±0.09 mS/cm, whereas in subclinical mastitis it was 4.83±0.06 mS/cm respectively. A significant increase ($p<0.0001$) in the concentration of EC in the milk of sub-clinically infected animals was observed as compared to the healthy group.

Table 1 Incidence of subclinical mastitis based on CMT scores

CMT score	Positive cases	Prevalence %
1	39	35.45
2	53	48.18
3	18	16.36

Where, 1, 2 and 3 indicate weak positive (+), distinct positive (++) and strong positive (+++)

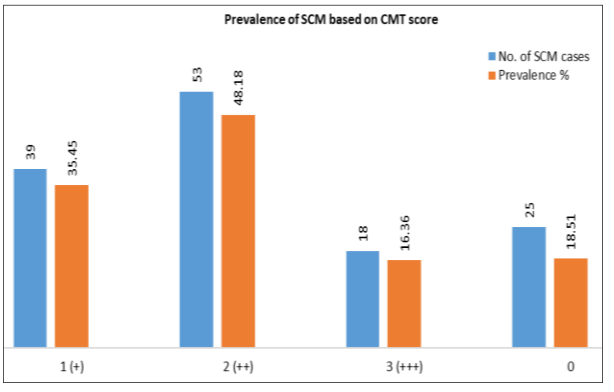

Fig 1 Incidence of subclinical mastitis with respect to CMT score

Relation between subclinical mastitis and pH

Sub-clinically infected Holstein-Friesian dairy cows showed numerically higher variation in levels of milk pH (CV= 11.67% vs 14.46%) as compared to the healthy control group. In addition, skewness statistics revealed a higher distribution of milk pH in subclinical mastitis as compared to healthy group, as scores clustered to the right. The mean concentration of milk pH in healthy and subclinical mastitis animals was 6.35±0.06 and 6.98±0.05 respectively. A significant increase ($p<0.0001$) in the concentration of milk pH was observed in the subclinical infected group as compared to the healthy group. The mean EC and pH concentration of milk has been presented in (Table 2). The box plot and multiple scatter data of EC and pH has been shown in (Fig 2a-d). The mean values of EC and pH in the milk samples of apparently healthy dairy animals and mastistis infected animals has been shown to vary significantly with respect to the health status.

Receiver operating characteristics (ROC) analysis

ROC curves represent the potential set of combinations of sensitivity and specificity possible for predictors. The analysis represented that the AUC for electrical conductivity in milk was 0.83 with the standard error of 0.04. The ROC curve analysis of electrical conductivity in milk is at a cut-off point of 4.44. The values of sensitivity and specificity for differentiating between mastitic and healthy cows were 70 % and 80% respectively. Similarly, AUC for milk pH was 0.87 with the standard error of 0.03 and at a cut-off value of 6.75. The value of sensitivity and specificity for differentiating between mastitic and healthy cows was 70 % and 90% respectively. Further a statistically significant difference ($p<0.0001$) was found in ROC curve analysis of both EC and pH in milk. The graphical representation of the ROC curve using EC and pH of milk as a potential indicator of mastitis is represented in (Table 2, Fig 2).

Table 2 Descriptive statistics of EC and pH in milk of healthy and Subclinical mastitis dairy cows

Parameter	Animal health status	Mean	SEM	SD	Skewness	Kurtosis	CV (%)	Optimal cut-off point	Sensitivity (Se)	Specificity (Sp)	Area under curve	P value
EC	Healthy	4.01	0.09	0.46	0.37	-0.00	11.67	4.44	70% (0.7)	80% (0.80)	0.83	<0.0001
	Subclinical	4.83	0.06	0.69	0.45	-0.39	14.46					
pH	Healthy	6.35	0.06	0.32	-0.09	-1.29	5.10	6.75	70% (0.7)	90% (0.90)	0.87	<0.0001
	Subclinical	6.98	0.05	0.53	1.60	5.65	7.72					

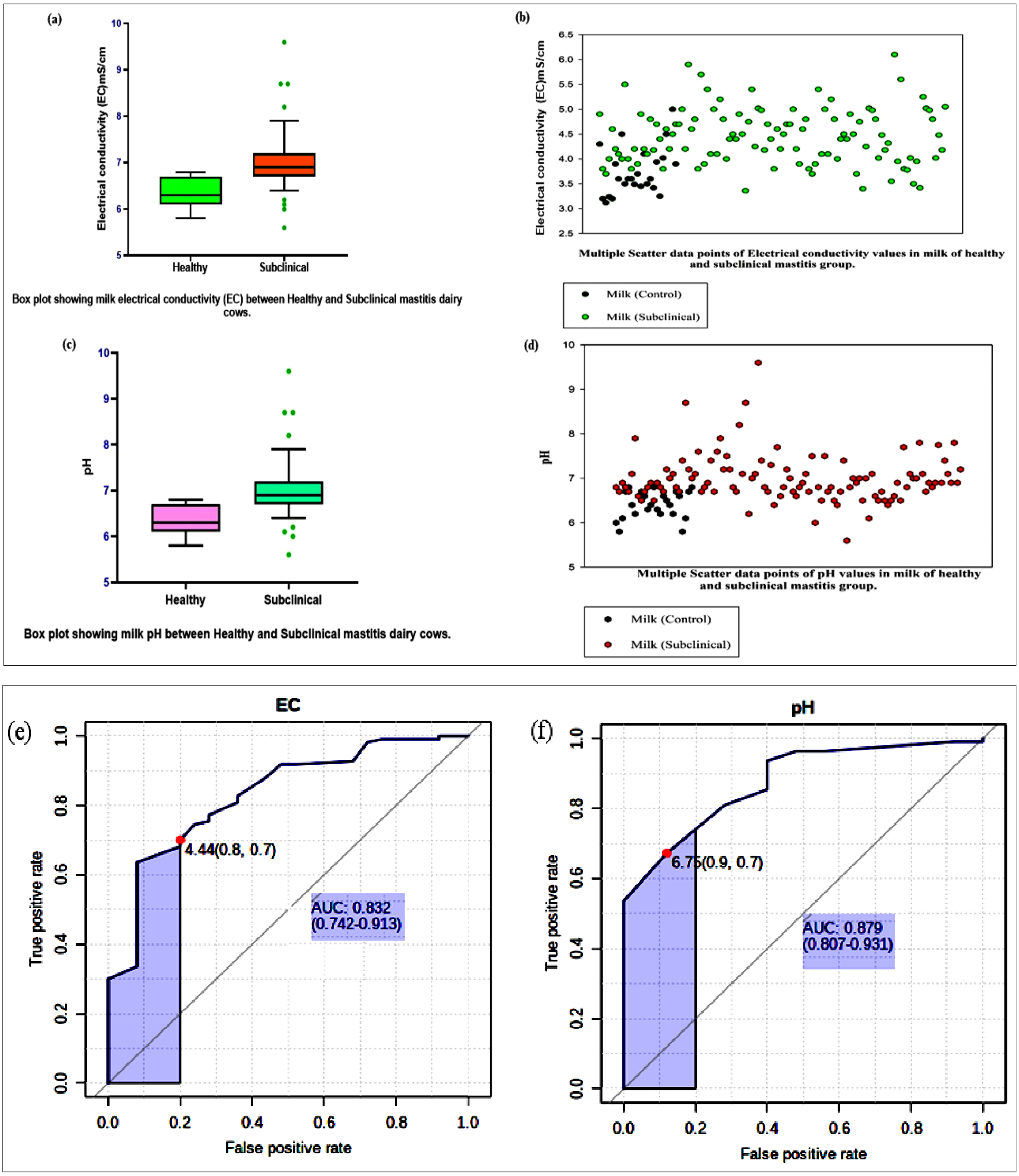

Fig 2 Box plot and multiple scatter of electrical conductivity (EC) (a, b) and pH (c, d) in milk of healthy and subclinical mastitis animals. The plots show the median (line within box), 25th and 75th percentiles (box), 10th and 90th percentiles (whiskers) and outliers (dots)

A graphical representation of ROC of EC and pH along with area under curve (AUC) as 0.83, 0.87 with sensitivity and specificity of 70% and 80% and 70% and 90% respectively.

Our study found the prevalence rate of mastitis to be 81.48% by CMT among Holstein Friesian dairy cows. This is in accordance with the studies of [9] who reported 81% prevalence of SCM on the CMT test. The results are comparable to the previous studies of 81.1% [10] and 82.9% [11] In our study, the rate of prevalence of CMT scores in +1, +2 and +3 cases was 35.45%, 48.18% and 16.36%. These findings are in agreement with [12]. This overall variation in the prevalence rate of mastitis as reported by authors indicates the complex nature of this disease which primarily suggests the interaction of many factors such as breed, milk production, age, stages of lactation, parity, farm management, hygiene practices, environment, causative agent and risk factors.

An important tool for detection of SCM in dairy cows before the appearance of clinical signs is the measurement of milk electrical conductivity. EC measures the presence of ions. In the present study the mean value of EC for healthy and subclinical mastitis milk was 4.01±0.09 mS/cm and 4.83±0.06 mS/cm respectively. The results of the present study do agree with the findings of [13] who have also reported elevated levels of EC in mastitic milk. In dairy cows with mastitis, the blood brain barrier is affected which facilitates movement of ions into the milk and as a result the concentration of ions particularly sodium (Na^+) and chlorine (Cl^-) is increased while as potassium (K) is decreased which ultimately raises the EC. Many other factors like breed, lactation stage, milking time etc. also affect EC [14].

In this study, the mean value of pH for healthy and SCM milk was 6.30±0.06 and 6.90±0.05 respectively. The findings of the present study are in accordance with the reports of [15-16] who also reported elevated levels of pH in mastitic milk. The increase in pH during SCM could be due to increased permeability of the membrane, which causes leakage of salts and different ions thereby into the milk [17]. The increase in milk leukocytes concentration can also result in higher pH values [18].

ROC analysis of EC revealed AUC was 0.83 with a cut-off value of 4.44. The value of sensitivity was found to be 70% while as specificity was 80%. At the beginning of cut-off point from <3.22 to <4.3 the sensitivity kept on increasing while from 4.44 the sensitivity and specificity were highest (100%). On the other hand from cut-off values of >3.22 to >6.50 the specificity decreased with each passing point. However, a threshold value of 4.44 could be used for differentiating between mastitic and healthy cows as the sensitivity and specificity were about 70% and 80% respectively which is good from a diagnostic purpose of view. The findings of this study agree with the previous studies i.e., 5 mS/cm [19]. In our study, both EC and pH showed the sensitivity of 70% respectively that is higher than previous studies. A study reported by [20] showed an accuracy of 69% for EC to differentiate healthy and SCM quarters in dairy cows.

Similarly, ROC curve analysis of pH revealed AUC of 0.87 with a cut-off value of 6.75. At this cut-off value, the sensitivity and specificity for differentiating between mastitic and healthy cows were 70% and 90% respectively. The values of sensitivity increased from cut-off values of 5.70 to 9.15, while values of specificity decreased within the same cut-off range. The sensitivity from cut-off points from 6.85 to 9.15 was highest but the specificity was lower, however a cut-off point of 6.75 could be ideal for differentiating mastitic and healthy cows as the sensitivity and specificity was 70% and 90% respectively. 59% accuracy for milk pH to differentiate between healthy and SCM quarters in dairy [20]. Also reported that composite milk samples have a threshold pH value of 6.63 for identifying SCM from non-mastitic cows. Different studies have reported that various factors such as breed, age, lactation stage and parity may also cause variation in threshold values of EC and pH.

CONCLUSION

The potential value of CMT, EC and pH measurement in milk samples as a screening test for detection of SCM was evaluated. With the establishment of infection in dairy cows, a significant increase in EC and pH of milk was observed. Therefore, it is possible to use these parameters for the detection of intramammary infection in dairy cows, particularly if EC and pH are measured daily in dairy cattle. Hence, CMT, EC and pH could be used as good indicators for detecting SCM in dairy cattle.

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Availability of data and materials

All data generated or analyzed during this study is presented in the main manuscript.

Conflict of interest

The authors declare no conflict of interest.

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