

*Original Research***Critical Thresholds of Milk SCC, EC and pH for Detection of Sub-Clinical Mastitis in Crossbred Cows Reared under Subtropical Agroclimatic Condition**

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Abstract

Early identification of mastitis in sub-clinical stage could be helpful for implementation of effective managerial interventions to reduce the economic losses. Milk samples were divided into two groups based on CMT score (CMT score 1 indicated healthy and CMT score 2-3 indicated SCM). Milk SCC, pH and EC value in SCM were significantly higher compared to healthy samples. Receiver Operator Characteristic (ROC) analysis revealed that milk SCC, pH and EC discriminated SCM quarters from healthy quarters with 99.97%, 93.42% and 99.09% accuracy. The critical threshold value of milk SCC, EC and pH were observed to be 245×10^3 cells/ml, 5.065 mS/cm and 6.605, respectively for identification of SCM quarters. ROC analysis revealed that milk SCC was more accurate to discriminate healthy and mastitic udder with critical threshold of milk SCC (245×10^3 cells/ml).

Key words: Sub-clinical Mastitis, CMT, SCC, EC, pH, ROC Analysis

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Introduction

The annual economic losses due to mastitis in India were calculated to be approximately INR 7165.51 crores; sub-clinical mastitis (SCM) is responsible for INR 4151.16 crores loss, which accounts about 57.93% of total losses due to mastitis in dairy animals (Bansal and Gupta, 2009). Therefore, early

identification of SCM could be helpful to implement effective management interventions to reduce the subsequent economic losses.

The common indicators of mastitis in dairy animals are somatic cell counts (SCC) and electrical conductivity (EC) of milk. In addition, change in milk pH is also positively associated with milk SCC (Shailja and Singh, 2002) and EC (Pyorala, 2003). California Mastitis Test (CMT) is considered as the oldest gold standard cow side test to identify SCM and positively associated with SCC (Viguier *et al.*, 2009). It has also been reported that both CMT and SCC are 100% accurate to differentiate mastitic milk from healthy milk samples (Kamal *et al.*, 2014). Although several studies indicated that the milk CMT score, SCC, EC and pH increased significantly during SCM (Syridion *et al.*, 2013; Kamal *et al.*, 2014), the threshold level varied with species and breed of animal. Threshold value of milk SCC was observed to be at 100,000 cells/ml in some studies (Bansal *et al.*, 2005) while in other studies it was 200,000 cells/ml (Viguier *et al.*, 2009). Similarly, threshold value for EC for identification of SCM was reported to be as low as 4.1 mS/cm (Bansal *et al.*, 2007) to as high as 6.5 mS/cm (Pyorala 2003) and for pH value was 6.63 (Bansal *et al.*, 2005), indicating that threshold values for different mastitic indicators are not consistent, which vary either depending on species or breed of dairy animals.

The present study reports the critical threshold level for milk SCC, EC and pH for early detection of sub clinical mastitis in crossbred cows. The critical threshold levels were established by ROC analysis considering the CMT as reference test.

Material and Methods

Experimental Animals and Sampling

The present study was conducted at Livestock Research Centre, National Dairy Research Institute, Karnal, Haryana, India. Milk samples (n=195) were collected from individual quarter of crossbred cows on 7th, 20th and 45th days post calving. Approximately 30 ml of milk from each quarter separately was collected aseptically in sterile milk container.

Estimation of Milk CMT, SCC, EC and pH

According to CMT score samples were divided in two groups; CMT score 1 indicated healthy udder quarters and CMT score 2-3 indicated SCM quarters. Milk SCC was estimated by Somatic Cell Counter (DeLaval International AB, Tumba, Sweden). Sample bottle containing milk was mixed well before recording pH using pH meter (Eu Tech, Korea) and Electrical conductivity (mili Simens/cm unit) in the pH-Conductivity Bench top (Orion 4 star Thermo Electron Corporation, USA).

Statistical Analysis

Milk SCC, pH and EC between healthy ($n = 128$) and SCM ($n = 67$) were tested for significance ($p < 0.05$) by t -test and values were presented as Mean $\pm$ SEM. Different threshold values of milk SCC, pH and EC were analyzed by ROC analysis (Patbandha *et al.*, 2015) taking CMT as reference test. Further, to confirm the thresholds of EC and pH, we used SCC thresholds as reference test using ROC analysis. Statistical analysis were done using Sigmaplot 11 software package (Systat software, Inc, California, USA). Though, ROC analysis produces range of potential threshold values, the value having highest combined Se and Sp called as critical threshold value of a diagnostic test.

Results and Discussion

Milk SCC, EC and pH

Mastitis is an inflammatory process where the inflammatory indicators such as SCC and EC of milk increase markedly and further positively associated with change in milk. Out of the 195 quarter milk samples collected, 67 samples (34.36%) were observed to be affected with SCM. Milk SCC, EC and pH of healthy and SCM milk samples are presented in Table 1.

Table 1: Milk SCC, EC and pH of Sub-Clinical Mastitic (SCM) and healthy quarters in crossbred cows

Samples	SCC ($\times 10^3$ cells/ml)	EC (mS/cm)	pH
SCM ($n=67$)	475.60 ^a $\pm$ 15.82	6.29 ^a $\pm$ 0.13	6.88 ^a $\pm$ 0.024
Healthy ($n=128$)	96.83 ^b $\pm$ 5.57	4.36 ^b $\pm$ 0.05	6.54 ^b $\pm$ 0.002

Means with different superscript (a, b) within a column differ significantly ($p < 0.05$)

The milk SCC ($\times 10^3$) of healthy and SCM milk samples were found to be 96.83 ± 5.57 cells/ml (ranged from 52 to 243 cells/ml) and 475.60 ± 15.82 cells/ml (ranged from 232 to 740 cells/ml), respectively, which was significantly ($P < 0.001$) higher in SCM compared to healthy quarters. Higher SCC in SCM milk samples observed in the present study is in agreement with the previous reports in crossbred and purebred dairy cows (Bansal *et al.*, 2005; Kasikci *et al.*, 2012; Syridion *et al.*, 2013; Kamal *et al.*, 2014). As per the findings of Skrzypek *et al.* (2004) milk SCC from healthy udders varies between 50,000 and 100,000 cells/ml which is in lower than our findings. The increase in SCC during infection might be due to the fact that bacterial invasion to mammary glands attracts circulating polymorph nuclear neutrophils (PMNs) which is addition to the dead and sloughed off mammary epithelial cells leads to high somatic cell counts in the milk (Viguier *et al.*, 2009).

Similarly, milk EC was observed to be 4.36 ± 0.047 mS/cm (ranged from 3.14 to 5.98) in healthy udder quarters which significantly increased in SCM milk samples i.e. 6.29 ± 0.13 mS/cm (ranged from 5.15 to 7.76 mS/cm). Generally, the EC value in normal milk at 25°C varies between 4.0 and 5.0 mS/cm

(Norberg *et al.*, 2004). However, milk EC value may go upto 5.5 mS/cm in normal milk at 25 °C and above 5.6 indicates mastitis (Kasikci *et al.*, 2012). Syridion *et al.* (2013) reported lower milk EC value in healthy samples compared to samples from infected quarters (5.63 vs. 6.71 mS/cm), but these values are comparatively higher than the values reported in our study. In similar line higher milk EC in SCM condition was reported by several researchers in dairy cows (Norberg *et al.*, 2004; Bansal *et al.*, 2005; Kamal *et al.*, 2014). The higher EC in SCM group may be attributed to change in ions particularly increase in milk sodium and chloride concentrations owing to inflammatory changes of mammary tissue (Pyorala 2003; Viguier *et al.*, 2009).

The pH of milk samples from healthy and SCM udder quarters were found to be 6.54 ± 0.019 (ranged from 6.5 to 6.59) and 6.88 ± 0.024 (ranged from 6.54 to 7.42), respectively which differed significantly. The increased milk pH during SCM is in agreement with previous findings (Ahmad *et al.*, 2005; Batavani *et al.*, 2007; Syridion *et al.*, 2013), but Bansal *et al.* (2005) did not observe any difference. The increased milk pH in the SCM may be associated with elevated concentration of alkaline blood constituents (sodium and bicarbonate ions) as permeability of the blood capillaries during inflammation of the mammary gland increase (Guha *et al.*, 2010). Additionally, the elevated milk pH value during mastitis may be due to lower acidity associated with reduced lactose contents in mastitic milk (Ahmad *et al.*, 2005).

Receiver Operator Characteristic (ROC) Analysis

The value of AUC of milk SCC, pH and EC are presented in Fig. 1. The milk SCC, pH and EC discriminated SCM quarters from healthy quarter with 99.97%, 93.42% and 99.09% accuracy.

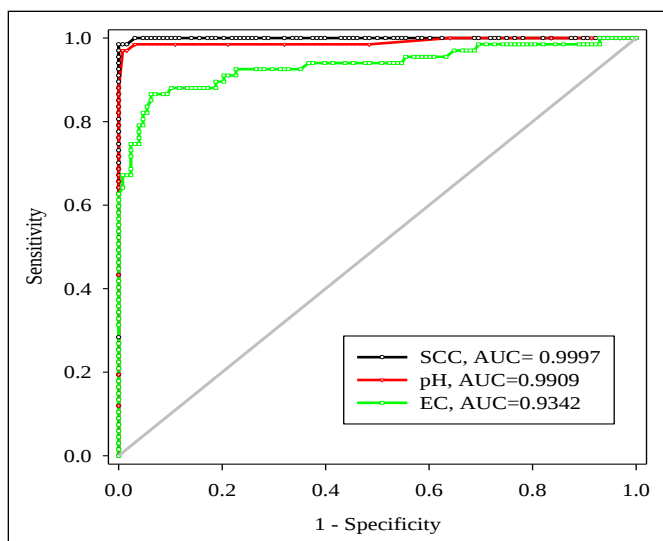


Fig. 1: Area under the receiver operating characteristic curve (AUC) of SCC, pH and EC for classification of mastitic and healthy quarters based on CMT as reference test

The AUC of a diagnostic test indicated the discrimination power or overall accuracy of a diagnostic marker to classify SCM and healthy quarters. Bansal *et al.* (2005) reported 75, 69 and 59% accuracy, respectively for SCC, EC and pH to discriminate healthy and SCM quarters in dairy cows which are comparatively lower than our results. Similarly, discrimination power of milk EC was observed to be 62.94% in buffalo (Bansal *et al.*, 2007). The fraction of milk used for detection of mastitis affects the accuracy for discrimination of healthy and SCM udder quarters (Bansal *et al.*, 2005), may lead to variation of accuracy in different studies. In addition, Bansal *et al.* (2005; 2007) used bacteriological identification and SCC to define mastitis which may also be another contributing factor for variation of accuracy.

The threshold values of milk SCC, EC and pH (established by ROC analysis using CMT as reference test) for identification of SCM and healthy quarter along with Se and Sp are presented in Table 2.

Table 2: Threshold values of foremilk SCC, EC and pH by ROC analysis based on CMT as reference test

Threshold value	Se (%)	95% CI for Se	Sp (%)	95% CI for Sp
SCC ($\times 10^3$ Cells/ml)				
245*	98.51	91.96-99.96	100	97.16-100
EC (mS/cm)				
5.065*	86.57	76.03-93.67	93.75	88.06-97.26
Ph				
6.605*	97.01	89.63-99.64	99.22	95.72-99.98

Se, Sensitivity; CI, Confidence Interval; Sp, Specificity; * Critical threshold value based on maximum combined Se and Sp.

Critical threshold of milk SCC, EC and pH was observed to be 245×10^3 cells/ml (Se=98.51% and Sp=100%), 5.065 mS/cm (Se=86.57% and Sp=93.75%) and 6.605 (Se=97.01% and Sp=99.22%) for identification of SCM quarters. The critical threshold values of milk EC and pH were further confirmed by ROC analysis using milk SCC thresholds (from 100,000 to 400,000 with increment of 50,000 cells/ml) as reference test and presented in Table 3 and 4, respectively.

Table 3: Critical threshold values of foremilk EC by ROC analysis based on SCC as reference test

SCC Thresholds ($\times 10^3$ Cells/ml)	AUC	Critical Thresholds	Se (%)	95% CI for Se	Sp (%)	95% CI for Sp
100	0.8136	4.665	68.38	59.13-76.66	79.49	67.41-86.76
150	0.8636	5.065	65.59	55.02-75.15	95.1	88.93-98.34
200	0.898	5.065	73.75	62.71-82.96	93.91	87.86-97.52
250	0.9456	5.065	89.23	76.06-95.56	93.85	88.23-97.31
300	0.9703	5.065	91.94	82.17-97.33	93.23	87.54-96.86
350	0.9741	5.095	93.33	83.30-98.15	92.59	86.80-96.39
400	0.9731	5.095	94.64	85.13-98.88	91.37	85.41-95.46

AUC, Area under Curve; Se, Sensitivity; CI, Confidence Interval; Sp, Specificity

Table 4: Critical threshold values of foremilk pH by ROC analysis based on SCC as reference test

SCC Thresholds (10^3 Cells/ml)	AUC	Critical Thresholds	Se (%)	95% CI for Se	Sp (%)	95% CI for Sp
100	0.8361	6.575	65.81	56.47-74.33	96.15	89.17-99.20
150	0.9128	6.575	80.65	71.15-88.11	95.1	88.93-98.39
200	0.9447	6.595	83.75	73.82-91.5	100	96.84-100
250	0.9989	6.605	98.46	91.72-99.96	98.46	94.55-99.81
300	0.9981	6.605	98.39	91.34-99.96	96.24	91.44-98.77
350	0.996	6.625	95	86.08-98.96	99.26	95.94-99.98
400	0.9961	6.625	98.21	90.45-99.95	97.84	93.82-99.55

AUC, Area under Curve; Se, Sensitivity; CI, Confidence Interval; Sp, Specificity

The threshold value having highest combined Se and Sp is called as critical threshold value. In the present study, critical threshold value of milk SCC was observed to be 245×10^3 cells/ml at which the Se was 98.51%. Similarly, Sp of 100% depicted that using milk SCC threshold. The sensitivity and specificity at optimum milk SCC threshold observed in our study are comparatively higher than Pyorala (2003), who reported that sensitivities ranged from 73-89% and specificities from 75-85% using threshold of SCC, as 200,000 cells/ml for identification of intra-mammary infections. The milk EC threshold value of 5.065 mS/cm had maximum combined Se and Sp, indicated that using this threshold 86.57% SCM quarters and 93.75% healthy quarters could be identified. The threshold values of EC (5.065 mS/cm) to detect SCM udder quarter observed in the present study is in agreement with previous reports i.e. 5.0 mS/cm (Norberg *et al.*, 2004; Kamal *et al.*, 2014). However, Kasikci *et al.* (2012) and Pyorala (2003) reported higher threshold value of EC (5.5 and 6.5 mS/cm, respectively). The threshold of pH for identification of SCM was 6.605, which is comparatively lower than Bansal *et al.* (2005), who reported threshold value of milk pH as 6.63 and 6.65 in composite and foremilk sample, respectively in dairy cows. The SCC, EC and pH values are not only affected by mastitis but also by non-mastitic factors such as species, breed, parity and stage of lactation, may be the reason for variation in critical threshold value by different studies. Further, the reference test used to define SCM also varies from study to study, might be another contributing factor for variation of threshold values. The threshold values of milk also depends on fraction of milk considered for identification of mastitis (Bansal *et al.*, 2005), might be another contributing factor for variation of threshold values.

Furthermore, considering the SCC as reference test the threshold values of milk EC and pH were produced by ROC analysis. The critical threshold of EC (5.065 mS/cm) for identification SCM was consistent between SCC lower thresholds of 150,000 to 300,000 cells/ml. However, the critical threshold of EC at thresholds of 350,000 cells/ml had higher accuracy (indicated by AUC) and maximum combined Se and Sp compared to others. Therefore, EC can't be considered as reliable diagnostic marker for identification sub-clinical mastitis. On the other hand, the critical threshold of milk pH (pH=6.605) at

250,000 cells/ml (as reference test) had maximum combined Se and Sp. The critical threshold of pH developed using SCC as reference test is comparable with that developed using CMT as reference test. Hence, pH could be considered as better indicator for detection of SCM as compared to EC.

Conclusion

From the findings of the above study, it was concluded that SCC had more accuracy to discriminate healthy milk from sub-clinical mastitic milk compared to EC and pH. The established critical threshold of milk SCC (245×10^3 cells/ml) had comparatively higher sensitivity and specificity to identify true positive and true negative cases, respectively as compared to EC and pH.

Conflict of Interest

Authors do not have any conflict of interest.

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