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Status of Subclinical Mastitis in Crossbred Cattle of Peri-urban Unorganized Herd of Middle Indo-gangetic Plains

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ABSTRACT

Background: Mastitis is the most common and economically important disease of dairy cattle. Subclinical mastitis is a more important form in India than clinical mastitis. Subclinical mastitis (SCM) detection done by periodic examination of the udder health by evaluation of milk at the herd level or the individual cow level by milk somatic cell count (SCC), followed by culture of random milk samples. The presented study was undertaken by survey and sampling of milk from lactating crossbred cattle of unorganized dairy farms and farmer's dairy of the peri-urban region of middle Indo-Gangetic Plains. The objective of the study was to monitor the status of SCM in crossbred cattle and associated changes in milk constitutes and oxidative stress in milk.

Methods: A total of 147 lactating crossbred cattle were screened for SCM using the modified California Mastitis Test (CMT) using detergent based CMT reagent and compared with conventional CMT reagent, followed by SCC, milk constituents, bacterial isolation and antibiotic sensitivity testing (ABST). General information was collected in pre-tested questionnaire. The data obtained were statistically treated to evaluate significance of the study.

Conclusion: The overall prevalence of subclinical mastitis in peri-urban cross-bred cattle was 36.74% which varied with parity and stage of milking. Prevalence of subclinical mastitis was highest (55.77%) in cross-bred cattle in mid-lactation and Parity 3-5. The mean SCC was significantly higher (7.21±0.27) in subclinical mastitis compared to CMT negative (3.66±0.06) milk sample. Somatic cell count was positively and significantly correlated with CMT reactions using conventional CMT reagent (rs=0.86) as well as modified CMT reagent (rs=0.815) attempted using Spearman rank-order correlation coefficient. The mean values of milk pH, fat and lactic acid acidity increased significantly ($P \leq 0.01$) in SCM compared to the milk of healthy cattle, except lactose which decreased significantly in SCM milk. The common contagious bacteria responsible for SCM isolate were coagulase-positive *Staphylococcus* spp. (64.82%) isolates from these SCM milk followed by *Streptococcus* spp. ABST test conducted on random CMT positive milk sample indicated that gentamicin as most sensitive, followed by enrofloxacin. The present finding indicates the suitability of enrofloxacin as the most useful antibiotic for the treatment of subclinical mastitis in cross-bred cattle of the peri-urban area of middle Indo-gangetic plains.

Key words: ABST, Cross-bred cattle, Detergent based CMT reagent, Milk constituents, Screening, Sub-clinical mastitis.

INTRODUCTION

Mastitis is a major global problem of lactating bovine population in which lack of timely diagnosis and treatment often results in complete loss of udder. Mastitis is inflammation of the parenchyma of the mammary gland regardless of the cause and characterised by a range of physical and chemical changes in the milk and pathological changes in the glandular tissue. Clinical mastitis can be grossly detected by the presence of flakes, clots, discoloration in milk and swelling, pain and induration in the udder. However, in most subclinical mastitis (SCM), these grossly visible changes in both milk and udder are not visible and thus making diagnosis very difficult. Subclinical mastitis is an important form in India than clinical mastitis (Joshi and Gokhle, 2006; Pitkala *et al*., 2004) as it is of longer duration and prevalent more than 15 to 40 times the clinical mastitis (Elango *et al*., 2010). Other adverse effects of SCM are deterioration of milk quality and loss of production of animals apart from a source of infection to other animals within the herd. SCM can be screened routinely based on California Mastitis test (CMT) along with somatic cell count (SCC) and bacterial isolation. Somatic cells are protective for udder health. SCC is a useful marker for intramammary infection and consist of leucocytes and epithelial cells. A high SCC in milk indicates inflammation in the udder (Dürr *et al*., 2008). Strategies designed to improve the immune

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cells of the diseased udder during immunosuppressive stages and target oxidative-anti-oxidative defence would greatly affect the ability of the animal to resist the pathogenic infection (Dimri *et al*., 2013). The present study has been undertaken to study the physiological risk factors for subclinical mastitis and associated changes in the milk through screening cross-bred cattle of unorganised commercial farmer's dairy.

MATERIALS AND METHODS

Ethics statement

The study involved sampling of milk during routine milking aseptically from cattle with the consent of the farmers. There is no specific law for milk sample collection and hence no approval was mandatory.

Animals

The work was undertaken in 147 crossbred (CB) lactating cattle of unorganised commercial farmer's dairy in the peri-urban region of middle Indo-Gangetic plains in 2016. All these CB cattle were in their early to mid-lactation phase and parity ranged from 1-5 as risk factors. The lactation period was considered early when animals were 15 to 60 days of lactation and mid between 60 to 120 days of lactation. All the 147 lactating cattle were apparently healthy with no gross clinical signs of mastitis in milk and udder. Dairy cattle with clinical mastitis were not included in the study. Management practices, health care, calf history, production record and marketing behaviour were also recorded. The laboratory works was carried out at ICAR Research Complex for Eastern Region, Patna.

Sample collection

Milk samples (50 ml) were collected from each quarter of the animals in a sterile container and were analysed the same day. Before milk collection, the udder was washed with a commercial disinfectant solution containing Sodium dichloroisocyanurate (klengard®) and dried with a sterile cloth towel. The quarters were disinfected using sterile swab dipped in 70% ethanol. Milk samples were collected after discarding 2-3 stripping from each teat and were properly labelled.

California mastitis test screening

Preliminary pen-side milk samples were screened for SCM by California mastitis test with modified CMT reagent and validated with conventional (teepol based) CMT reagent. Modification in the reagent was attempted by replacement of teepol (Sigma) by commercial available detergent Ezee® (Godrej) to make the reagent cheaper and validated the results with anionic detergent Teepol® (Sigma-Aldrich) based CMT reagent. The final composition of modified CMT reagent was homogenous solution prepared by mixing 1.5 g Sodium, 0.5 ml Ezee, 0.01 g bromothymol purple and distilled water up to 100 ml. CMT diagnostic paddles were procured from Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. CMT reaction is based upon the amount of cellular nuclear protein present in the milk sample (Greiner *et al*., 2000). The test was conducted and scored according to Schneider and Jasper (1964). An equal volume of milk and CMT reagent was mixed in each of the four cups of the CMT paddle. Each testing carried out in duplicate using conventional and modified CMT reagent. Mixing was accomplished by gentle circular motion in a horizontal plane. The reaction developed almost immediately with milk containing a high concentration of somatic cells. The peak of the reaction was obtained within 10 seconds and scored from 0 to +++ depending upon the severity of reaction (Fig 1 a and 1 b).

Somatic cell count

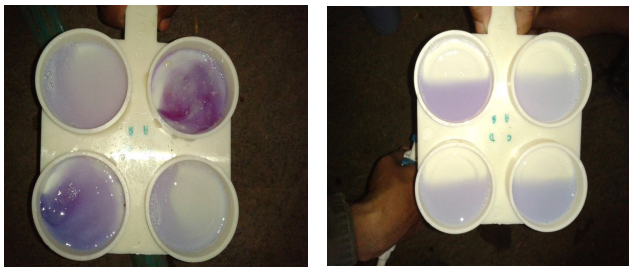
Somatic cell count was estimated manually by direct microscopy using the method described by Schalm *et al*., 1971. The milk samples were continuously mixed several times. The glass slide was placed over the template to outline four 1 sq.cm areas using a glass marker. Ten micro-liter of milk was spread evenly over the 1 sq.cm template. The films were dried at room temperature. The dried slide was flooded with modified Newman-Lampert stain (Himedia) for 2 min. The excess stain was drained off and air-dried. The slide was rinsed under tap water and air-dried. Stained films were examined under oil immersion objective and the number of cells in 10 fields were counted.

Milk parameters

The pH of fresh milk samples were estimated using electronic pH meter (Eutech pH 2700, Thermo Scientific) after proper calibration. Lactose and fat content in milk was analysed by an automated milk analyser (Lactostar, Funke-Gerber). The titratable acidity of milk expressed as percentage lactic acid was estimated as per the procedure described by the Bureau of Indian Standards (1960).

Total oxidant and antioxidant capacity in milk

Total oxidant capacity in both CMT negative and CMT positive milk was measured using the method of Erel (2005). Similarly, total antioxidant capacity was measured by the method described by Erel (2004). Dimri *et al*., (2013)



a b

Fig 1: Modified California mastitis test for indirect detection of subclinical mastitis in CMT paddle observed with 30 secs of mixing equal volume of milk and modified CMT reagent.
a). CMT testing of +2 to traces in different cups of CMT paddle.
b). Negative CMT results in all the cups.

explained both the methods in detail. In this study, 25 samples of both CMT negative and CMT positive milk samples were randomly selected for evaluation for the total oxidant and antioxidant capacity.

Bacterial isolation and antibiotic sensitivity test (ABST)

The positive samples of milk were subjected to bacterial isolation in blood agar (Himedia) followed by selective media for *Staphylococcus* spp.

The identification of the causative organism in collected milk samples was carried by inoculating 10 µl of milk on 5% bovine blood agar plates with a sterile 'L' shaped loop by the quadrant streaking method. The causative organism of the milk samples was identified based on colony morphology and haemolytic pattern on 5% blood agar after anaerobic incubation of the streaked plates at 37°C for 24-48 h (Cowan and Steel, 1965). A gram staining of isolates was undertaken to assist the identification of the causative organism. Further identification of isolate at genus level was made using selective media such as Mannitol salt agar, Edward medium and MacConkey agar. Colonies with gross culture characteristics of *Staphylococcus* which grew on both 5% blood agar and Mannitol salt, were considered as *Staphylococcus* spp. Colonies with the gross characteristic of streptococci on blood agar and negative growth on Mannitol salt and MacConkey agar and positive growth on Edward media were categorized as *Streptococcus* spp. Culture growth on MacConkey agar was considered as gram-negative bacteria and was considered as coliforms based on staining characteristics.

Random CMT positive milk samples (n=20) were subjected to ABST using Mastitest ABST test kit TM (Himedia) as per manufacturer's instruction for assessing antibiotic resistance and sensitivity. Colour change in the vials inoculated for 16-18 hrs at room temperature indicates resistance to a particular antibiotic while a minimal colour change in vial indicates sensitivity to a particular antibiotic.

Statistical analysis

The prevalence of subclinical mastitis by CMT and SCC was calculated as the proportion of affected cow/quarters of the base population. Statistical analysis of the data was done as per the standard procedures of Snedecor and Cochran (1994). Cohen's kappa coefficient was used for validating modified CMT test reagent with conventional CMT test reagent and interpreted by the methods described by McHugh (2012). All the results are expressed as the mean±S.E. and statistical significance was accepted for $p \leq 0.05$ or greater. The multivariate statistical analysis like principal component analysis (PCA) is used (Miekley *et al*., 2013) on the four categories of cow milk and milk components parameters like SSC, pH, acidity, fat and lactose. PCA was performed on the normalised data sets to assess which factors in the milk were more variable with different risk factors.

RESULTS AND DISCUSSION

The study conducted in the peri-urban region of Patna covering small dairy farmers, as they are the backbone of milk supply to individual consumer and gross dairy requirements in the city. These peri-urban dairy animals were cross-bred cattle brought from villages after recent parturition or purchased from the local market. These peri-urban cross-bred cattle were kept with the sole objective of liquid milk sale. They are either sent back to the village area or sold when they become dry or in the last trimester of pregnancy. These cattle were kept on a stall-fed system on a high concentrate diet and with two times hand-milking. Udder and teat cleaning was carried out before milking with clean water but the practice of wiping after washing or use of teat disinfectants was not practiced by any of the farmers. The practice of feeding during milking was dependent on milking place. When the milking was done at the customer's door or any other market place, milking was done without feeding while feeding was practiced during milking at cattle shed. Dry cow therapy was not known to any of the farmers. Farmers were unaware of the state of subclinical mastitis. Calf health care and management were not considered with were high (74.15%) calf mortality below six months of age. The calf mortality was higher in males (97.10%; n=69) compared to females (53.85%; n=73) within the same age group, indicating negligence of the farmers. This negligence was identified as restricted colostrum and milk feeding, poor care and supplemental feeding and poor health management. The government policy on restriction of sale and culling of male calves and decreasing demand for bullock for farm work were also important factors responsible for their negligence (Ranjan, 2017). Similar reports of high calf mortality in commercial dairy farms of Andhra Pradesh and Uttar Pradesh have been observed for calf mortality and gender difference (Sreedhar and Sreenivas, 2015; Tiwari *et al*., 2007). Most farmers used manual teat massage for milk let down. This may be one of the possible reasons associated with calf mortality restricting natural milk let down by calf suckling.

The prevalence of subclinical mastitis in peri-urban cross-breed cattle based on CMT and associated changes in somatic cell count, milk pH, acidity, fat and lactose are depicted in Table 1. The overall prevalence of subclinical mastitis in peri-urban cross-bred cattle was 36.74% which varied with parity and stage of milking. Our finding of moderate SCM in peri-urban cattle of Bihar corroborates with the study carried out by International Livestock Research Institute in rural, peri-urban and urban households in Bihar (Hardenberg, 2016). However, a similar study carried out in buffaloes of the peri-urban region of Patna indicated a very high (61.87%) prevalence percentage of subclinical mastitis (Kumar *et al*., 2012). Our finding of lower prevalence of subclinical mastitis may be due to the selection of only apparently healthy lactating cattle of early and mid-lactation, assuming physiologically higher somatic cell count in late lactation mostly due to increasing in epithelial cells

(Dohoo and Meek, 1982). Prevalence of subclinical mastitis was highest (55.77%) in cross-bred cattle in mid-lactation and Parity 3-5 and lowest (20%) in cattle in early lactation and parity 1-2. Exposure to pathogens increases with age and physiological increase in udder size. Higher parity increases the risk of trauma to the teats and udder, therefore the prevalence of mastitis increase with parity and stage of lactation (Ghosh and Prasad, 1998; Modh *et al*., 2017). A similar finding of increased incidence of mastitis with an increase in parity has been reported in a study conducted on the prevalence of subclinical mastitis in Swedish dairy (Jingar *et al*., 2014). Hiitiö *et al*., (2017) also reported in corroboration with our findings that increase parity as significant cow and herd factors associated with subclinical mastitis. Higher incidence recorded in parity 3 to 5 may be attributed to an increase in milk yield pressure and lowered immunity influencing an increase in somatic cell count (Mukherjee and Dang, 2011).

Subclinical mastitis and somatic cell count

The somatic cells include leucocytes of the blood and epithelial cells of the mammary gland and are measured as the number of cells per milliliter of milk (Fig 2 a,b). Somatic cell count was positively and significantly (P≤0.01, 2-tailed) correlated with CMT reactions using Teepol based conventional CMT reagent (rs= 0.86) as well as modified CMT reagent (rs=0.815) attempted using Spearman rank-order correlation coefficient. Similarly, the Cohen's Kappa coefficient for measuring the reliability of modified CMT with conventional CMT was 0.7 indicating a substantial measure of agreement as described by McHugh (2012). The results of the Spearman correlation and Kappa coefficient indicated that the modified CMT reagent was equally effective in detecting milk SCC and subclinical mastitis. The result indicates that CMT screening is a suitable indirect test to assay SCC and so the status of infection in the mammary gland. It was observed that mean SCC was significantly (P≤0.01) higher (7.21±0.27) in subclinical mastitis compared to CMT negative (3.66±0.06) milk samples (Table 1). The mean SCC of CMT negative and positive milk samples increased with parity which corroborates with findings of Saravanan *et al*., (2015). However, a significant difference was observed in CMT negative samples between groups 2 and 3 (P≤0.01) and between 2 and 4 (P≤0.05). In CMT positive milk samples, the mean SCC difference between groups 2 and 3 was significant (P≤0.05), however, mean SCC was non-significantly higher with increasing parity in other groups. The SCC was non-significantly different between different stages of lactation though it was slightly higher in early lactation compared to mid-lactation.

Table 1: Status of subclinical mastitis based on CMT results in crossbred cattle in early and mid-lactation and different parity and corresponding changes in milk parameters (means±S.E).

Groups	Risk factors	CMT score results	SCC ($\times 10^5$/ml)	Milk pH	Acidity (% lactic acid)	Fat (%)	Lactose (%)
Group 1	Early lactation	Traces (n=3)	$5.95^{a}\pm0.44$	$6.47^{ab}\pm0.03$	$0.15^{ab}\pm0.00$	$3.63^{a}\pm0.13$	$4.38^{a}\pm0.02$
(n=25)	and parity 1-2	+ to ++ (n=2)	$10.02^{b}\pm0.77$	$6.65^{a}\pm0.05$	$0.17^{a}\pm0.01$	$3.55^{a}\pm0.15$	$4.29^{a}\pm0.00$
		Mean positive (n=5)	$7.57^{A}\pm1.05$	$6.54^{A}\pm0.05$	$0.16^{A}\pm0.00$	$3.60^{A}\pm0.09$	$4.34^{A}\pm0.03$
		Mean negative (n=20)	$3.57^{Bc}\pm0.12$	$6.34^{Bb}\pm0.03$	$0.14^{Bb}\pm0.00$	$3.55^{Aa}\pm0.05$	$4.67^{Bb}\pm0.02$
Group 2	Mid lactation	Traces (n=7)	$5.84^{a}\pm0.14$	$6.63^{a}\pm0.05$	$0.15^{a}\pm0.00$	$3.59^{a}\pm0.06$	$4.38^{a}\pm0.02$
(n=31)	and parity 1-2	+ to ++ (n=3)	$6.92^{a}\pm0.41$	$6.63^{a}\pm0.09$	$0.17^{a}\pm0.06$	$3.50^{a}\pm0.06$	$4.24^{a}\pm0.03$
		Mean positive (n=10)	$6.16^{A}\pm0.22$	$6.63^{A}\pm0.04$	$0.16^{A}\pm0.00$	$3.56^{A}\pm0.05$	$4.34^{A}\pm0.03$
		Mean negative (n=21)	$3.23^{Bb}\pm0.11$	$6.26^{Bb}\pm0.02$	$0.12^{Bb}\pm0.00$	$3.66^{Aa}\pm0.03$	$4.70^{Bb}\pm0.03$
Group 3	Early lactation	Traces (n=7)	$7.38^{a}\pm0.68$	$6.50^{a}\pm0.03$	$0.15^{a}\pm0.00$	$3.50^{a*}\pm0.05$	$4.44^{a*}\pm0.03$
(n=39)	and parity 3-5	+ to ++ (n=3)	$11.49^{b}\pm0.51$	$6.57^{a}\pm0.03$	$0.16^{a}\pm0.01$	$3.60^{b*}\pm0.06$	$4.32^{b*}\pm0.01$
		Mean positive (n=10)	$8.61^{A}\pm0.79$	$6.52^{A}\pm0.02$	$0.15^{A}\pm0.00$	$3.53^{A}\pm0.04$	$4.40^{A}\pm0.03$
		Mean negative (n=29)	$3.95^{Bc}\pm0.11$	$6.34^{Bb}\pm0.02$	$0.13^{Bb}\pm0.00$	$3.34^{Bc}\pm0.02$	$4.67^{Bc}\pm0.02$
Group 4	Mid lactation	Traces (n=20)	$5.97^{a}\pm0.15$	$6.44^{a}\pm0.03$	$0.16^{a}\pm0.00$	$3.56^{a*}\pm0.02$	$4.42^{a*}\pm0.02$
(n= 52)	and parity 3-5	+ to ++ (n=9)	$9.38^{b}\pm0.38$	$6.61^{b}\pm0.06$	$0.17^{b}\pm0.00$	$3.62^{b*}\pm0.04$	$4.34^{b*}\pm0.03$
		Mean positive (n=29)	$7.03^{A}\pm0.33$	$6.49^{A}\pm0.03$	$0.16^{A}\pm0.00$	$3.58^{A}\pm0.05$	$4.40^{A}\pm0.02$
		Mean negative (n=23)	$3.78^{Bc}\pm0.09$	$6.33^{Bc}\pm0.02$	$0.13^{Bc}\pm0.00$	$3.41^{Bc}\pm0.04$	$4.70^{Bc}\pm0.03$
Overall		Mean positive (n=54)	$7.21^{A}\pm0.27$	$6.53^{A}\pm0.02$	$0.16^{A}\pm0.01$	$3.57^{A}\pm0.02$	$4.38^{A}\pm0.01$
(n=147)		Mean negative (n=93)	$3.66^{B}\pm0.06$	$6.32^{B}\pm0.01$	$0.13^{B}\pm0.01$	$3.47^{B}\pm0.02$	$4.69^{B}\pm0.01$

Capital superscripts have been used to denote the significant difference between mean positive and mean negative between CMT results. Small superscripts have been used to denote the significant difference between, traces, positive and negative CMT score results.

*has been used to denote the significance at 5% level.

Mean SCC of negative milk samples between groups 2 and 3 and 2 and 4 was significantly different at 1% and 5% level, respectively. Mean SCC of positive milk samples between groups 2 and 3 was significantly (p≤0.01) different.

The findings corroborate with reports of past-published work that documents slightly higher somatic cells in the first month of lactation and then decrease in the second month of lactation followed by fluctuation till 300 days of milking (Singh and Dang, 2002).

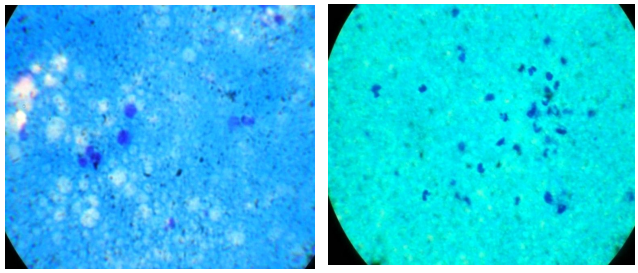
Subclinical mastitis and associated changes in milk components

Inflammation and associated changes in the mammary gland due to pathogens during the subclinical stage of mastitis affect milk components and characteristics of the milk. However, gross physical changes are in general absent or not noticed in subclinical mastitis. The changes observed in milk pH, acidity as percentage lactic acid, lactose (%) and milk fat (%) associated with CMT results and SCC of 147 apparently healthy crossbred cattle are presented in Table 1. The results indicated that the mean values of all these parameters in milk increased significantly ($P \leq 0.01$) in SCM compared to the milk of healthy cattle except lactose, which decreased significantly in SCM cattle. These associated changes in milk parameters suggest that acidity, lactose and fat in milk may have some potential for monitoring SCM in crossbred cattle. These findings corroborate with the published reports that SCC has a significant effect on milk fat and lactose (Paixão *et al*., 2017; Zecconi *et al*., 2019). Milk parameters were non-significantly variable with the severity of CMT results and SCC except milk fat and lactose was significantly ($P \leq 0.05$) varied with the severity of SCM in groups 3 and 4. Among different milk parameters, acidity was significantly ($P \leq 0.05$) different between groups 1 and 2 in CMT negative milk sample while fat was significantly different between all groups. The mean milk fat percentage difference between groups 1 and 3; 2 and 3; 2 and 4 were highly ($P \leq 0.01$) significant while groups 1 and 4 differed significantly at 5% level. The findings indicate that the fat percentage in milk varies physiologically with different stages of milking and parity, while other parameters vary non-significantly with the different physiological states of cattle. Milk acidity also showed an increasing trend with the advancement of lactation in early parity healthy animals. These findings are in agreement with the earlier reports of Bhoite and Padekar (2002) who observed a significant effect of the stage of lactation on fat in crosses involving Jersey. Contrary to our findings, Sarkar *et al*., (2006) reported that the lactation stage had no influence on fat content but a significant effect observed on lactose content was in conformity with our report. Similarly, Radhika *et al*., (2012) observed a non-significant effect of parity on fat percentage; however, the increase in fat with parity was similar to our findings.

The two principal components explained 79.93% variation in the data in which first component showed maximum 60.20% and second component showed 17.92% variation in the data sets. Here principal component 1 is dominated by group 4 and 3 respectively in order of their contribution and group 1 dominates principal component 2. The PCA biplot showed that group 4 is maximum affected while group 1 is less affected. The acidity of the milk as lactic acid observed maximum for the cross breed cow's with SCM in group 4 while minimum in group 1 (Fig 4). The SSC, pH and acidity showed the reverse relation with the lactose contents in the milk during the mastitis.

Subclinical mastitis and oxidant-antioxidant capacity in milk

Reactive oxygen species (ROS) are natural products of cellular metabolism. During the peripartum period of lactating cattle, mammary gland cells have a high metabolic rate and produce large amounts of reactive oxygen species (Jin *et al*., 2014). Increased ROS or decreased antioxidants can disrupt the balance and refer to as oxidative stress (Sordillo and Aitken, 2009). Association between oxidative stress and inflammation during intra-mammary infection and their role in the pathogenesis of mastitis has also been studied in the past (Turk *et al*., 2017, Weiss *et al*., 2004). Total oxidant and antioxidant capacity in the milk of cross -bred cattle were estimated to evaluate the role of oxidative stress in disease pathogenesis of SCM (Table 2). The total oxidant capacity was significantly ($P \leq 0.01$) high in CMT positive milk samples. Similarly, the total antioxidant capacity in SCM affected milk was significantly compromised ($P \leq 0.01$) compared to CMT negative milk samples. Oxidative stress



a b

Fig 2: Somatic cell count in milk stained with modified Newman-Lampert stain visualized under oil immersion (100x).
a). Somatic cells in a field of milk testing negative by CMT,
b). Somatic cells in a field of milk testing +1 by CMT.

a b

Fig 3: ABST of CMT positive milk sample using commercial Mastitest kit (Himedia) visualized by colour changes.
a). Preparation and milk inoculation in different vials for observation of ABST results.
b). Colour changes observed after 16 hours inoculation at room temperature in different vails containing antibiotics.

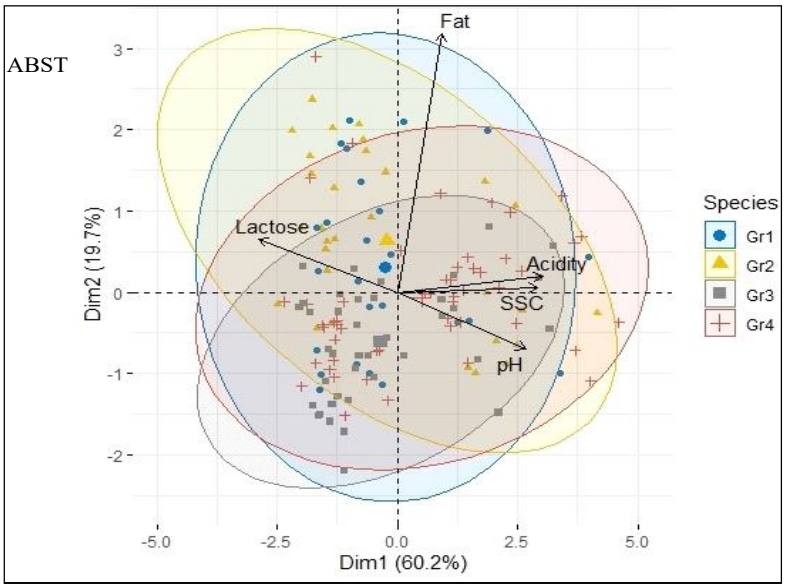


Fig 4: PCA biplot between four groups of mastitis diagnosed cow milk and milk components showing group 1 (Early lactation and parity 1-2) is less affected and group 4 (mid-lactation and parity 3-5) is most affected of the studied samples.

Table 2: Total oxidant and antioxidant status in CMT positive and negative mastitis milk of cross-bred cattle (Mean±S.E.).

Oxidant-antioxidant property	CMT positive milk	CMT negative milk
Total oxidant capacity (micromoles H_2O_2 equivalent per liter)	18.83^{a}±0.67	13.76^{b}±0.45
T value and significance	6.26; 0.00	
Total antioxidant capacity (millimoles Trolox equivalent per liter)	0.33^{a}±0.02	0.70^{b}±0.04
T value and significance	-7.91; 0.00	

Values with different superscript varies significantly (P≤0.01).

Table 3: Bacterial isolates from CMT positive (n=54) samples.

Bacterial isolates	Frequency	Prevalence %
Staphylococcus spp. (Coagulase positive)	35	64.82
Streptococcus spp.	8	14.81
Coliforms	3	5.56
Mixed isolate (Gram positive and gram negative bacilli)	5	9.26
No growth	3	5.56

due to an imbalance in oxidant and anti-oxidants in SCM milk indicates its role in the pathogenesis of the disease. It also indicates the possibilities of antioxidants to ameliorate the pathogenesis of inflammation in the mammary gland. Similar findings of higher total oxidant capacity and nitrous oxide in milk from SCM affected quarters compared to milk from healthy quarters of cow and buffaloes have been reported (Atakisi *et al*., 2010; Dimri *et al*., 2013). Thus, monitoring total oxidant and antioxidant capacity in milk could be used as an alternative diagnostic tool to screen subclinical mastitis in cross-bred cattle.

Bacterial isolate and antibiogram in SCM milk

All CMT positive milk samples (n=54) were inoculated on 5% bovine blood agar plates for bacterial isolation. Three milk samples did not yield any culture growth. Bacterial culture of milk samples were identified using the methods described above. Distribution of pathogens changes over time and dependent upon many factors, therefore, a bacteriological examination should be carried out to monitor udder health and plan treatment protocol (Pankaj *et al*., 2012). The different isolates identified from these milk samples are depicted in Table 3 along with their frequency. The most common contagious bacteria responsible for SCM was coagulase-positive *Staphylococcus* spp. (64.82%) isolates from these SCM milk followed by *Streptococcus* spp. (14.81%) and mixed isolates (9.26%). Our findings corroborate with the findings of Hardenberg (2016) who reported *Staphylococcus aureus* as the predominant bacteria (28.3%) followed by other *Staphylococcus* species (21.3%) and *Streptococcus* species (17.9%) in cattle samples across rural, peri-urban and urban households in Bihar. Similar findings of a high prevalence of staphylococci have been reported from cattle milk affected with mastitis in India (Jena *et al*., 2015; Hegde *et al*., 2013; Pankaj *et al*., 2012). The low frequency of Coliforms (5.56%) suggests that environmental contamination causing subclinical mastitis, as a factor was lest responsible for SCM in the study area. The possible reason may be due to the awareness of livestock farmers of peri-urban areas towards environment hygiene.

Table 4: Antimicrobial susceptibility of random CMT positive milk samples (n=20) using Masti Test kit.

Antibiotics	Sensitive	Intermediate	Resistance
Gentamicin	14	4	2
Enrofloxacin (8 mcg)	11	9	0
Ciprofloxacin (8 mcg)	9	5	6
Ampicillin-cloxacillin (128/128 mcg)	5	9	6
Amoxicillin-cloxacillin (128/128 mcg)	8	10	2
Tetracycline (128 mcg)	2	5	13
Chloramphenicol (8 mcg)	0	2	18
Streptomycin-penicillin (128/128 mcg)	0	1	19

ABST test conducted on random CMT positive milk sample indicated that gentamicin as most sensitive, followed by enrofloxacin, ciprofloxacin, Ampicillin/cloxacillin, tetracycline (Table 4). However, chloramphenicol, Streptomycin-penicillin were found to be non-sensitive to most samples. Gentamicin showed the highest sensitivity and least resistance but its use was restricted due to possibilities of its residue in milk (Martins *et al*., 2014). The present finding indicates the suitability of enrofloxacin as the most useful antibiotic for the treatment of subclinical mastitis. Pati and Mukherjee (2016) also showed a sensitivity pattern of *S. aureus* with maximum susceptibility towards fluoroquinolones, glycopeptide and extended-spectrum beta-lactam inhibitors class of antibiotics. The sensitivity results were similar to those for *Staphylococcus aureus* ABST since the majority of the isolate identified were *Staphylococcus* spp. in the present study. A similar type of pattern of antimicrobial drug resistance of *Staphylococcus aureus* isolated from subclinical bovine mastitis to penicillin and streptomycin and least to gentamicin (Mubarack *et al*., 2012; Schmidt *et al*., 2015).

CONCLUSION

Peri-urban dairy crossbred cattle are susceptible to SCM with moderate prevalence due to managemental practices discussed above. The modified CMT reagent prepared based on commercial detergent to reduce the cost of preparation was comparable to conventional CMT reagent, thus can be recommended for SCM screening. Staphylococcus spp. was the most prominent pathogen isolated from SCM milk. We recommend the use of enrofloxacin for treatment based on ABST and the cost of the treatment. Dry cow therapy needs to be practiced by dairy farmers to further reduce the prevalence of SCM.

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