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Research Article

COMPARATIVE EFFICACY OF DIFFERENT MASTITIS MARKERS FOR DIAGNOSIS OF SUB-CLINICAL MASTITIS IN COWS

Anil Langer[1], Sunanda Sharma[2], Narendra Kumar Sharma[3]* and DS Nauriyal[4]

[1]College of Veterinary and Animal Science, RAJUVAS, Bikaner, India
[2]Department of Veterinary Gynaecology & Obstetrics, CVAS, RAJUVAS, Bikaner, India
[3]Divisional Movile Veterinary Surgery and Infertility Unit, Animal Husbandry Department, Bikaner-334001, India.
[4]College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Gujrat

*Corresponding author email: naren21761@gmail.com

Abstract

Seven hundred ninety six milk samples from 266 quarters of 69 lactating cows were subjected to microbiological investigations for identification of pathogens. One hundred ninety bacterial isolates were recovered from 89 infected quarters, among these monomicrobial infection was found in 50 (56.2%) quarters, whereas, mixed infection was observed in 39 (43.8%) quarters. Bacterial isolates identified were Staph. chromogenes (49.47%), Staph. hyicus (21.1%), Staph. epidermidis (11.05%), Str. agalactiae (5.8%), Staph. aureus (4.2%), Staph. intermedius (3.1%), Enterobacter sp. (1.5%), Klebsiella sp., E. coli (1.05%), Micrococcus sp. (1.05%) and Serratia marcescens (0.52%). Milk samples from every quarter of each cow were also subjected to 6 mastitis marker tests named Somatic cell count (SCC), California mastitis test (CMT), electrical conductivity (EC) by EC-meter as well as by hand-held mastitis detector, pH detection by impregnated paper strip and also by pH meter. Efficacy of mastitis markers for diagnosis of sub-clinical mastitis was determined by comparing results of mastitis marker tests with microbiological findings. Mean value of SSC in milk from healthy quarters was significantly lower ($p \leq 0.01$) than that in milk from infected quarters. Significantly higher ($p \leq 0.01$) value of SSC was observed in milk samples having coagulase positive staphylococci as compared to that in milk from quarter with coagulase negative pathogens. The mean electrical conductivity (EC) in milk samples from infected quarters was significantly higher ($P<0.05$) than that from healthy quarters. Numbers and percentages of samples showing true positive, true negative, false positive and false negative results with SSC, CMT, EC by EC-meter, EC by hand-held meter, pH by impregnated strips, pH by digital pH-meter tests were evaluated and compared. The sensitivity and specificity of impregnated pH paper strip, CMT, pH-meter test, SCC, electrical conductivity by EC-meter and the same by hand-held mastitis detector were evaluated The compatibility between the results of SCC, impregnated pH paper strip, CMT, EC-meter, pH-meter, hand-held mastitis detector and bacteriological culture examination (reference test) was found to be 64.4, 63.4, 61.5, 59, 59 and 53 respectively.

Key words: Subclinical mastitis; bacteriological culture examination; somatic cell count; California mastitis test; electrical conductivity; pH strip test

Introduction

Sub clinical mastitis (SCM) is a major cause of economic loss in dairy herds that shows no gross inflammatory changes in udder, hence remains unnoticed unless investigated by employing laboratory tests. There are several direct and indirect tests with varying efficacies for detection of subclinical mastitis viz. culture, isolation and identification of causal agents, somatic cell count, California mastitis test, modified white side test (WST), bromothymol blue card test, electrical conductivity of milk, Cl^- estimation in milk, Modified Aulendorfer Mastitis Probe (MAMP) test, N-Acetyl-β-D-Glucosaminidase (NAGase) enzyme activity and ELISA etc., among these tests, bacterial culture from the milk has been considered as standard method for confirming subclinical udder infections in dairy cows (IDF, 1991 and Sudhan and Sharma, 2010). Somatic cell count (SCC) is a useful predictor of subclinical udder infection therefore it is considered as an important component for assessing the quality and milk hygiene for mastitis control protocols (Sharma *et al*., 2011). California mastitis test (CMT) is a simple, inexpensive, rapid and highly sensitive test that accurately predicts the inflammatory cell counts in milk from individual quarters or pooled milk samples (Madut *et al*., 2009). Electrical conductivity (EC) and pH of milk have been used as indicators of mastitis since last two decades. Present study was instituted for comparing the sensitivity, specificity, and accuracy of several mastitis markers named SSC, EC by EC-meter as well as by hand-held mastitis detector, CMT and pH by impregnated strip as well as by pH meter. Efficacy of these tests was evaluated by comparing the results with reference test conducted by microbial culture,

isolation and identification in milk samples from individual quarter of lactating indigenous (Kankrej and Gir) and crossbred (Kankrej x Jersy x HF) cows from an organized herd.

Materials and Methods

Present study was conducted on 796 milk samples (40 ml each) collected for 3 days from 266 healthy quarters of 69 lactating cows (26 Kankrej, 8 Gir and 35 triple crosses of Kankrej x Jersey x HF) maintained under identical management conditions at Livestock Research Station (LRS) of university. Each sample was marked with cow's identification number and teat from which sample was collected i.e. fore-left (FL), fore-right (FR), rear-left (RL) or rear-right (RR) teat. Milk samples were immediately transported to laboratory over ice pack where these were kept at room temperature for 15-20 minutes before investigations. Mammary infections were investigated by subjecting individual milk samples for microbial culture, isolation and identification of microbes. Other tests employed on each milk samples were SCC, CMT, EC and pH detection tests.

Diagnostic Tests / Mastitis Markers

Bacteriological Culture Examination

Milk samples from each quarter were inoculated and incubated (37^0C/24 hours) for microbial culture on blood agar (containing 5% sheep blood) as well as in MacConkey agar, thereafter examined for growth and morphological characteristics of bacterial colonies. Identical colonies were further isolated, inoculated and incubated (37°C/24-48 hours) on nutrient/glucose agar, thereafter identification and characterization of bacteria was performed as per the method described by Cowan and Steel (1970).

Somatic Cell Count (SCC)

SCC was estimated with Fossomatic™ Minor cell counter (Foss Electric, Hillerod, Denmark) as per technique described by Gonzalo *et al*. (2003).

Identification of infected quarters

Results of SCC were correlated with those of microbiological investigations. Infected quarters were identified as per following guidelines of IDF.

Quarter health status	Culturing of milk samples	SCC of milk samples (Cells/ml)
Healthy	2 times negative	All 3 times < 5 lakh
Latent infection	2 times positive	All 3 times < 5 lakh
Non-specific mastitis	2 times negative	Minimum 1 time > 5 lakh
Specific mastitis	2 times positive	Minimum 1 time > 5 lakh

California Mastitis Test (CMT)

The CMT was performed as per the method described by Schalm and Noorlander (1957).

Measurement of pH in Milk

The pH of each milk sample was estimated by Digital pH-meter (Khodke *et al*., 2009) as well as by impregnated pH-strips (Davis, 1999).

Electrical Conductivity (EC) Test

Electrical conductivity of milk samples was detected by Hand held mastitis detector (Draminski™) as well as by EC-meter (Janzekovic *et al*., 2009). Milk samples with EC ≥ 300 were considered to be from healthy and uninfected quarters, whereas, those with EC ≤ 250 were considered to be from SCM suspected quarters. (www.draminski.com).

Results and Discussion

Microbiological investigations on milk samples revealed that out of 266 quarters, 89 were sub-clinically infected wherein 190 isolates were recovered; Among these infected quarters, mono-microbial infection was observed in 50 (56.1%), whereas mixed infection was found in 39 (43.8%) quarters. Numbers and percentage of cows showing sub-clinical infection in one, two, three and all four quarters were 15 (32.6%): 22 (47.8%): 6 (13%) and 3 (6.5%) respectively. These findings are in close approximation to those reported by Patel (2001). Higher numbers of cows having infection in one quarter have also been reported by Dhote *et al*. (1999) and Patil *et al*. (2000).

Somatic cell counts (SCC) in 796 milk samples from 266 quarters revealed true positive, true negative, false positive and false negative cases of SCM in 144 (18%), 369 (46.3%), 66 (8.2%) and 217 (27.2%) samples respectively. Contrary to our results, Lather et. al., (2010) reported almost double numbers of milk samples showing true positive results by SCC. Infection and inflammation of mammary tissue evokes infiltration of polymorphonuclear cells (PMNs) at the site of infection (Schalm *et al*., 1971). Leucocytes are normally present in milk, damage/inflammation of mammary tissue incites release of chemotactic agents or chemical messengers from the leukocytes or damaged tissue. These chemical messengers/chemotactic agents are responsible for presence of large number of PMNs into the milk (Nickerson and Pankey, 1984). These PMNs act to engulf and digest the invading bacteria as self-defense mechanism of udder (Harmon, 1994).

Mean SCC in milk from healthy (155.59 x 10^3 cells/ml) and latent quarters (243.36 x 10^3 cells/ml) was significantly ($P<0.01$) lower than that in milk from quarters having non-specific (978.18 x 10^3 cells/ml) and specific infection (1949.48 x 10^3 cells/ml). These findings are in accordance with those reported by Leitner *et al*. (2003) and Verma (2008).

Mean SCC in milk from quarters infected with coagulase positive staphylococci (Staph. aureus, Staph. hyicus and Staph. intermedius) was significantly ($P<0.05$) higher (1239.04x 10^3/ml) than that (689.27 x 10^3/ml) in milk samples from quarters infected with coagulase negative

staphylococci (Staph. chromogenes and Staph. epidermidis). Our findings are in close approximation to those of Patel (2001) who reported significantly higher (16.29 x 10^5/ml) mean SCC in quarters infected with coagulase positive pathogens as compared to that (10.78 x 10^5/ml) in udder infected with coagulase negative pathogens. Vianna *et al*. (2005) reported 10.94×10^5/ml somatic cells in milk from quarters infected with coagulase negative pathogen.

Results of CMT revealed that out of 796 milk samples, the number of samples showing true positive, true negative, false positive and false negative were 217 (27.2%), 273 (34.2%), 162 (20.3%) and 144 (18.0%) respectively. Dubal *et al*. (2010) and Saluja *et al*. (2004) also diagnosed subclinical infections in 24.6 and 27.5% samples by employing CMT, whereas, Sharma *et al*. (2010) reported higher rate of SCM diagnosis (32 to 92%) with CMT test.

Electrical conductivity (EC) tests with EC-meter revealed true positive, true negative, false positive and false negative cases of SCM in 90 (11.3%), 380 (47.7%), 56 (7.0%) and 270 (33.9%) samples respectively. Mean vale of EC in milk samples from infected quarters (4.96 ± 0.07 mS/cm) was significantly higher ($P<0.05$) than that (4.26 ± 0.03 mS/cm) in milk samples from healthy quarters. Chahar (2007) and Jain *et al*. (2009) have reported higher percentages of true positive cases (38 and 22.5% respectively) detected by EC.

Number and percentages of milk samples showing true positive, true negative, false positive and false negative detection of SCM by EC test using hand-held EC-meter (Dramiński 4QMast) were 61 (7.6%), 364 (45.7%), 55 (6.9%) and 316 (39.6%) respectively. Janzekovic *et al*. (2009) observed higher values of EC (>6.5 mS/cm) in 80% quarters with increased count of somatic cells. Muhamed *et al*. (2011) reported 65.2% true positive cases of SCM with electronic EC detector. In our study, detection of SCM by EC test using hand-held EC-meter was very low (7.6%). High electrical conductivity in infected quarter is attributed to opening up of the alveolar junction and increased permeability of capillaries due to infection that in turn results into high Na^+, K^+ and Cl^- ions in extracellular fluid poured into lumen of alveolus thereby increased levels of these ions in the milk of infected gland. Electrical conductivity of milk thus depicts its ionic contents (Linzell and Peaker, 1975).

Results of pH detection (paper strip method) revealed true positive, true negative, false positive and false negative cases of SCM in 224 (28.1%), 155 (19.4%), 136 (17.0%) and 218 (27.3%) samples respectively. Estimation of pH in milk samples by digital pH meter revealed true positive, true negative, false positive and false negative cases of SCM in 220 (27.6%), 250 (31.4%), 159 (19.9%) and 167 (20.9%) samples respectively. Tiwari and Sisodia (2000) also detected 23.1% milk samples positive for SCM by employing impregnated pH paper strip. However, Kumari and Gupta (2002) reported 71 (87.65%) quarters positive for SCM using BTB card test.

Increased pH of milk from SCM quarter has also been reported by Sood *et al*. (2008) and Hussain *et al*. (2011). Increased alkalinity/pH in milk from SCM cases has been attributed to increased permeability of the blood capillaries due to inflammation of mammary gland that causes entry of alkaline blood constituents (Na^+ and bicarbonate ions) into the milk (Muhamed *et al*., 2011).

Sensitivity and Specificity of SCC, CMT, EC and pH Tests

The sensitivity of SCC as a mastitis marker was observed to be 39.8%. This was much lower than that (56-100%) reported by Muhammad *et al*., 2009 and Sharma *et al*., 2010. The specificity of SCC observed in present study (84.8%) is in close approximation to that reported by Jain *et al*. (2009) and Choudhari (2000) who documented the same as 79.6 and 84.4% respectively, however, Sharma *et al*. (2010) recorded 97.76%.

Sensitivity of CMT observed in present study (60.1%) is comparable with that reported by Chahar (2007). However, it was much lower than that (71-86%) observed by Muhammad *et al*. (2009) and Sharma *et al*. (2010). The specificity of CMT in present stud was 62.7% that concurs with the observations of Sharma *et al*. (2010).

In present study, sensitivity of EC-meter (25%) was much lower than that (56%) reported by Chahar (2007). The specificity of EC-meter in present study (87.1%) was in agreement with the observation of Choudhari (2000).

The sensitivity of hand-held EC-meter (12.7%) was much lower than that (51%) reported by Mansell and Seguya (2003). The specificity of hand-held EC-meter (89.0%) was much higher than that (71%) reported by Mansell and Seguya (2003).

The sensitivity of impregnated pH paper (62.2%) was comparable with that reported by Chahar (2007), however it was lower than that (69.3 and 71.29) reported by Tiwari and Sisodia (2000) and Verma (2008). The specificity of pH strip test in present study (64.4%) was much lower than that (85.8 to 94.2 %) reported by Ghose *et al*. (2004) and Verma (2008).

The sensitivity of digital pH meter in detecting SCM was found to be 56.84 %. However the specificity of pH measured by pH digital meter was a bit higher at 61.1 per cent.

Compatibility between reference test and various mastitis markers

In present investigation, bacteriological culture examination was considered as the reference test and the compatibility of different 6 mastitis markers was calculated by comparing their results with that of the reference test. The analysis of results obtained in the present study revealed that SCC, as a mastitis marker, showed 64.4%

compatibility with the results of bacteriological culture examination, which was close to the findings of Patel (2001). Higher compatibility between the results of SCC and bacteriological culture examination as reported by earlier workers has been 75.4% (Nauriyal, 1996) and 85.5 % (Pachauri *et al*., 2001). The results of CMT revealed 61.5% agreement with the results of culture examination. Higher compatibility between the results of CMT and the reference test has been reported by earlier workers at 72.0% (Patel, 2001), 78.4% (Nauriyal, 1996) and 88.8% (Pachauri *et al*., 2001). CMT is an indirect estimation of SCC and therefore in the present study the agreement between the results of reference test and SCC as well as CMT was pretty close at 64.44 and 61.55 % respectively. This justifies the results obtained in our study. The compatibility between the results of EC, as determined by EC-meter and hand-held mastitis detector, to detect qms with SCM, and the reference test was noted at 59.0 and 53%, respectively. The agreement between the results of impregnated pH paper test and digital pH meter with bacteriological examination for detection of SCM was observed to be 63.4 and 59.0%, respectively. Earlier, Pachauri *et al*. (2001) and Verma (2008) reported the compatibility of pH paper strip with reference test to be 84.4 and 90.7%, respectively, which was much higher than the present findings. There was no published report which could be traced on the agreement between the results of digital pH meter and EC-meter and /or hand-held mastitis detector with the reference test.

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