DETECTION AND THERAPY OF SUBCLINICAL MASTITIS IN GOATS

SANDEEP HALMANDGE

DEPARTMENT OF VETERINARY MEDICINE

VETERINARY COLLEGE, BANGALORE

KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR

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SANDEEP HALMANDGE

DEPARTMENT OF VETERINARY MEDICINE

VETERINARY COLLEGE, BANGALORE

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KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR
DEPARTMENT OF VETERINARY MEDICINE
VETERINARY COLLEGE, BANGALORE

CERTIFICATE

This is to certify that the thesis entitled "***Detection and therapy of subclinical mastitis in goats***" submitted by **Mr. SANDEEP HALMANDGE,** ID. No. **DVHK- 1219** in partial fulfillment of the requirements for the award of **DOCTOR OF PHILOSOPHY** in **VETERINARY MEDICINE** of Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar is a record of the bonafide research work carried out by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bangalore
June, 2016

(Prof. H.A. UPENDRA)
Dept. of TVCC, Vet. College, Bangalore
Major Advisor

Approved by:

Chairperson : _____
(Prof. H.A. UPENDRA)

Nominated External Examiner : _____
(Prof. R. RAMPRABHU)

Members : **1.** _____
(Prof. P.T. RAMESH)

2. _____
(Prof. VIVEK R. KASARALIKAR)

3. _____
(Prof. D. RATHNAMMA)

4. _____
(Dr. SHRIKRISHNA ISLOOR)

5. _____
(Dr. V.V.S. SURYANARAYANA)

To,

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LIST OF ABBREVIATIONS

%	:	Per cent
^{0}C	:	Degree Celsius
ABST	:	Antibacterial sensitivity test
ALT	:	Alanine Transaminase
AST	:	Aspartate Transaminase
ATP	:	Adenosine Triphosphate
b.wt.	:	body weight
Cl	:	Chloride
CMT	:	California Mastitis Test
dL	:	deci litre
DW	:	Distilled Water
EC	:	Electrical conductivity
ELISA	:	Enzyme linked immunosorbent assay
et al.	:	et alia
etc.	:	Etcetera
FAO	:	Food and Agricultural Organization
Fig.	:	Figure
g	:	grams
GGT	:	Gamma Glutamyl Transferase
GPx	:	Glutathione-peroxidase
i/m	:	intra muscularly
IL	:	Interleukin
INR	:	Indian Rupees
IU	:	International unit
kg	:	kilo grams
L	:	Litre

LDH	:	Lactate Dehydrogenase
LPB ELISA	:	Liquid phase blocking Enzyme linked immunosorbent assay
Ltd.	:	Limited
M	:	Molar
mg	:	milli gram
min	:	Minute
ml	:	milli litre
mS/cm	:	milli siemens per centimeter
Na	:	Sodium
NAGase	:	N-Acetyl-β-D-glucosaminidase
OD	:	Optical density
OPD	:	Ortho phenylene diamine dihydrochloride
PBS	:	Phosphate Buffer Saline
PBST	:	Phosphate Buffer Saline Tween
PIBB	:	Polyimmunoreactive bovine biomarker
PMN cells	:	Polymorphonuclear cells
rpm	:	Revolution per minute
s.i.d	:	Once a day
SAA	:	Serum Amyloid A protein
SCC	:	Somatic cell count
SCM	:	Subclinical mastitis
SE	:	Standard error
U/L	:	Units per litre
viz.	:	videlicet (namely)
w/w	:	Weight by weight
µl	:	Micro litre

INTRODUCTION

I. INTRODUCTION

Goats are multi-purpose animals, producing meat, milk, skin and hair. Goat is known as the 'poor man's cow' and has a tremendous potential to be projected as the 'Future Animal' for rural prosperity. Goat production is a rapidly growing agricultural industry in many countries. Goats are becoming important suppliers of meat and milk to rural population in different parts of the world, particularly arid and semi-arid regions (Haenlein, 2004). This could be attributed to the comparatively low input of goat herds, substantial value and ability of the species to be raised in adverse conditions with little rainfall or agricultural potential (FAO, 2010). Additionally, smaller sizes of goats allow large herds to be reared in small areas (Kyozaire, 2003).

According to FAO (2010), 95 per cent of goats worldwide are concentrated in developing countries, in which they provide multiple economic opportunities that contribute to food security and poverty alleviation. Goats provide a leaner meat in comparison to other domestic ruminants and their milk and milk products have several scientifically unexplored medicinal and health promoting properties that need to be validated scientifically and promoted commercially.

India possesses 135.2 million goats and 65.1 million sheep (Livestock Census 2012). They contribute about INR 47.3 million to national economy through various products and by-products (Rekib and Vihan, 1997). World goat milk production has reached 15.5 million tons in 2009, most of it being used by households or families rearing goats (termed auto consumed) or sold to the neighborhood or used for kid suckling. In India, goat farming is well established but unorganized and mostly reared by landless

farmers (Prasad *et al.*, 2005). India ranks first in the world with the total goat milk production of about 4.1 million MT in 2009. However, goat milk is not only 3.2 per cent of the national milk production, but most of it is auto-consumed by the rural population (Kumar *et al.*, 2012).

Goat milk differs from cow or human milk in having better digestibility, alkalinity, buffering capacity and certain therapeutic values in medicine and human nutrition. Therefore, it is imperative to create awareness about advantage of consumption of goat milk so that production and utilization of goat milk could be enhanced.

The small ruminant population in our country is frequently exposed to ravages by various infectious diseases *viz.* bacterial, viral, parasitic diseases and nutritional disorders. Among them, mastitis plays a major role. Mastitis is usually classified into two forms *viz.*, clinical and subclinical. In clinical forms it could be diagnosed visually or by palpation of mammary gland, whereas subclinical form is diagnosed via assessment of somatic cells and /or bacterial agents (Keisler *et al.*, 1992; Radostits *et al.*, 2000). Subclinical form of goat mastitis is considered as a great threat as it causes great reduction in milk production and also causes kid mortality due to feeding of mastitis milk (Mhase *et al.*, 2007).

Hence, assessment of udder health in goats should be prioritized. Several diagnostic tests such as Somatic Cell Count (SCC), Electrical Conductivity (EC), California Mastitis Test, White Side Test, Surf Field Milk Test, Tests to detect the pH, specific gravity, compositional changes of milk and microbiological status of milk, estimation of enzymes and lastly the detection and quantification of biomarkers, have

been employed for diagnosis of subclinical mastitis (SCM), with an intention to diagnose the condition as early as possible so that suitable treatment can be initiated at the earliest.

Recently various biomarkers have been reported to be early indicators of various infections in animals including mastitis. During intramammary infection (IMI), many enzymes do increase in milk. This is more so with enzymes secreted by phagocytes or leukocytes. One such enzyme is N-Acetyl-β-D-Glucosaminidase (NAGase). NAGase being a biomarker, has been proven to be a reliable indicator of inflammation (Sharma *et al.*, 2007)

Besides these indirect tests, biomarkers like adenosine triphosphate, bovine serum albumin, antitrypsin and acute phase proteins *viz.* haptoglobin and milk/serum amyloid A protein also are found to increase during intramammary infection and hence act as good indicators in diagnosis of SCM. Earlier workers have also standardized sensitive tests such as Enzyme Linked Immunosorbent Assay (ELISA) for the diagnosis of mastitis caused by common pathogens namely *S. aureus, Listeria* species etc., and were proved to be helpful in early and specific diagnosis of SCM.

It is essential to study haematological and biochemical alterations associated with SCM in goats for proper understanding of the pathogenesis which will be very useful in formulating the proper therapeutic measures.

Treatment of SCM in goats plays a major role in milk production. Researchers have used antibiotics or antibacterials either alone or in combination and further with supportive or additional chemotherapeutic agents (anti-inflammatory drugs, vitamins,

etc.,) or chemicals. In order to hasten recovery from mastitis, to trigger immune response or to ensure 100 per cent normalcy, supportive therapy has been advocated by Veterinary practitioners. Among the supportive treatments adopted in the treatment of mastitis, Vitamin E and Selenium tops the list.

Though researchers have undermined the information regarding metabolism, activity and beneficial effects of supportive therapy, there is a need for a systematic therapeutic study using antibacterial in combination with supportive therapy for the treatment of SCM in goats and evaluation of their response. Further, there is a need for a highly sensitive and specific laboratory test to detect the cytokines which are present in minute quantities and noticed very early in intramammary infection. With this background, the present study was undertaken with the following objectives:

1. Detection of subclinical mastitis in goats based on Somatic cell count, Electrical conductivity, NAGase activity and LPB ELISA.
2. To study the prevalence of subclinical mastitis in goats.
3. To study certain haematological and biochemical alterations in subclinical mastitic goats.
4. To evaluate the efficacy of Vitamin E and Selenium combination, Trisodium citrate and organic Selenium in treatment of subclinical mastitis in goats

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Scientific information pertaining to diagnosis, prevalence, hemato-biochemical alterations and treatment of mastitis has been reviewed and presented in this chapter.

2.1. Mastitis diagnostic tests

2.1.1. Somatic cell count (SCC)

Poutrel and Lerondelle (1983) reported that presence of $1x10^6$ somatic cells per ml of goat milk to be considered as positive for mastitic condition.

Drake *et al.* (1992) while evaluating the bulk tank goat milk samples opined that the increase in neutrophils may probably be due to increase in milk yield during early lactation and thus contribute to high SCC in milk .

Lerondelle *et al.* (1992) studied somatic cell count of 94 goat milk samples and found many factors affect cell numbers. They opined increase of somatic cells due to mammary infection and the susceptibility of goat mammary gland to various stress must be taken into account before using somatic cell count as diagnostic of mammary infections.

Contreras *et al.* (1995) studied 369 milk samples from half udders of 188 Murciano-Granadina goats in the second and third month of lactation, from ten commercial dairy goat herds. They concluded that the best SCC threshold for defining subclinical mastitis was 500 x 10^3 cells per ml. They opined that this threshold had a poor

positive predictive value (28.5%) and only 62.3 per cent of samples were correctly classified.

Contreras *et al.* (1996) recorded that the SCC threshold for defining subclinical mastitis in goats was 500 x 10^3 cells per ml of milk.

Wilson *et al.* (1995) reported involution of uterus tends to be spontaneous and is associated with an increase of SCC independently of intramammary infection.

Boscos *et al.* (1996) reported that mean SCC numbers in infected gland was 1252x10^3 cells/ml and in uninfected gland was 1115x10^3 cells/ml of milk.

Das and Singh (2000) opined that the variations in SCC between goats and between different experimental periods were highly significant because SCC is influenced by season, stage of lactation and productive stage of the goats and inversely related to milk yield.

Gambo and Etchike (2001) assessed the prevalence of subclinical mastitis in lactating zebu cattle by direct Somatic Cell Count (SCC) method and the California Mastitis Test (CMT). Monitoring was carried out for a period of six months using bulk milk (37 dairy farms) and individual milk samples (504) at monthly interval. Mean direct SCC was 834+204 x 10^3 cells/ml of bulk milk, equivalent to a CMT 2 score (sign of mastitis). Monthly differences were not significant. They concluded that parity and the stage of lactation significantly affected SCC and SCC was a useful tool for small or larger scale integrated control programme to detect mastitis systematically and regularly.

Paape *et al.* (2001) opined that goat milk contains high SCC and most of them were neutrophils.

Rupp and Boichard (2001) conducted three studies for a period of two and a half years in Western France, to find out the relationship between SCC and clinical mastitis in French Holstein dairy cows. The first two studies focused on the putative risk of clinical mastitis associated with very low SCC. They were of the opinion that lower the SCC, lower was the risk of clinical mastitis and hence selection of animals based on lower SCC level was relevant and should increase resistance to both clinical and subclinical mastitis.

Samore *et al.* (2001) carried out research to set up test day model to genetically evaluate primiparous cow for SCC. The aim was to identify bull that tends to transmit susceptibility to mastitis to their progenitor. They opined that SCC could be used as an indicator of mastitis in selection and the advantages of using SCC were: (1) the higher heritability when compared to direct traits; (2) the low cost of collecting data; (3) the objectivity of the measurements; and (4) SCC is an indicator for both clinical and subclinical mastitis.

Haenlein (2002) examined the non-pathological influences on somatic cell counts and found that variation can be as much as 90 % and also found that goat milk contains 45 to 74 per cent neutrophils.

Laurinaviciute *et al.* (2003) opined that the somatic cell count in goat milk is commonly used as an effective index of udder health in dairy goats. It was shown that SCC increased in the milk samples of 38.23 per cent goats in the second month of

lactation, in 42.85 per cent goats in the fifth month of lactation and in 74.19 per cent goats during late lactation. Compared to local crossbred goats, SCC was mostly increased in the milk of Saanen goats during the second and fifth months of lactation by 32.14 per cent ($P<0.05$) and 17.85 per cent ($P<0.05$) of cases, respectively. The difference in SCC between Saanen and local crossbred goats (5.05% cases) were not significant ($P>0.05$) in late lactation stage.

Leitner *et al.* (2004) found that effect bacteriological infection effect on somatic cell count ($x10^3$) was significant and the mean values of uninfected (n=327) and infected (n=273) Israeli dairy goats were 338 and 922 respectively. Further, they also observed that the lactation number, time of milking and days in milk did not affect SCC significantly.

Diana and Rotaru (2006) analysed somatic cell counts (SCC) of raw milk samples from three lactations to determine the healthy and diseased mammary glands of goats. Results showed that the highest SCC values were observed during mid and late lactation. A significant increase in epithelial cells was observed in animals with catarrhal desquamate or catarrhal purulent mastitis. They also found that there was an intense desquamation of the necrotic and apoptotic mammary epithelium at all levels of lactation.

Gomes *et al.* (2006) conducted trails in 40 goats to evaluate the effect of stage of lactation on SCC on monthly collections. The mean SCC ($x10^5$) during first to eighth month of lactation was reported to be 2.56, 4.42, 3.45, 8.52, 5.96, 5.84, 6.41 and 6.51 cells/ml of milk respectively.

Samanta *et al.* (2006) reported that SCC and the pH of the milk had a very high positive association with the SCM infection among Karan Swiss and Karan Fries breed of cows. The quarter-wise counts of somatic cells per ml of milk increased with the severity of infection in both the breeds.

Bagnicka *et al.* (2007) opined that the economics of goat milk production influenced by both clinical and subclinical mastitis and the somatic cell count of goat milk is influenced by pathogens and other factors. Thus, SCC is not the only indicator of state of mammary gland health.

Hall and Rycroft (2007) estimated the mean somatic cell counts during a study on the bacterial causes of subclinical mastitis in 159 goats, revealed that the mean SCC was 4,28,000 and 27,85, 000 cells/ml in uninfected and infected milk samples respectively. They also reported that 93 per cent of uninfected samples had counts less than 10,00,000 cells/ml.

Maurer and Schaeren (2007) opined that somatic cell count differed significantly between herds and with stage of lactation and concluded that the limiting value for somatic cell count is much higher in goat milk (1 million/ml) than in cows (350 000/ml).

Paape *et al.* (2007) opined that the European Union has set the SCC threshold for raw cow milk at 400x 10^3 cells mL^{-1} and so far no limit values has been set for goat milk.

Raynal-Ljutovac *et al.* (2007) compared that while the health of udder quarters of cows are confirmed by SCC up to 100 x10^3 cells /mL, the maximum SCC for goats ranged from of 200 x10^3 cells ml^{-1} up to a few million cells ml^{-1}.

Sharma *et al.* (2007) observed that the average SCC of milk samples from normal goats (0.467 ± 0.023 million cells/ml) and those having subclinical mastitis (2.291 ± 0.178 million cell/ml). The maximum SCC was observed in the 5^{th} lactation. Lactation had an insignificant effect on SCC.

Vasiu *et al.* (2007) examined 94 primiparous goats for somatic cells in milk and opined an increased number of somatic cells was correlated with the presence of intramammary infections.

Zafalon *et al.* (2007) compared CMT and SCC as auxiliary methods for diagnosis of the bovine subclinical mastitis caused by *Staphylococcus aureus* and Corynebacterium spp. CMT and SCC showed higher diagnostic sensitivity when *S. aureus* was the microorganism isolated from milk samples compared to Corynebacterium. The diagnostic efficiency of both the tests was higher when subclinical mastitis was caused by *S. aureus* compared to the Corynebacterium.

Contreras *et al.* (2008) performed cross-sectional study on dairy goat herds designed to establish the relationship between the presence of *Mycoplasma* spp. in bulk-tank milk samples from different farms and the bulk-tank milk somatic cell count (BTMSCC) in an area where contagious agalactia (CA) is endemic. Somatic cell counts for bulk-tank samples containing mycoplasmas were higher than those recorded for negative samples (1,176,000 cells/ml *vs.* 875,000 cells /ml). Analyses revealed that presence of Mycoplasma in bulk-tank milk increased the risk of surpassing all SCC thresholds considered, with the highest risk for *Mycoplasma* positive bulk-tank milk samples exceeding the threshold of 1500×10^3 cells/ml.

Chul *et al.* (2008) performed investigations to study the best staining method for the somatic cell classification of dairy goat milk. The samples were stained and analysed with direct microscopic method, using five different staining methods: Wright's stain, Giemsa stain, Diff-quik stain, Newman's stain and Pyronin Y-Methyl Green stain. Among them, the Newman's staining was found to be the most rapid and effective method, which required the shortest time for staining and provided the easiest way to classify somatic cells.

Petzer *et al.* (2008) evaluated udder health in dairy goats by SCC and reported infected udder halves had significantly higher log SCC than non-infected udder halves before and after treatment, but not during treatment. There was a moderate positive correlation between stage of lactation and SCC (R^2=0.438).

De and Mukherjee (2009) studied Somatic Cell Count (SCC) pattern in different breeds of cattle and reported that for subclinical mastitis, the milk SCC was significantly highest in Holstein-Friesian (F) x Haryana (H) and lowest in F x B x H cows.

Nielsen *et al.* (2009) studied the relationship between SCC and milk yield in different stages of lactation, in non mastitic cows and reported that the magnitude of daily milk loss associated with increased SCC depended on stage of lactation and parity, and was most extensive in late lactation irrespective of parity and daily milk loss at an SCC of 500,000 cells/ml ranged from 0.7 to 2.0 kg (3 to 9 %) in primiparous cows, depending on stage of lactation. In multiparous cows, corresponding loss was 1.1 to 3.7 kg (4 to 18 %).

Lievaart *et al.* (2009) reported that Bulk Milk Somatic Cell Count (BMSCC) was a frequently used parameter to estimate the prevalence of subclinical mastitis in a dairy herd.

Diaz *et al.* (2011) recorded SCC in 105 Murciano-Granadina goat milk samples, collected monthly for seven months. A significant increase in SCC was determined with parity; SCC was significantly lower in milk collected from primiparous goats (556,000 cells/ml) than multiparous goats (1,335,000 cells/ml).

Oliveira *et al.* (2011) collected bulk milk samples from 96 randomly selected, small-scale, extensively managed dairy goat farms to provide a baseline investigation to support intervention measures for prevention and control of food borne pathogens. They recorded the somatic cell counts which ranged from 275,860 cells/ml to 11,055,620 cells/ml and the mean somatic cell count was 2,494,097 cells/ml. They considered that one million somatic cells per ml as reference value, and found that 87.3 per cent (62/71) samples in the present study resulted in SCCs greater than 1,000,000 cells/ml.

Stuhr *et al.* (2012) observed that a significant influence of the lactation week excluding first week and lactation number on $\log_{10}$ SCC (5.140 ± 0.040 in uninfected halves and 5.332 ± 0.060 in infected halves) was noticeable which underlines that this parameter should not be used as a single indicator for IMI detection in early lactation.

2.1.2. Electrical conductivity (EC)

Juarez and Ramos (1986) estimated the physical properties of goat milk and recorded the conductivity 0.0043-0.0038 $ohms^{-1}$ cm^{-1} which is higher compared to cow's milk.

Das and Singh (2000) studied the variation in electrical conductivity of milk of ten Alpine x Beetal and Saanen x Beetal crossbred goats at biweekly intervals from day 14 post-kidding for 22 weeks. They recorded a low electrical conductivity of milk during first two periods (Mean and SE of 2.10 ± 0.03 and 2.01 ± 0.01 moles) and increased thereafter till the ninth experimental period of lactation ($P < 0.01$) (Mean and SE of 4.10 ± 0.13 moles). They concluded that changes in EC ($P<0.01$) was noticed during different periods of experiment and no changes in EC of two breeds of goats and between the goats. Also, they indicated that EC of milk, changes with stages of lactation.

Singh *et al.* (2000) conducted a study to compare the efficacy of different tests for the detection of SCM in cows in which they collected 200 milk samples from 50 healthy crossbred cows and subjected them to electrical conductivity (EC), estimation of lactose, Bromothymol blue card test, Sodium lauryl sulfate test, , estimation of sodium, chloride and potassium. The percentage of agreement of various tests was calculated by comparing results of different tests with bacteriological findings and reported that the percentage of agreement of EC was 69.38, 71.47, 77.66, 76.15, 67.63, 70.87 and 55.0 respectively.

Mele *et al.* (2001) reported that EC data of composite milk samples were useful to detect both clinical and SCM in cows, with a good sensitivity.

Spakauskas *et al.* (2001) collected 1014 samples from Lithuanian cows at the end of milking and subjected it to several tests including EC which was measured using a Mastiindikaator device made in Estonia. EC of the samples taken from healthy cows was 4.5-6.0 mS/cm, while EC in the samples taken from cows with subclinical mastitis was 6.1-8.5 mS/cm. Even though EC of milk produced by the cows with mastitis tends to increase, it does not always correlate with SCC.

Ying *et al.* (2002) analysed the weekly milk samples of two groups of Alpine goats in their early lactation (n=36 samples) for 4 weeks and late lactation (n=240) for 16 weeks of second lactation for various physicochemical properties. The study revealed that the Mean ± SD was 5.18 ± 0.30 and 6.26 ± 0.95 (mS /cm) in early lactation and late lactation respectively.

Mansell and Seguya (2003) evaluated the accuracy of a hand-held EC meter for the detection of subclinical mastitis in an Australian dairy herd in late lactation that had a high prevalence of *S. agalactiae* and *Staph. aureus* infection. Milk samples collected from 233 quarters from 59 cows revealed that the EC was higher in infected quarters than uninfected quarters.

Pyorala (2003) reported that SCM should be efficiently detected and informed that indicators of inflammation in the milk which can be determined using rapid, reliable and easy routine techniques should be used for early detection of SCM such as EC, SCC, estimation of milk lactate, lactose, NAGase, acute phase protein, haptoglobin and serum and milk amyloid A. EC does not perform well enough alone and hence, should be supplemented with other parameters like temperature, milk yield and properties of milk.

Ying *et al.* (2004) observed that EC increased with infection in Saanen goats (from 5.6 mS/cm in healthy animals to 5.8 mS/ cm in infected ones), whereas for Alpine goats the EC decreased (from 6.1 mS/cm for healthy goats to 5.4 mS/cm in infected ones).

Bogdan *et al.* (2005) compared the milk and mammary health by monitoring EC, pH and temperature of the fresh milk from each mammary quarter and reported the relevance of individual testing and interpretation of EC in determining early stage of subclinical and clinical mastitis in cows.

Zafalon *et al.* (2005) studied EC measured by a hand-held meter and chloride concentration of milk, as auxiliary methods for the diagnosis of bovine subclinical mastitis in the identification of affected mammary quarters from where *Staphylococcus aureus* and Corynebacterium sp. were later isolated. Tests were performed for 2 years in Holstein cows in a dairy farm producing type C milk where milking was performed once a day. The sensitivity of EC and estimation of chloride concentration in mammary quarter, where *Corynebacterium sp.* was isolated (65.3 % and 78.3 % respectively), were superior to the mammary quarters from where *S. aureus* was isolated (55.4 % and 68.2 %, respectively). The efficacies of the two diagnostic tests were similar and statistically significant.

Naschif-Junior *et al.* (2006) reported that mastitis was the most prevalent infectious disease in dairy herds. Subclinical form of mastitis played an important role in decreasing milk yield and milk quality. As physical examination of udder or milk could not detect disease process, a diagnostic test was required. Early detection of infection

reduced transmission. In their study, they used 1584 samples from clinically healthy quarters and evaluated the sensitivity and specificity of CMT and EC in detecting subclinical mastitis throughout lactation. CMT showed the highest sensitivity (100 %) and MEC had the highest specificity (86.98 %). Results suggested that physiological changes occurring in early and late lactation may interfere with CMT and MEC results. This problem can be minimized using both tests so the results can be more reliable.

Chahar (2007) in a study collected 300 quarter milk samples from 78 apparently healthy cows of different lactation stage and subjected to various diagnostic tests, like somatic cell count (SCC), Electrical Conductivity (EC), Modified California mastitis test (CMT), Trypsin inhibitor activity, White Side Test (WST) and milk pH and reported that the sensitivity of these tests were 100 per cent, 84.37 per cent, 63.80 per cent, 55.55 per cent, 62.74 per cent and 29.41 per cent respectively. Since SCC was cumbersome and required laboratory facilities, EC test was the best screening test under field conditions. This test allowed daily monitoring of the cows and identification of infected quarters at the early stage.

Chen *et al.* (2008) found that the value of electrical conductivity in goat milk was over 6.8 mS/cm or the ratio of electrical conductivity between different halves was over 1.1, the animal had mastitis.

Tangorra *et al.* (2010) highlighted that the milk electrical conductivity test is not a good mastitis indicator in goats and found that the average of the 20 highest electrical conductivity half udder values recorded within milking in early lactation and the highest

half udder X_{20} value within goat and milking in mid lactation could be potentially used in monitoring udder health of dairy goats.

Diaz *et al.* (2011) concluded that EC was associated with the chemical composition of milk, particularly of chloride ions explained most of the variance in EC (R2 = 0.91).

Romero *et al.* (2012) found that mean EC of multiparous goats (5.45 mS/cm) was significantly higher than in primiparous dams (5.07 mS/cm). EC was highest in F-1 (5.43 mS/cm) and decreased significantly with as milking progressed in F-2 (5.28 mS/cm) and F-3 (5.07 mS/cm). The only significant differences between healthy and infected glands were obtained in F-3, with EC being higher in infected ones (5.01 vs 5.14 mS/cm).

2.1.3. N-Acetyl-β-D-Glucosaminidase (NAGase) activity

Obara (1985) found that NAGase activity in infected goat udder was significantly higher than in control udders and suggested that NAGase activity was the best to monitor udder epithelial cell damage.

Mean NAGase activities in milk of uninfected and infected goat udder halves was 1.51 and 2.58 and cow quarters uninfected and infected was 4.58 and 6.00 nmol/min/ml, respectively. The NAGase in cow milk was 2 to 3 times higher than in goat milk. The mean plasma NAGase activity was similar on all days as well as within each goat (2.91 to 3.0 nmol/min/ml). The mean NAGase in plasma of cows and goats were approximately estimated as 30 and 3 nmol/min/mL, respectively, or a 10-fold higher than in cows (Timms and Schultz, 1985).

NAGase as a suitable parameter for the diagnosis of SCM in dairy goats although the level of NAGase activity in goat milk is nearly 4-fold lower as compared to bovine milk (Maisi and Riipinen, 1988 and Vihan, 1989).

The range of NAGase activity of the herd A and B in infected halves varied from 0.012 to 0.41 and 0.028 to 0.036 µmoles/ml milk, while the milk of negative cultures showed values of 0.0054 ± 0.0001 and 0.0029 ± 0.00007 µmoles/ml respectively. He pointed out that at 0.020 µmoles/ml, 90-100 per cent of the infection was detectable and was reliable for diagnosing subclinical mastitis with acceptable proportion of errors (Vihan, 1989).

Maisi (1990a) recorded the NAGase values of 0.9 ± 1.5 units in healthy halves were measured in contrast to infected halves with 10.3 ± 6.3 units.

The mean and SE of NAGase activity of uninfected (n=327) 15.6 ± 0.8 and infected halves (n=273) were 59.2 ± 5.3. Here, a value of 100 corresponds to the release of substrate-derived product at 5 mmol l^{-1} min^{-1} at 25 °C (Leitner *et al.*, 2004).

Chagunda *et al.* (2006) found that increased activities of NAGase in dairy cows are related to the immune defense during udder infections.

Bagnicka *et al.* (2007) opined that the economics of goat milk production are influenced by both clinical and subclinical mastitis and the somatic cell count of goat milk is influenced by pathogens and other factors. Thus, SCC is not the only indicator of the state of mammary gland health. The most promising indicators of subclinical mastitis

are the N-acetyl-beta-D-glucosaminidase activity, followed by electrical conductivity and lactose content of the milk during the first stage of lactation.

The lactation week and infection status showed a significant effect of infection status on log10 NAGase whereas the lactation number had no significant effect on log 10 NAGase (Stuhr *et al.*, 2012).

2.1.4. Biomarkers

Emanuelson *et al.* (1987) analyzed quarter milk samples for somatic cell count, Adenosine Triphosphate (ATP), NAGase, Bovine serum albumin (BSA), antitrypsin, conductivity and bacteriological findings, for the diagnosis of bovine mastitis and compared these tests with respect to their ability to predict the infection status of the quarter. They were of the opinion that all these components were increased in the presence of infection. Predictive ability was higher for ATP, SCC and NAGase and they suggested that combination of two diagnostic tests increased the predictive ability in most cases.

Grove and Jones (1992) developed an ELISA to detect specific antibodies that bind to a purified antigen fraction of *S. aureus.* This *S. aureus* milk antibody test was 97% accurate in detecting IMI by *S. aureus* when compared with bacterial culture methods on a panel of 30 samples submitted by four Universities. The test had 92 per cent sensitivity for positive cultures and 100 per cent specificity for negative cultures. The Pro Staph test offered the dairy industry a fast, accurate diagnostic tool that could screen large numbers of milk samples for *S. aureus* IMI.

Nickerson and Owens (1993) used an indirect ELISA to determine anti staphylococcal serum IgG titers in cows vaccinated with a cell-toxoid adjuvanted preparation of *S.aureus* strain. Mean anti *S.aureus* IgG titers in serum across the trial for vaccinates remained elevated approximately 4.7 fold over those of controls and pre treatment titers throughout the trial. At week 8 and 10 (2 and 4 weeks after booster injections), titers in vaccinates tended to be higher than at other times during the trial and were elevated over those at 4^{th} week.

Herbeline *et al.* (1997) used an indirect ELISA to measure the antibodies in sera and milk samples of the dairy cows immunized with *S.aureus* toxin. Sera were diluted at 1:2000 and 1:4000 whereas milk samples were diluted at 1:100. The antibody titres in sera and milk samples were increased after immunization. Ten lactating Holstein cows that were free of intramammary infection received systematic immunization by subcutaneous injection of Freund's Incomplete Adjuvant with a toxin mixed with type 5 capsular polysaccharide. The magnitude of antibody response was similar for all cows that had been immunized either with a toxin alone or with a toxin that was conjugated with CP5.

Winter and Colditz (2002) studied the responses of five lactating ewes to experimental mammary infection with *S. epidermidis.* Cytokines in milk were measured by ELISA. IL-8 was elevated in infected glands at 2 hr, peaked at 24 hr and remained elevated until the final sampling at 144 hr. IL-6 was transiently elevated at 4 and 8 hr while IL-1 beta remained elevated from eight until 144 hr. The results suggested that the intense early neutrophil infiltrate eliminated most but not all bacteria and a state of

subclinical infection ensued. After 24 hr, leukocyte numbers in milk declined while cytokines, especially IL-8 remained elevated, suggesting that sensitivity or responsiveness of gland to inflammatory signals decreased as infection progressed. This attenuation of the host defense response might have contributed to the failure of the gland to eliminate bacteria and might be an important feature of the development of chronic and subclinical mastitis.

Pyorala (2003) reported that SCM should be efficiently detected and informed that indicators of inflammation in the milk which can be determined using rapid, reliable and easy routine techniques should be used for early detection of SCM such as EC, SCC, milk lactate, lactose, NAGase, acute phase protein, haptoglobin and serum and milk amyloid A Protein. Milk lactate, lactose and NAGase were significantly higher when compared to the healthy quarter. He reported that NAGase in foremilk and lactose in the composite milk could be considered as best candidates for detection of SCM and acute phase proteins like haptoglobin and serum amyloid A proteins were most potential candidates.

Gronlund *et al.* (2005) evaluated the potential of Haptoglobin (HP), ATP, Serum Amyloid A protein (SAA) and bacteriological culture as indicators of subclinical mastitis using 41 quarter milk samples. They reported that HP and SAA concentrations increase substantially in milk samples with chronic subclinical mastitis when compared to healthy ones and hence conluded that both HP and SAA were considered as best indicators of subclinical mastitis in cows.

Hiss *et al.* (2007) quantified acute phase proteins - Haptoglobin (HP) and Lactate dehydrogenase (LDH) in milk samples in healthy and subclinically affected quarters by using ELISA and directly in the milk parlour respectively. They reported that both the parameters were suitable to distinguish between sterile sample and culturally positive sample and correlated well with SCC, but as far as the ability to differentiate between minor and major pathogens was considered it was better in HP than in LDH. They concluded that both HP and LDH are useful parameters for the diagnosis of SCM. LDH activity in raw milk was less sensitive and specific than HP but the method described offers the opportunity to measure LDH activity directly in the milk parlor and might therefore be suitable for an online system development.

Kalorey *et al.* (2007) standardized and evaluated indirect and Avidin Biotin ELISA using highly purified listeriolysin for detecting *Listeria monocytogenes* antibodies in bovine milk samples and compared with bacteriological examination. The sensitivity of both ELISA was 100 per cent whereas specificity of indirect and Avidin Biotin ELISA was 97.1 and 99.9 per cent respectively.

Gerardi *et al.* (2009) assessed the potential value of measuring amyloid A in serum and milk by ELISA and the correlation with somatic cell count and total microbial count (TMC), in the diagnosis of subclinical mastitis. The reliability of serum ELISA kit for the measurement of amyloid A in serum (SAA) and milk (mAA) and milk ELISA kit for measurement of amyloid A protein in quarter milk (MAA) was also tested. They reported that TMC was significantly higher in cows with clinical mastitis, while no differences were observed between healthy and subclinical mastitic cows. SCC and milk

Amyloid A protein measured by milk ELISA kit (MAA) levels were significantly different among the three groups. Whereas mAA concentrations were similar between cows with subclinical and clinical mastitis, and SAA was not affected by mastitis. A significant correlation between SCC and MAA or mAA was detected, while no correlation was recorded between SAA and mAA. A close relationship between MAA and mAA was noticeable even at low concentrations, suggesting that irrespective of the type of ELISA kit used, milk Amyloid A protein serves as a potential physiological marker of subclinical mastitis.

Moyes *et al.* (2009) conducted a study to identify specific blood markers as risk factors for the development of mastitis during early lactation, in 634 lactations from 317 cows. Cows of three different breeds and parity were used in the study. Blood samples were collected weekly from 56 days before expected calving date through 90 days in milk and were analyzed for SCC, nonesterified fatty acid, beta - hydroxybutyrate, aspartate aminotransferase, energy intake and energy balance. The researchers reported that nonesterified fatty acids and aspartate amino transferase, might be potential markers for the risk of mastitis in early lactation.

Safi *et al.* (2009) compared the accuracy of Acute Phase Proteins measured in milk and in serum with bacterial culture for the diagnosis of bovine subclinical mastitis in 175 randomly selected cows from 7 dairy farms of Iran. Milk samples were analyzed using CMT and SCC and for milk haptoglobin (MHp) and amyloid A (MAA) concentrations and were also subjected for bacterial culture. Serum samples obtained concurrently were analyzed for serum haptoglobin (SHp) and amyloid A (SAA). They

compared SCC, MAA, MHp, SAA, and SHp concentrations between culture-positive and culture-negative animals and assessed the performance of each test using bacterial culture as the reference method. They reported that determination of MAA concentration was most accurate of the five tests, with a sensitivity of 90.6 per cent and specificity of 98.3 per cent. MAA and MHp had significantly larger areas under the curve than the respective serum proteins, SAA and SHp. It was concluded that measuring haptoglobin and amyloid A in milk was more accurate than serum analysis for the diagnosis of subclinical mastitis in Holstein cows.

Vyavahare *et al.* (2009) conducted an experiment to investigate the change in the activity of lactate dehydrogenase in the milk whey and blood-plasma associated with udder health status of cows. On the basis of clinical examination of udder and California Mastitis Test (CMT) score, animals were grouped as normal, subclinical (1+, 2+, 3+) and clinical. The pH and somatic cell count (SCC) were determined in the whole milk and lactate dehydrogenase (LDH) activity was estimated in the milk-whey and blood-plasma. They reported that average values of pH and SCC in whole milk samples differed significantly among different udder health groups and revealed a direct relationship with CMT score and LDH activity in milk-whey. The LDH activity in milk-whey differed significantly among different udder health status, however, it did not differ significantly in blood-plasma and they concluded that assay of LDH activity in milk whey was a useful parameter to assess the udder health status of the cows.

Ying *et al.* (2009a) collected milk samples from 220 lactating Holstein cows. SCC and lactate dehydrogenase (LDH) activity were determined in the milk samples. The

results showed that, LDH activity in the milk of subclinical mastitic cows increased significantly. There was a positive correlation between SCC and LDH activity. The LDH activity of more than 100 IU/L was taken as the threshold to diagnose subclinical mastitis, and its sensitivity and specificity were 70 per cent and 82.3 per cent respectively. These results suggested that LDH could be used as an indicator for early diagnosis of subclinical mastitis of cows.

Ying *et al.* (2009b) detected the activity of milk enzymes quantitatively and in real time for the diagnosis of subclinical mastitis in 220 randomly selected lactating Holstein cows. They studied the variations of SCC, N-acetyl- beta -D- glucosaminase (NAGase), lactate dehydrogenase (LDH), myeloperoxidase (MPO), alkaline phosphatase (ALP) and lactoperoxidase (LP) between healthy cows and those with subclinical mastitis. SCC threshold of $50x10^4$ mL^{-1} in milk was used as the classification standard of subclinical mastitis. They reported that activities of five milk enzymes in cows with subclinical mastitis were significantly higher than the healthy cows ($P<0.05$) and were positively correlated with SCC. Correlation coefficient was 0.98 between SCC and activity of NAGase, and 0.95 between activity of NAGase and LDH and concluded that subclinical mastitis influenced the activities of five milk enzymes significantly ($P<0.05$) and NAGase and LDH could be used as indicators in the early diagnosis of bovine subclinical mastitis.

Katsoulos *et al.* (2010) investigated changes occurring in activities of the enzymes lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) in sheep and goat milk as a result of subclinical intramammary

infections (IMI) and evaluated use of these enzymes for the diagnosis of subclinical IMI in dairy sheep and goats, using 206 samples of sheep milk and 162 samples of goat milk, obtained from equal udder halves. They reported that activities of LDH, ALP and AST were significantly higher in the subclinical infection group than in the no-infection group ($P<0.05$) in both sheep and goats. The activity of LDH was identified as indicator for subclinical IMI in both sheep and goats. The optimum cut-off values for LDH activity, offering the highest diagnostic sensitivity (DSn) and diagnostic specificity (DSp), determined by receiver operating characteristic (ROC) analysis, were 197 U/l, 185 U/l and 197 U/l for sheep, goats and both species, respectively. DSn for sheep, goats and both species at these cut-off values was 92.8 per cent, 98.2 per cent and 94.0 per cent whereas DSp was 95.4 per cent, 96.3 per cent and 96.3 per cent, respectively. They concluded that the determination of LDH activity in milk serum is a sensitive and reliable method for the detection of subclinical IMI in dairy sheep and goats.

Suryanarayana (2012) standardized Liquid Phase Blocking ELISA using antiserum raised against recombinant polyimmunoreactive bovine biomarker (PIBB) antigen comprising immunogenic epitopes of five cytokines (IL-8, Pentraxine-3, L-selectin, protease peptone-3 and complement component C3) to detect cytokines released during intramammary infection. Hyperimmune sera against PIBB antigen was raised in rabbits. Commercially available goat antirabbit IgG conjugated with horse raddish peroxidase and orthophenylene diamine dihydrochloride were used as detection system. A threshold of 0.55 OD at a wavelength of 492 nm was considered for declaring a sample as positive or otherwise. He reported that LPB ELISA was found to have high sensitivity

and specificity and could differentiate significantly between healthy and subclinical mastitic cows.

Jing *et al.* (2012) investigated the dynamics of IL-17 in sera and milk in goat mastitis induced with *E. coli* experimentally. The results indicated IL-17 is one of important mediators in mammary gland inflammation for bacterial clearance in dairy goat mastitis and IL-6 and TGF-β play an important role or help for development in IL-17 producing cells in goat.

Sripad (2013) conducted a study to detect SCM in dairy cows by employing different diagnostic tests namely SCC, EC, NAGase activity and LPB ELISA and reported LPB ELISA and NAGase activity as sensitive diagnostic tests for detection of SCM in dairy cows.

2.2. Prevalence of mastitis

Manser (1986) examined five goat herds to determine the prevalence of subclinical mastitis. In 170 samples taken from the pairs of mammary glands of 85 goats, the prevalence of infection in the different herds ranged from 15 to 79 per cent of halves.

Cruz *et al.* (1994) studied the prevalence of subclinical mastitis in 466 Manchega sheep, was determined at 3 and 4 month of lactation. A high proportion of glands and ewes (26.8% and 36.7%, respectively) showed bacterial infection, which was attributable to *Staphylococcus* in 83.2% of cases.

Lafi *et al.* (1998) determined the prevalence of intramammary infections in Awassi sheep flocks. Of the 3472 udder halves examined, 29.8% had over 10^6 SCC/ml

and 0.03% had dry teats due to chronic mastitis. The incidence of clinical mastitis (expressed as the number of clinical cases per 100 ewe-months) were 2.1 ± 1.9 (SD), 1.9 ± 1.1, and 1.2 + 2.1 for small, medium and large flocks size strata, respectively. The overall population estimate was 1.7 ± 0.02 cases per 100 ewe-months.

Contreras *et al.* (1999) studied the prevalence in 138 lactating goats that had a high somatic cell count in bulk tank milk and the prevalence of intramammary infection reported to be 34%.

Gupta *et al.* (1999) recorded the incidence of mastitis from 1990-1997 in goats kept under a semi-intensive system of management and reported higher incidence of clinical mastitis in Barbari goats (3.02%) as compared to the Jamunapari goats (1.22%). The overall incidence was found to be 2.18 per cent. On a yearly basis, the incidence was highest (14.76%) in 1993, whereas the lowest incidence (4.68%) was recorded in 1991. It was also observed that the highest cases of mastitis (40%) were reported in the rainy season, followed by winter (33.75%) and summer (26.25%). The relationship between mastitis incidence and meteorological data revealed that the incidence of mastitis in goats was inversely proportional to the environmental temperature, and directly correlated with the increase in relative humidity.

Sanchez *et al.* (1999) observed a positive statistical association between subclinical intramammary infections and greater than fifth parity and concluded that tendency for prevalence to increase was seen as the parity increased.

Ndegwa *et al.* (2000) reported the prevalence of subclinical mastitis in dairy goats comprising a mixed population of German Alpine, Toggenberg, Saanen and Galla crosses. California mastitis test (CMT), direct leukocytes counts and bacteriological examination were performed on 630 milk samples from apparently healthy mammary glands. The prevalence of subclinical mastitis was 9.8% according to CMT, 9.7% according to direct leukocyte counts and 28.7% by bacterial isolation.

In sheep and goats, mastitis episodes are the main reason for culling because of sanitary problems, which occur mainly during the first 2–3 months of lactation (Malher *et al.*, 2001).

Bergonier *et al.* (2003) reviewed on mastitis of dairy small ruminants and concluded that the incidence of clinical mastitis in small ruminants tends to be lower than 5 per cent per year and in lower percentage of herds, the incidence was higher and may exceed 30-50 per cent animals, causing mortality or culling up to 70 per cent of the herd.

Al-Majali and Jawabreh (2003) investigated the period prevalence of subclinical mastitis in Awassi ewes from the southern part of Jordan. The period prevalence of subclinical mastitis was 18.3% and the prevalence within each flock remained relatively constant throughout the study period. A significant association was found between the occurrence of subclinical mastitis and the age of ewe.

Leitner *et al.* (2004) found that the effect of bacteriological infection on somatic cells count (SCC) was significant ($P>0.001$) and the percentages of udder halves with subclinical intramammary infection in the flocks ranged from 35 to 71 per cent and

overall prevalence was 52 per cent. The lactation number did not significantly influence either infection rate of udder halves or SCC, although the percentage of udder halves with no bacteriological findings was higher at the first lactation than at the third lactation.

Bachaya *et al.* (2005) determined the quarter-wise and animal-wise prevalence of subclinical mastitis in cattle in Attock district of Punjab by using Surf Field Mastitis Test (SFMT). The overall quarter-wise prevalence was 44.17 per cent, while animal-wise prevalence was 44 per cent.

Schaeren and Maurer (2006) examined three herds for SCC values for goat milk and found that about 40 per cent of mammary halves and 30 per cent goats were infected.

The parity had no significant effect on SCC, but infected udder halves had significant higher levels of SCC than uninfected halves (2.35 vs. 3.02). It was observed that the response of SCC between udder halves was not independent. If one half was infected with CoNS the SCC of the non-infected udder half was influenced and tended to increase (Aulrich and Barth, 2008).

Kostelic *et al.* (2009) conducted study on 20 French alpine goat farms in Croatia. The results of the study lead to the conclusion that the subclinical mastitis in goats had a prevalence of 20% on average which increases with higher lactation number.

Diaz *et al.* (2011) studied the effect of farm, parity, stage of lactation and health status of glands on EC and SCC in 105 Murciano-Granadina goats. The incidence of mastitis in various farms was 34.6, 59.3 and 52.6 per cent and the overall incidence was 48.6 per cent.

Gebrewahid *et al.* (2012) conducted a study on 390 lactating animals comprising 255 goats and 135 sheep to assess the prevalence of subclinical mastitis in lactating small ruminants. The overall prevalence of subclinical mastitis was found to be 18.03% and 28.14% in goats and sheep, respectively. There was no statistically significant association between risk factors such as age, parity and stage of lactation.

Islam *et al.* (2012) screened 462 milk samples of Black Bengal goats for subclinical mastitis using California Mastitis Test (CMT), White Side Test (WST) and Surf Field Mastitis Test (SFMT) simultaneously. Integrated test results yield the prevalence of caprine subclinical mastitis as 44.59%. Animal level and udder half level prevalence of subclinical caprine mastitis were 39.83, 38.96, 38.10% and 35.05, 34.85, 31.60% by CMT, WST and SFMT, respectively noticed when tests were interpreted individually.

Milk samples of 60 clinically healthy lactating goats between their first (n = 14) and eighth lactation (2^{nd}: 11, 3^{rd}: 12, 4^{th}: 9, 5^{th}: 6, 6^{th} and above: 8) were investigated during the first 6 weeks of lactation. The workers observed that a significant influence of the lactation week and lactation number on $\log_{10}$ SCC was noticeable. The infection status must be acknowledged as the most dominating predictor of $\log_{10}$ SCC, but also the lactation number had a highly significant effect on $\log_{10}$ SCC. The lactation week showed the highest significant effect on log10 NAGase (Stuhr *et al.*, 2012).

Mishra *et al.* (2013) carried out the study to assess the status of subclinical mastitis in Jamunapari and Barbari goats. The overall prevalence rate of SCM among the

goats was determined as 19.9 %. Barbari breed showed higher prevalence of SCM (24.2%) than that of Jamunapari (15.1%).

Prabavathy (2013) reported an incidence of subclinical was 72.5 per cent, clinical mastitis was 8.5 per cent and overall mastitis was 81 per cent. Incidence of subclinical mastitis was highest in summer season.

Mishra *et al.* (2014) carried out the study to assess the status of subclinical mastitis in different breeds of goats. The overall prevalence rate of SCM among the goats was determined as 24.2%. Jakhrana breed showed highest prevalence of SCM (36.36%) followed by Barbari (28.18 %) and Jamunapari (8.18 %).

Zhao *et al.* (2015) investigated the prevalence of subclinical mastitis in dairy goats in China. Out of 683, 313 (45.82%) goats were detected distinct or strong positive for SCM by using California mastitis test.

2.3. Haematological and biochemical alterations associated with mastitis

Singh *et al.* (1998) inoculated 10 goats with *Candida albicans* intramammary in their experimental study resulted in the development of mastitis. Haematology revealed leucocytosis and neutrophilia. Further an increase in total immunoglobulins in the milk and plasma along with increase in total plasma proteins were observed.

Smith *et al.* (2001) examined medical records of 142 dairy cows with clinical mastitis for haematological and serum biochemical results to distinguish between gram negative and gram positive bacterial mastitis episodes. Cows with gram negative mastitis had significantly lower blood leucocyte, segmented neutrophil, monocyte and

lymphocyte counts and had higher blood haemoglobin concentrations, haematocrits and serum urea nitrogen than cows with gram positive mastitis. They opined that routine haematologic analysis is useful for predicting pathogen type in dairy cows with clinical mastitis, therby facilitating treatment decisions.

Ajuwape *et al.* (2005) study on mastitis in goats reported that the erythrocytic values of mastitic does were significantly lower than that of the non-mastitic does. The total and differential leukocyte counts of the mastitic does were significantly higher than that of the non-mastitic does. Total and differential leucocyte counts in lactating does are true reflection of mastitis and will even assist the clinicians in predicting the prognosis of mastitic animals.

Zaki *et al.* (2008) investigated the effect of subclinical mastitis on some biochemical and clinicopathological changes in buffaloes. The results indicated that there is a significant elevation of cortisol, SGOT, PCV, LDH activity in milk while a notable decrease in total protein, serum calcium and haemogram was observed. However serum phosphorus level did not exhibit obvious changes in subclinical mastitis buffaloes.

Tabrizi *et al.* (2008) reported the potential use of Acute phase proteins in the assessment of mammary gland health by examining the level of fibrinogen, ceruloplasmin in plasma and milk from dairy cows with different grades of mastitis. The concentration of fibrinogen in the plasma, fibrinogen and ceruloplasmin in the milk of cows with subclinical and clinical mastitis were higher than in the healthy cows. There was no significant difference in plasma concentration of ceruloplasmin between healthy and subclinical groups. They opined that measurement of fibrinogen in plasma and milk

and ceruloplasmin only in milk might be suitable for early diagnosis of mastitis in dairy cows.

Matei *et al.* (2010) reported the changes that occur in serum metabolic parameters in healthy and subclinical mastitis cows. The total serum protein (9.14±2.74 g/dl) and serum globulin (5.76 ±1.82 g/dl) from subclinical mastitis cows were higher compared to healthy cows. They opined that an increased protein and globulin in the blood of cows indicate an activation of immune response following infection of the mammary gland. Further reported low values of the two enzymes, AST (87.20±30.16 U/l) and GGT (18.80±8.90 U/l), along with decrease activity of CK (221.80±80.53 U/l) in cows with subclinical mastitis. Contrarily, ALP activity (71.80±50.70 U/l) increased in cows with subclinical mastitis compared with healthy cows.

Pandey *et al.* (2012) in a study evaluated the influence of subclinical mastitis on biochemical components of blood in dairy cows. The biochemical analysis of blood samples revealed a significant elevation in serum AST, total serum protein and globulin whereas significant decline was observed in levels of AG ratio, Na, K and Cl in cows having subclinical mastitis as compared to healthy cows. They opined that these noticeable changes are due to the damage caused to ductal and secretory epithelium of udder by invading pathogenic organism.

Jain *et al.* (2013) conducted a study to investigate the changes in various biochemical parameters during mastitis of cows. Albumin was found to be lower in the mastitis cows (3.49±0.28 g/dl) as compared to the healthy ones (3.98±0.3 g/dl). The value for SGPT in mastitis cow (78.92±5.98 U/L) was found to be significantly higher than the

control (38.5±4.94 U/L). Total serum protein and serum globulin were non-significant but higher in mastitis cows. They concluded that biochemical parameters were altered in mastitic cows.

Singh *et al.* (2014) studied the effect of subclinical mastitis on haematological and biochemical profile of crossbred cattle. Haematology of animals revealed significantly higher average values of TLC in sub-clinically infected animals (9.20x 103/µl) than healthy animals (6.87x 103/µl). Differential leucocytic count revealed neutrophilia and lymphopenia in sub-clinical mastitis. Biochemical estimations of plasma samples from sub-clinically infected animals revealed higher average values of TPP (8.44 g/dl), globulin (5.82 g/dl) and fibrinogen (0.74 g/dl) compared with control group (7.44 g/dl, 4.41 g/dl and 0.60 g/dl, respectively), but lower average values of albumin (2.44 g/dl) compared with control group (2.94 g/dl).

Fasulkov *et al.* (2014) assessed the changes in acute phase proteins fibrinogen and haptoglobin, as well as hematological and blood biochemical in a study on local Bulgarian goats with experimentally induced *Staphylococcus aureus* mastitis. Blood haptoglobin in goats was considerably higher as early as 8 h after the pathogen's inoculation. Plasma fibrinogen increased significantly 8 h after infection and reached the highest mean concentration (9.12 g/L) by 72 h. Leukocytosis was established as early as 4 h after the experimental infection, when leukocyte counts were significantly higher than baseline. The analysis of results indicated that in experimentally induced *Staphylococcus aureus* mastitis, significantly higher levels of leukocyte, fibrinogen and haptoglobin are established as early as 8 h after the infection.

Gain *et al.* (2015) evaluated blood leucocyte together with serum enzyme profile during sub-clinical mastitis in crossbred cows. There was no significant alteration in TLC and DLC between normal and sub-clinical mastitic cows. The alteration in serum protein content was non-significant but albumin : globulin ratio decreased significantly during subclinical mastitis. Serum AST and ALP level in sub-clinical mastitic cows was significantly higher but no significant variation observed in serum AST level in normal and sub-clinical mastitic cows.

Chandrasekaran *et al.* (2015) had undertaken the study to know the haemato-biochemical alterations of drug resistant mastitis in dairy cows. Haematological changes observed were reduced Hb, PCV, TEC and increased TLC with neutrophilia and lymphopenia. Biochemical changes noticed were hypoalbuminemia and hyperglobulinemia and a significant increase in ALP and AST enzymes in mastitic cows compared to control group.

2.4. Treatment of mastitis

Malbe *et al.* (1995) compared the effects of organic Se (selenized yeast) and sodium selenite in 100 dairy cows at the Estonian Agricultural University which appeared to have an extremely low Selenium status (Se 5.6 µg/litre in whole blood and 3.2 µg/litre in milk) and blood glutathione peroxidase (GSH-PX). Se uptake, udder health and in vitro function of blood neutrophils were monitored. Supplementing the feed with organic Se 0.2 mg or sodium selenite for 8 weeks increased blood Se within this period from the background level of 5.6 µg to 167 (Se-yeast) and 91 µg/litre (selenite). Corresponding values for GSH-PX were 0.22, 3.0 and 2.3 µKat/g Hb. Blood GSH-PX continued to

increase up to 10 weeks after the supplementation had ceased. The bioavailability of yeast Selenium was superior to selenite. They concluded that Selenium supplementation had a positive effect on udder health. The percentage of quarters harboring mastitis pathogens dropped from 22.9 to 13.0 in Selenium-yeast group and from 18.4 to 7.4 in the selenite group during supplementation. The effect of Selenium on mastitis was also reflected as a decrease in the number of milk somatic cells and N-acetyl-beta-D-glucosaminidase (NAGase) activity.

Ndiweni and Finch (1996) indicated the potential benefits of supplementation of dairy cows with Vitamin E and Selenium in terms of enhancing their natural resistance to mastitis.

Ali-Vehmas *et al.* (1997) supplemented the feed of Selenium-deficient dairy cows with Selenium (Se)-yeast or selenite at a level of 0.2 ppm. They opined that supplementation of organic Selenium improved the correlation between infection and the respective inflammatory response, as indicated by the somatic cell count (SCC) and the N-acetyl-beta-D-glucosaminidase activity (NAGase) of milk. Further they reported supplementation of Selenium yeast would improve recruitment of phagocytes to the infected milk compartment of the udder and thus inducing self cure of subclinical mastitis and decreasing the prevalence of quarters harbouring subclinical mastitis to about one half during the eight week supplementation period. Organic Selenium supplementation induced an unspecified antibacterial activity in milk lactoserum (whey), restricting in vitro growth of the mastitis pathogens.

Dhillon *et al.* (2000) treated buffaloes suffering from mastitis using 15 g of trisodium citrate dissolved in 250 ml of water given orally once a day till recovery and they reported that milk pH and consistency were restored after the treatment.

Langoni *et al.* (2000) evaluated the efficacy of Enrofloxacin and Amoxycillin (alone or in combination) for the treatment of bovine clinical and subclinical mastitis in primiparous and multiparous cows in lactation, by intramammary infusion after milking, in the morning and afternoon, for three consecutive days. The recovery rates for subclinical and clinical cases after treatment with Amoxycillin, Enrofloxacin or a combination of both drugs were: 84.6 and 75.0; 83.3 and 75.0; 87.5 and 84.0 per cent, respectively.

Sreeramachandramurthy (2001) in an experimental study used trisodium citrate and different combination of antibiotics in the treatment of bovine mastitis and assessed the cure rate based on EC and SCC. He reported that the cure rate as assessed on 21st day post treatment, was 62.5 per cent with trisodium citrate and was equivalent to the cure rate of a combined therapy with Enrofloxacin + Amoxycillin-cloxacillin + trisodium citrate.

Gupta *et al.* (2002) conducted *in vitro* therapeutic studies on sub clinical mastitis in goats indicated that Cloxacillin, Kanamycin, Ciprofloxacin, Gentamicin and Chloramphenicol were most effective antibiotics. *In vivo,* Amoxicillin-cloxacillin and Chloramphenicol injection cured all animals with or without incorporation of ascorbic acid in mastitis therapy.

Ram-Naresh *et al.* (2002) evaluated ascorbic acid treatment in clinical and subclinical mastitis of Indian dairy cows. Ascorbic acid was administered in clinical and subclinical cases even after cure considering its immunostimulatory and healing inducing effects. The recovery rate was faster in cases of clinical mastitis treated with ascorbic acid along with an intramammary infusion than the other group. Subclinical mastitis treated with ascorbic acid showed 83.33 per cent recovery. However, they reported that the pharmacology of ascorbic acid with particular reference to health of mammary gland was required to be investigated.

Paes *et al.* (2003) challenged primiparous Saanen goats with *S. aureus* on 10 days postpartumn to mammary gland after supplementing goats with Vitamin E (2 gm in 20 ml oil vehicle qs) intramuscularly at birth and same dose repeated after a week. The result revealed that Vitamin E injected group showed lower SCC and recovery of less microorganisms after challenging with *S. aureus,* indicating Vitamin E supplementation could able to maintain the udder health and reduce the occurrence of mastitis in goats.

Mohan *et al.* (2004) studied the causative organisms associated with goat mastitis and their antibiogram by in vitro sensitivity test. Based on the results, authors suggested that Ciprofloxacin, Enrofloxacin, Chloramphenicol, Gentamicin, Amoxicillin, Cloxacillin and Oxytetracycline may be selected in decreasing order of their efficacy for treating mastitis in goats. The variation in the sensitivity may be attributed to the indiscriminate use of these drugs, which contribute to the increased resistance of different bacterial strains.

Saluja *et al.* (2005) evaluated the systemic therapy of lactating cows affected with mastitis, using Enrofloxacin, Enrofloxacin with Levamisole, Enrofloxacin with Vitamin E and Selenium, Levamisole alone and Vitamin E and Selenium. They reported that Enrofloxacin alone was found to be most efficacious in systemic therapy on day three post treatment, whereas on day 14 post treatment the combination of Enrofloxacin with Vitamin E and Selenium was found to be more efficacious.

Awasthi and Upadhyay (2006) screened and treated the subclinical mastitic cows and concluded that Enrofloxacin was the most effective antibiotic (72.73 %) followed by Ciprofloxacin (63.64 %). The microorganisms were resistant to Penicillin and Nitrofurantoin.

Paschoal *et al.* (2006) evaluated the influence of prepartum supplement of Selenium and Vitamin E on milk somatic cell count in 80 HF cows during summer. Blood Selenium level and milk somatic cell counts were estimated before and up to 60 days after the supplementation. They reported that Selenium and Vitamin E did not decrease ($P>0.05$) SCC up to week 12 of lactation.

It is opined that Selenium supplementation in diet has beneficial effect in animals by enhancing the mammary gland resistance against infectious diseases. Hence, Sanchez *et al.* (2007) has attempted to supplement Selenium (Barium selenate) by injecting goats @ 1mg/kg b.wt. 15 days before mating. Subsequently the goats were monitored to assess the occurrence of mastitis. It was recorded that SCC and incidence of clinical mastitis were significantly lower in the treated group of goats than the control group. The result

revealed that Se injection significantly increased the glutathione peroxidise activity and had beneficial effect in the subsequent lactation.

Peer and Bhattacharyya (2007) studied the caprine gangrenous mastitis and found that gentamicin has got highest sensitivity (60%) *in-vitro* but unable to cure the condition *in-vivo*. Hence, gentamicin was ineffective and this may be due to involvement of multi-etiological factors in gangrenous mastitis.

Pyorala (2009) reported that rapid bacteriological diagnosis would facilitate proper selection of the antimicrobial. Treating subclinical mastitis with antimicrobials during lactation was seldom economical, because of high treatment costs and generally poor efficacy. All mastitis treatment should be evidence-based, i.e., the efficacy of each product and treatment length should be demonstrated by scientific studies. Use of an arm written protocol for mastitis treatment would promote a judicious use of antimicrobials and reduce indiscriminate use of antimicrobials.

Dogruer *et al.* (2010) studied the treatment of subclinical mastitis in Damascus goats during lactation concluded that the intramammary Ampicillin Dicloxacillin treatment had the best treatment rates, the combination of intramuscular Amoxycillin clavulanic acid was also successful. Intramuscular Amoxycillin clavulanic acid as the sole treatment was not as effective as intramammary therapy

Marin *et al.* (2010) conducted *in-vitro* experiment to know minimum inhibitory concentration of fluoroquinolones (DAN, MAR and ORB) by MIC tests against *Staph. aureus* strains isolated from milk. The test results revealed that Danofloxacin was the

most active compound followed by Marbofloxacin and Orbifloxacin as a therapy for control of clinical mammary infection in goats

McDougall *et al.* (2010) treated subclinical mastitis in dairy goats (n=57 glands) with three intramammary infusions of 75 mg Sodium Ampicillin and 250 mg Sodium Cloxacillin at 12 hours interval. They observed that there was no significant difference among two treatment groups. However, increased overall cure proportion and reduced SCC among the treatment groups was recorded when compared to control group.

Suresh *et al.* (2010) conducted a study in Jersey crossbred cows with a history of reduced milk yield, using long acting Enrofloxacin. Cure was assessed by milk pH, SCC, EC, MWST and MCMT, which were significantly elevated during the pretreatment stage and reported that 100 per cent quarter cure was observed.

Sar *et al.* (2012) assessed the efficacy of Fibrosin® a poly herbal drug against mastitis in Black bengal goats. In their study, a total of 18 goats were divided in to 3 groups in which G-I was treated with single dose of ceftriaxone @ 50 mg/kg b.wt., G-II was treated with ½ boli (1.9 g) of Fibrosin® a poly herbal drug and G-III was treated with a boli of Fibrosin® one hour prior to ceftriaxone administration. All the groups were monitored for enzymatic activities in milk. Based on the enzymatic activity, administration of Fibrosin® as co-therapy with ceftriaxone has resulted better treatment than other groups.

Prabavathy (2013) in a study on *invitro* sensitivity pattern of isolates of caprine mastitis reported Amikacin, Ciprofloxacin and Enrofloxacin were found to be highly sensitive and all the isolates were least sensitive to Clindamycin.

Sreeja *et al.* (2013) attempted to treat acute goat mastitis as per the *in-vitro* culture and sensitivity test with Ceftriaxone @10 mg/kg b.wt. for 5 days and found clinical cure was 58.33 per cent whereas bacteriological cure was only 50 per cent. The correlation among *in-vitro* and *in-vivo* could not able to establish in their study.

Chetan-Kumar *et al.* (2014) evaluated the efficacy of Ceftriaxone and Tazobactam combination along with Meloxicam, Vitamin E, Selenium and Trisodium citrate in 24 clinical cases of mastitis and assessed recovery based on reduction in the clinical signs, reduction in EC and SCC. He reported that there was disappearance of symptoms, reduction in EC and SCC within 5 days post treatment and concluded that treatment with combination of Ceftriaxone + Tazobactam with Vitamin E and Selenium was found superior over other three treatment regimes.

Sripad *et al.* (2016) reported that Enrofloxacin + organic Selenium, Enrofloxacin + inorganic Selenium and Enrofloxacin alone, can be the order of preference or choice for treatment of SCM in dairy cows and concluded that oral organic Selenium along with Enrofloxacin, is more efficacious and beneficial in the treatment of SCM in dairy cows.

Jena and Gupta (2016) ensured complete recovery from mastitis in a goat, treated with Gentamicin, Vitamin- A, D3, H and E parenterally and Trisodium citrate and NSAID orally for 5 days. They opined that Trisodium citrate alters the milk pH making it unfavourable for growth of bacteria. Supplementation of Vitamin- A, D3, H and E help to ameliorate the altered milk oxidant / antioxidant balance towards normalcy.

MATERIALS AND METHODS

III. MATERIALS AND METHODS

3.1. Materials

3.1.1. General considerations

The glasswares used in this study were of neutral glass of Corning, Borosil India Ltd. or Scott Durham (Germany) make. Plasticwares including syringes, micro centrifuge tubes, centrifuge tubes, micropipette tips, cryovials, and autoclave bags were procured from M/s Tarson Products Pvt. Ltd., Kolkata and Axygen Inc., USA. ELISA plates were procured from Nunc Pvt Ltd., Denmark. The chemicals of Analar and Laboratory reagent grade were used for the preparation of various solutions and reagents. The buffers and other biochemical reagents were prepared in Milli Q water. The buffers, chemicals, stain, enzymes, conjugate and other requirements were obtained from M/s HiMedia, Mumbai; BD, BBL and DIFCO, USA; Sisco Research Laboratories Pvt. Ltd., Mumbai; Sigma Aldrich, USA.

3.1.2. Equipments

Following equipments were procured and used in this study:

ECO Testr® EC High (M/s Eutech Instruments, Singapore)

Micropipette (M/s Ependorf (I) Pvt. Ltd., Bangalore)

Water bath (M/s Remi Motors Pvt. Ltd., Thane)

Refrigerated Centrifuge (M/s Remi Motors Pvt. Ltd., Thane)

Incubator (M/s Remi Motors Pvt. Ltd., Thane)

Shaker incubator (M/s Remi Motors Pvt. Ltd., Thane)

Epoch ELISA Reader (M/s Biotek, USA)

Erma Auto Haematology Analyser, Model: PCE-210 Vet, manufactured by M/s ERMA INC., Tokyo, Japan.

Semi Autochemistry Analyser, Model: ARTOS, manufactured by M/s SWEMED Biomedicals Pvt. Ltd., Bangalore, India.

REMI Centrifuge, Model; C-852, manufactured by M/s Remi Motors Ltd. Ugai (E), Dist: Thane, Maharashtra.

Microscope procured from the Industrial and Laboratory Equipment Co. (Mysore), Bangalore was used in this study.

3.1.3. Chemicals and stains

The chemicals used in this study were of analytical grade, triple glass distilled water (pH 7.0 to 7.2) were used for preparation of reagents. Following chemicals and stains were procured and used in this study:

3.1.3.1. Modified Newman's stain

Modified Newman's stain was used for SCC – procured from M/s HiMedia Pvt. Ltd., Mumbai

3.1.3.2. Potassium chloride

Potassium chloride calibration solution (12.88 mS) was used for electrical conductivity – procured from M/s Eutech Instruments, Singapore

3.1.3.3. N-acetyl-β-D-glucosaminidase

N-acetyl-β-D-glucosaminidase (substrate) was obtained from M/s Sigma Aldrich Co. Pvt. Ltd., USA.

Glycine - procured from M/s HiMedia Laboratories, Mumbai.

Sodium deoxycholate - procured from M/s HiMedia Laboratories, Mumbai.

Following LR grade chemicals were procured from M/s SD Fine Chemicals Pvt. Ltd., Mumbai and used in this study:

Sodium citrate, Citric acid and Chloroform.

3.1.3.4. Liquid Phase Blocking ELISA

Orthophenylene diamine dihydrochloride (OPD) – procured from M/s Sigma Aldrich, Pvt. Ltd, USA.

Horse Raddish Peroxidase conjugated anti rabbit IgG – procured from M/s Sigma Aldrich, Pvt. Ltd, USA.

Hydrogen peroxide - procured from M/s Nice Chemicals Pvt. Ltd., Cochin.

Poly immune bovine bioreactive (PIBB) antigen – prepared in the laboratory

Hyper immune serum against PIBB antigen – raised in the laboratory

Tween -20 - procured from M/s Sisco Research Laboratories Pvt. Ltd., Mumbai

Following chemicals were procured from M/s SD Fine Chemicals Pvt. Ltd., Mumbai and used in this study:

Sodium carbonate, Sodium bicarbonate, Potasium dihydrogen phosphate, Dipotasium hydrogen phosphate, Sodium chloride, Potasium chloride, Sulphuric acid and Skim milk powder.

3.1.4. Biochemical kits

3.1.4.1. ALT (Alanine Transaminase) biochemical kit

Swemed ALT kit, manufactured by M/s Swemed Biomedicals Pvt. Ltd., Bangalore was procured from M/s Siscon Chemicals, Bidar and used in this study.

3.1.4.2. AST (Aspartate Transaminase) biochemical kit

Swemed AST kit, manufactured by M/s Swemed Biomedicals Pvt. Ltd., Bangalore was procured from M/s Siscon Chemicals, Bidar and used in this study.

3.1.4.3. GGT (Gamma Glutamyl Transferase) biochemical kit

Swemed GGT kit, manufactured by M/s Swemed Biomedicals Pvt. Ltd., Bangalore was procured from M/s Siscon Chemicals, Bidar and used in this study.

3.1.4.4. Total protein biochemical kit

Swemed Total Protein kit, manufactured by M/s Swemed Biomedicals Pvt. Ltd., Bangalore was procured from M/s Siscon Chemicals, Bidar and used in this study.

3.1.4.5. Albumin biochemical kit

Swemed Albumin kit, manufactured by M/s Swemed Biomedicals Pvt. Ltd., Bangalore was procured from M/s Siscon Chemicals, Bidar and used in this study.

3.1.5. Therapeutic study

Following therapeutic agents were used in this study:

Enrofloxacin – Quinintas® containing Enrofloxacin 100 mg/ml marketed by M/s Intas Pharmaceuticals Ltd., Ahmedabad

Vitamin E and Selenium – E-care Se® containing DL-α-Tocopheryl acetate IP 50 mg and Sodium selenite USP 1.5 mg per ml marketed by M/s Tetragon chemie Pvt. Ltd., Vet Care Division, Bangalore

Trisodium citrate – 500g marketed by M/s Nice Chemicals Pvt. Ltd., Cochin

Organic Selenium – 0.3 mg/g w/w marketed by M/s Indian Herbs Pvt. Ltd., Bangalore

3.1.6. Glass wares and plastic wares

Polysterene ELISA plates – procured from M/s Nunc Pvt. Ltd., Denmark

Petriplates - were of neutral glass, procured M/s Borosil India Ltd., Mumbai

Following plastic wares were procured from M/s Tarson Products Pvt. Ltd., Kolkata and used in this study:

Sterile milk collection tubes, Disposable syringes, Hypodermic needles, Storage vials/bullets, Rubber bulbs and Microtips.

3.2. Methods

3.2.1. Preparation of glass wares

The glass wares used in the study were prepared by soaking them in detergent (Teepol) solution overnight. The following day, they were washed thoroughly in running

tap water, then rinsed in deionised / distilled water. The oven dried glassware were packed and sterilized in hot air oven for one hr at 160°C as per Collee *et al.* (1996).

3.2.2. Preparation of plastic wares

The new plastic wares including centrifuge tubes, micro centrifuge tubes and micropipette tips were sterilized by autoclaving at 121°C for 15 min at 15 psi.

3.2.3. Study Animals

3.2.3.1. Prevalence Study

Two hundred goats from unorganized farms of various demographic locations in and around Bidar, Karnataka formed the source of animals for the study. The study animals were grouped based on the parity (lactation number) and lactation stage. The prevalence was expressed in per cent positive by taking into account the number positive out of number of goats tested for SCM.

To know the stage of lactation wise prevalence of SCM in goats, the average lactation length of 120 days (Shettar, 2011) was divided into three stages viz., Early stage (0 – 40 days), Mid stage (41 – 80 days) and Late stage (81 – 120 days) lactation. The details regarding parity and stage of lactation are depicted in the tables A and B.

Table A. Parity wise classification of goats

Sl. No.	Groups based on parity	No. of goats
1	1st parity	34
2	2nd parity	31
3	3rd parity	34
4	4th parity	30
5	5th parity	30
6	6th parity	27
7	7th parity	14
	TOTAL	**200**

Table B. Lactation stage wise classification of goats

Sl. No.	Groups based on lactation stage	No. of goats
1	Early lactation	80
2	Mid lactation	75
3	Late lactation	45
	TOTAL	**200**

3.2.3.2. Haematological and biochemical study

Eight healthy adult goats and eight goats positive for SCM allocated into healthy and affected group, formed the source animals for haematological and biochemical study.

3.2.3.3. Therapeutic study

Forty animals which were detected positive for SCM were randomly allocated into five groups namely Group I, Group II, Group III, Group IV and Group V with each group comprising of eight animals, formed the source animals for the therapeutic study.

3.2.4. Collection of milk samples

3.2.4.1. Collection of milk samples for prevalence study

To study the prevalence of SCM, a total of 200 goats composite milk samples were collected from various demographic locations in and around Bidar. The relevant information with respect to parity and stage of lactation were also collected. Approximately 20 ml of milk was collected in sterile tubes following strict aseptic measures and was immediately transported to laboratory on ice.

3.2.4.2. Collection of milk samples for therapeutic study

To study the response to the treatment, post treatment milk samples were collected from all the forty animals belonging to control and different treatment groups on zero, 7^{th}, 14^{th}, 21^{th}, and 30^{th} day post treatment.

3.2.5. Screening for subclinical mastitis

All the 200 milk samples were subjected to SCC, EC, NAGase activity and Liquid phase blocking Enzyme Linked Immunosorbent Assay (LPB ELISA), for detecting SCM.

3.2.5.1. Somatic Cell Count (SCC)

Freshly collected milk samples were used for SCC estimation by Direct Microscopic Somatic Cell Count method.

3.2.5.1.1. Direct microscopic somatic cell count in milk

The procedure described by Prescott and Breed cited by Schalm *et al.* (1971) was followed.

a) Preparation of milk films

- The milk samples were mixed 15 to 20 times to obtain an uniform distribution of cells and were allowed to stand for 2 to 5 minutes to permit air bubbles to rise and foam to disappear.
- A clean, grease free microscopic slide was placed over the template to outline one sq.cm area.
- Ten µl of milk was placed exactly in the centre of the one sq.cm template and was spread evenly to cover all the area delineated by the template.
- From each sample, two films were prepared using successive areas of the slide. The films were dried at room temperature and then stained.

b) Staining by modified Newman's staining technique

- The slides with milk smears were placed on the slide rack and were flooded with modified Newman's stain (HiMedia) for 2 min.

- The excess stain was drained off by standing the slides on absorbent paper and air-dried.
- The slides were rinsed in tap water and air dried.

c) Counting of cells

Stained films were examined under oil immersion lens and the number of somatic cells in 20 fields were counted (Plate 3). The fields were selected by moving the slide horizontally from one edge of the film through the centre to the opposite edge and then, repeated in a vertical direction. The average number of cells per field was multiplied by the microscopic factor.

d) Calibration of the microscope / Calculation of microscopic factor

The diameter of the microscopic field seen through oil immersion objective was measured using a stage micrometer slide ruled 0.1 and 0.01 mm. The diameter of the field was measured up to two decimal points and the area of the field was calculated using the formula πr^2.

$$\text{Microscopic factor (MF)} = \frac{\text{Area of the smear (in mm}^2\text{)}}{\text{Area of the microscopic field}}$$

The diameter was, 0.16, then r = 0.08

$$\text{So, MF} = \frac{100}{3.14\text{X } 0.08^2} = 4976 \approx 5000$$

Since, the milk sample taken on the slide was 0.01 ml, the total number of cells per ml of milk was given by the formula;

Cell count per ml of milk = Average no. of cells per field x MF x 100

The SCC value of 5,00,000 cells/ml of milk and above was taken as criteria to declare the milk / animal as subclinically mastitic (Contreras *et al.*, 1996).

3.2.5.2. Electrical Conductivity (EC)

Initially the ECO testr EC instrument calibration was checked with Potassium chloride calibration solution (12.88 mS). About 5 ml of milk sample is drawn into the receptacle of milk checker and the instrument is switched on to read the conductivity in milli siemens per centimeter (mS/cm) (Plate 4). EC of 6.8 milli Siemens (mS)/cm and above was taken as criteria to declare the milk / animal as subclinically mastitic / infected (Chen *et al.*, 2008).

3.2.5.3. N-acetyl-β-D-glucosaminidase (NAGase) activity

NAGase enzyme activity was estimated as per the method described by Kitchen and Middleton, (1976).

Step-1. 0.2 ml of milk sample was mixed with 0.3 ml of the substrate in 0.33 M Citrate buffer, pH 4.6 (appendix) in a small polypropylene tube.

Step-2. The tubes were incubated for 15 minutes at 50^0C in a water bath.

Step-3. Reaction was stopped by adding 1 ml of 1 M glycine containing 1% deoxycholate, (pH adjusted to 10 with sodium hydroxide) (appendix).

Step-4. One ml of chloroform was added and shaked vigorously for 5 sec.

Step-5. Tubes were centrifuged at 2000 G for 10 minutes.

Step-6. Top aqueous layer was removed with pasteur pipette.

Step-7. Absorbance was recorded at 405 nm

Calculation of NAGase enzyme activity

Enzyme activity was calculated by using Beer-Lambert law as follows,

$$\textbf{Calculation}: \text{Enzyme activity } (\mu\text{moles/min/ml}) = \frac{V\ (\mu l) \times OD\ 405nm\ cm^{-1}}{e \times \text{incubation time (min)} \times V\ enz\ (ml)}$$

where,

V is the assay volume - 0.5 ml (500 µl)

OD 405nm (cm^{-1}) is the absorbance at 405 nm divided by the light-path length (cm).

e is the molar extinction coefficient (M-1 × cm-1).

For *p*-nitrophenol, e = $1.78 \times 10^4\ M^{-1} \times cm^{-1}$.

V enz (ml) – volume of sample containing the enzyme

NAGase activity of 14.04 µmoles/min/ml (equivalent to the OD value 0.27 at 405 nm) and above was taken as positive index for SCM (Kitchen, 1976).

3.2.5.4. Liquid Phase Blocking ELISA (LPB ELISA)

The test was carried out as per the method described by Suryanarayana (2012).

Step-1. Ninety-six well flat bottomed immunoplate was incubated with PIBB antigen (300 ng/well) in coating buffer (100 µl/well) at 4^0C overnight (Appendix).

Step-2. After incubation the contents were discarded and the plate was washed with washing buffer (PBS containing 0.05% Tween-20) three times at three minute intervals (Appendix).

Step-3. The plate was tapped thoroughly to clear off any residual washing buffer

Step-4. The leftover sites in the wells were blocked with 100µl/well of blocking buffer (PBST with 3% skimmed milk powder) and incubated at 37^0C in the shaker incubator for one hour (Appendix).

Step-5. Simultaneously milk samples (150 µl of 1:8 dilution) were incubated for 1hr at 37^0C with equal quantity of diluted hyper immune serum (at 1:50 dilution in Blocking buffer) raised against biosynthetic protein (PIBB antigen).

Step-6. After one hr incubation the plate was washed with washing buffer as described in Step 2 and the plate was tapped thoroughly.

Step-7. 100 µl of the serum - milk sample mixture was added to each well in the plate and incubated at 37^0C for 1 hr.

Step-8. The plate was washed three times with washing buffer as described in Step 2 and tapped thoroughly.

Step-9. Later the wells were charged with 100 µl/well of anti-rabbit HRP conjugate (1:5000 dilution in blocking buffer) and incubated for 1 hr at 37^0C.

Step-10. The plate was washed three times with washing buffer at three minute interval, to remove the unbound conjugate and tapped thoroughly.

Step-11. The antigen antibody reaction was monitored by the addition of 100 µl/well orthophenylene diamine dihydrochloride (OPD) (chromogen) solution containing 0.4% hydrogen peroxide (substrate solution).

Step-12. Reaction was allowed to proceed for 15 minutes for reddish brown colour development.

Step-13. Reaction was stopped with 50 µl/well of stopping solution consisting of 1M sulphuric acid.

Step-14. Colour development as obsersved by the absorbance at 492 nm was measured in an ELISA reader (Plate 6)

LPB ELISA OD value of 0.55 and less was taken as positive index for SCM (Suryanarayana, 2012).

3.2.6. Haematological and biochemical studies associated with subclinical mastitis in goats

3.2.6.1. Haematological study

Blood samples from healthy goats and goats positive for SCM were collected and subjected for haematological study.

3.2.6.1.1. Total Leucocyte Count (TLC) ($\times 10^3/\mu L$)

TLC in blood sample was enumerated using ERMA Auto Haematology Analyser as per the procedure prescribed by the manufacturer.

3.2.6.1.2. Differential Leucocyte Count (DLC) (per cent)

DLC in blood sample was enumerated using ERMA Auto Haematology Analyser as per the procedure prescribed by the manufacturer.

3.2.6.2. Biochemical study

Serum/ plasma samples from healthy goats and goats positive for SCM were subjected for biochemical study.

3.2.6.2.1. Alanine Transaminase (ALT / SGPT) (U/L)

ALT / SGPT activity in serum sample was measured using ALT kit provided by the manufacturer. Semi auto chemistry analyzer was utilized and procedure as indicated by the manufacturer was employed.

3.2.6.2.2. Aspartate Transaminase (AST / SGOT) (U/L)

AST / SGOT activity in serum sample was measured using AST kit provided by the manufacturer. Semi auto chemistry analyzer was utilized and procedure as indicated by the manufacturer was employed.

3.2.6.2.3. Gamma Glutamyl Transferase (GGT) (U/L)

GGT activity in serum sample was measured using GGT kit provided by the manufacturer. Semi auto chemistry analyzer was utilized and procedure as indicated by the manufacturer was employed.

3.2.6.2.4. Total protein (g/dL)

Total protein in plasma sample was measured using total protein kit provided by the manufacturer. Semi auto chemistry analyzer was utilized and procedure as indicated by the manufacturer was employed.

3.2.6.2.5. Albumin (g/dL)

Albumin content in plasma was measured using albumin kit provided by the manufacturer. Semi auto chemistry analyzer was utilized and procedure as indicated by the manufacturer was employed.

3.2.6.2.6. Globulin (g/dL)

Globulin content in plasma was calculated by subtracting the serum albumin value from total protein value, as per the method described by Brar *et al.* (2000)

3.2.6.2.7. Fibrinogen (mg/dL)

Fibrinogen content in plasma was determined by heat precipitation test as per the method described by Brar *et al.* (2000)

3.2.7. Therapeutic study

Forty animals which were positive for SCM based on SCC and LPB ELISA were randomly allocated into five groups namely Group I, Group II, Group III, Group IV and Group V with each group comprising of eight animals and were used for the study. The following treatment regimens were followed for different groups:

Group I: Goats in this group were maintained as control.

Group II: Goats in this group were administered with Enrofloxacin @ 5 mg/kg b.wt. i/m for 5 days.

Group III: Goats in this group were administered with Enrofloxacin @ 5 mg/kg b.wt. i/m for 5 days along with Vitamin E and Selenium combination (E CARE Se®) @ 1 ml/25 kg b.wt. i/m on zero, 3rd and 7th day.

Group IV: Goats in this group were administered with Enrofloxacin @ 5 mg/kg b.wt. i/m along with Trisodium citrate @ 30 mg/kg b.wt. orally for 5 days.

Group V: Goats in this group were administered with Enrofloxacin @ 5 mg/kg b.wt. i/m along with organic Selenium @ 0.3 mg/animal orally for 5 days.

Milk samples from all the forty goats belonging to different groups were collected on zero, 7th, 14th, 21st and 30th day post treatment and were subjected to SCC and LPB ELISA.

3.2.8. Statistical Analysis

The data generated in the study were statistically analyzed by Student's t-test and ANOVA, by using statistical software Statistical Package for Social Sciences (SPSS) version 11, to arrive at the conclusion.

RESULTS

IV. RESULTS

The present work was undertaken to detect SCM, prevalence of SCM, to study the haematological and biochemical alterations and to evaluate the efficacy of various supportive therapies along with antibacterial for treatment of SCM in goats. The results of the study are presented and depicted in Tables 1 to 19, Fig. 1 to 18 and Plates 1 to 6.

4.1. Diagnosis of subclinical mastitis in goats based on different diagnostic tests

A total of two hundred composite milk samples collected from goats in and around Bidar, Karnataka were subjected to SCC, EC, NAGase activity and LPB ELISA.

SCC of 5.0 lakh/ml and above (Contreras *et al.*, 1996), EC of 6.8 mS/cm and above (Chen *et al.*, 2008), NAGase activity of 14.04 µmoles/min/ml and above (Kitchen, 1976) and LPB ELISA OD value of 0.55 and less (Suryanarayana, 2012) were considered as cut off values to declare a sample as positive for SCM.

The mean ± SE values of the results of four screening tests viz., SCC, EC, NAGase and LPB ELISA for all the 200 milk samples are given in Table 1 and Fig. 1.

The mean ± SE values of four screening tests viz., SCC, EC, NAGase and LPB ELISA of all the 200 samples were 10.15 ± 0.55 lakh/ml, 7.22 ± 0.17 mS/cm, 16.91 ± 0.73 µmoles/min/ml and 0.54 ± 0.01 respectively. A total of 115 milk samples had SCC of more than 5 lakh/ml with a mean ± SE value of 15.28 ± 0.61 lakh/ml. A total 105 milk samples had EC value of more than 6.8 mS/cm with a mean ± SE value of 9.12 ± 0.14 mS/cm. A total of 92 milk samples had NAGase activity of more than 14.04 µmoles/min/ml with a mean ± SE value of 26.26 ± 0.81 µmoles/min/ml. A total of 70

milk samples had LPB ELISA OD value of less than 0.55 with a mean ± SE of 0.41 ± 0.01. The mean ± SE values of positive and negative milk samples of SCM in goats were found to be significantly different ($P \leq 0.01$) in all diagnostic tests.

Out of 200 samples screened, based on the number of milk samples detected positive by each individual test / a combination of two or more tests, the percentage positivity was calculated.

When diagnostic tests were evaluated individually, the per cent positivity of SCM in goats based on SCC, EC, NAGase activity and LPB ELISA tests were 57.50, 52.50, 46.00 and 35.00 per cent respectively, indicating ELISA as the most specific diagnostic test for detection of SCM. The per cent positivity was highest in SCC (57.50 %), followed by EC (52.50 %) and NAGase (46.00 %) (Table 2 and Fig. 2).

Sensitivity and specificity of SCC, EC and NAGase activity were calculated by considering LPB ELISA as standard diagnostic test to detect SCM in goats. Sensitivity and specificity of SCC were 91.43 and 60.77 per cent respectively. Sensitivity and specificity of EC were 87.14 and 66.15 per cent respectively. Sensitivity and specificity of NAGase were 71.43 and 67.69 per cent respectively (Table 3).

When different combinations of two / three diagnostic tests were evaluated, the percentage positivity of SCM for SCC and EC combination, SCC and NAGase combination, EC and NAGase combination and SCC, EC and NAGase combination was 49.00, 43.50, 39.50 and 39.50 per cent respectively. It was observed that a combination

of SCC and EC revealed highest per cent positivity followed by SCC and NAGase, EC and NAGase and SCC, EC and NAGase combinations (Table 4).

The present study indicated that LPB ELISA was the most specific diagnostic test to detect SCM in goats as compared to SCC, EC and NAGase activity.

Test of correlation between the four tests used for diagnosis of SCM in goats was carried out and it was observed that SCC had positive correlation with EC (r = 0.66) and NAGase activity (r = 0.65), EC had positive correlation with NAGase activity (r = 0.59) but only LPB ELISA had negative correlation with SCC (r = -0.86), EC (r = -0.56) and NAGase (r = -0.58). The coefficient of correlations were highly significant ($P \leq 0.01$) (Table 5).

4.2. Prevalence of SCM in goats

For the purpose of studying the prevalence of SCM, the goats under study two hundred numbers were grouped based on the parity and lactation stage.

4.2.1. Prevalence of SCM in goats based on SCC

The prevalence of SCM in goats based on SCC as diagnostic test was found to be 57.50 (115/200) per cent (Table 2 and Fig. 2).

4.2.1.1. Parity wise prevalence of SCM in goats based on SCC

The per cent prevalence of SCM in goats based on SCC as diagnostic test was 14.71 (5/34), 12.90 (4/31), 47.06 (16/34), 76.67 (23/30), 93.33 (28/30), 92.59 (25/27) and

100.00 (14/14) per cent during first, second, third, fourth, fifth, sixth and seventh parity respectively.

The mean ± SE SCC (lakh/ml) in positive milk samples were 15.30 ± 3.53, 14.44 ± 5.37, 10.92 ± 0.90, 11.92 ± 0.80, 14.21 ± 0.93, 19.18 ± 1.42 and 21.14 ± 1.58 in first, second, third, fourth, fifth, sixth and seventh parity respectively and the mean ± SE SCC of positive samples differed significantly from negative samples during first, third, fourth, fifth and sixth parity (Table 6 and Fig. 3).

The per cent prevalence of SCM in goats based on SCC as diagnostic test was found to be highest during seventh parity and least prevalence was noticed during second parity. Further, the per cent prevalence of SCM in goats based on SCC as diagnostic test was found to be increased from second to seventh parity.

4.2.1.2. Lactation stage wise prevalence of SCM in goats based on SCC

The per cent prevalence of SCM in goats based on SCC as diagnostic test was 63.75 (51/80), 64.00 (48/75), and 35.56 (16/45) per cent during early, mid and late lactation respectively.

The mean ± SE SCC (lakh/ml) in positive milk samples were 15.16 ± 0.95, 14.76 ± 0.89 and 17.19 ± 1.76 in early, mid and late lactation respectively and the mean ± SE SCC of positive samples differed significantly from negative samples (Table 7 and Fig. 4).

The per cent prevalence of SCM in goats based on SCC as diagnostic test was found to be highest during mid lactation followed by early lactation and least in late lactation.

4.2.2. Prevalence of SCM in goats based on EC

The prevalence of SCM in goats based on EC as diagnostic test was found to be 52.50 (105/200) per cent (Table 2 and Fig. 2).

4.2.2.1. Parity wise prevalence of SCM in goats based on EC

The per cent prevalence of SCM in goats based on EC as diagnostic test was 26.47 (9/34), 9.68 (3/31), 35.29 (12/34), 66.67 (20/30), 83.33 (25/30), 81.48 (22/27) and 100.00 (14/14) per cent during first, second, third, fourth, fifth, sixth and seventh parity respectively.

The mean ± SE EC (mS/cm) in positive milk samples were 8.52 ± 0.25, 9.30 ± 0.26, 8.81 ± 0.42, 9.26 ± 0.38, 8.79 ± 0.30, 9.46 ± 0.28 and 9.59 ± 0.42 during first, second, third, fourth, fifth, sixth and seventh parity respectively and the mean ± SE EC of positive samples differed significantly from negative samples during first, second, third, fourth, fifth and sixth parity (Table 8 and Fig. 5).

The per cent prevalence of SCM in goats based on EC as diagnostic test was found to be highest during seventh parity and least prevalence was noticed during second parity. Further, the per cent prevalence of SCM in goats based on EC as diagnostic test was found to be increased from second to seventh parity.

4.2.2.2. Lactation stage wise prevalence of SCM in goats based on EC

The per cent prevalence of SCM in goats based on EC as diagnostic test was 57.50 (46/80), 58.67 (44/75) and 33.33 (15/45) during early, mid and late lactation respectively.

The mean ± SE EC (mS/cm) in positive milk samples were 9.03 ± 0.23, 9.17 ± 0.21 and 9.24 ± 0.43 during early, mid and late lactation respectively and the mean ± SE EC of positive samples differed significantly from negative samples (Table 9 Fig. 6).

The per cent prevalence of SCM in goats based on EC as diagnostic test was found to be highest during mid lactation followed by early lactation and least in late lactation.

4.2.3. Prevalence of SCM in goats based on NAGase activity

The prevalence of SCM in goats based on NAGase activity as diagnostic test was found to be 46.00 (92/200) per cent (Table 2 and Fig. 2).

4.2.3.1. Parity wise prevalence of SCM in goats based on NAGase activity

The per cent prevalence of SCM in goats based on NAGase activity as diagnostic test was 8.82 (3/34), 6.45 (2/31), 26.47 (9/34), 63.33 (19/30), 76.67 (23/30), 85.19 (23/27) and 92.86 (13/14) per cent during first, second, third, fourth, fifth, sixth and seventh parity respectively.

The mean ± SE NAGase activity (µmoles/min/ml) in positive milk samples were 15.83 ± 0.30, 17.39 ± 1.34, 20.73 ± 1.95, 22.67 ± 1.48, 27.22 ± 1.49, 30.47 ± 1.67 and

29.94 ± 1.57 during first, second, third, fourth, fifth, sixth and seventh parity respectively and the mean ± SE NAGase activity of positive samples differed significantly from negative samples during first, third, fourth, fifth, sixth and seventh parity (Table 10 and Fig. 7).

The per cent prevalence of SCM in goats based on NAGase activity as diagnostic test was found to be highest during seventh parity and least prevalence was noticed during second parity. Further, the per cent prevalence of SCM in goats based on NAGase activity as diagnostic test was found to be increased from second to seventh parity.

4.2.3.2. Lactation stage wise prevalence of SCM in goats based on NAGase activity

The per cent prevalence of SCM in goats based on NAGase activity as diagnostic test was 56.25 (45/80), 46.67 (35/75) and 26.67 (12/45) per cent during early, mid and late lactation respectively.

The mean ± SE NAGase activity (µmoles/min/ml) in positive milk samples were 26.22 ± 1.23, 25.64 ± 1.19 and 28.20 ± 2.52 during early, mid and late lactation respectively and the mean ± SE NAGase activity of positive samples differed significantly from negative samples (Table 11 and Fig. 8).

The per cent prevalence of SCM in goats based on NAGase activity as diagnostic test was found to be highest during early lactation followed by mid lactation and least in late lactation.

4.2.4. Prevalence of SCM in goats based on LPB ELISA

The prevalence of SCM in goats based on LPB ELISA as diagnostic test was found to be 35.00 (70/200) per cent (Table 2 and Fig. 2).

4.2.4.1. Parity wise prevalence of SCM in goats based on LPB ELISA

The per cent prevalence of SCM in goats based on LPB ELISA as diagnostic test was 5.88 (2/34), 12.90 (4/31), 29.41 (10/34), 23.33 (7/30), 53.33 (16/30), 66.67 (18/27) and 92.86 (13/14) per cent during first, second, third, fourth, fifth, sixth and seventh parity respectively.

The mean ± SE LPB ELISA OD values in positive milk samples were 0.43 ± 0.10, 0.46 ± 0.05, 0.49 ± 0.01, 0.47 ± 0.03, 0.41 ± 0.02, 0.36 ± 0.02 and 0.37 ± 0.03 during first, second, third, fourth, fifth, sixth and seventh parity respectively and the mean ± SE LPB ELISA OD values of positive samples differed significantly from negative samples during third, fourth, fifth and sixth parity (Table 12 and Fig. 9).

The per cent prevalence of SCM in goats based on LPB ELISA as diagnostic test was found to be highest during seventh parity and least prevalence was noticed during first parity. Further, the per cent prevalence of SCM in goats based on LPB ELISA as diagnostic test was found to be increased from first to third parity and decreased in fourth parity and then again increased from fifth to seventh parity.

4.2.4.2. Lactation stage wise prevalence of SCM in goats based on LPB ELISA

The per cent prevalence of SCM in goats based on LPB ELISA as diagnostic test was 41.25 (33/80), 34.67 (26/75) and 24.44 (11/45) during early, mid and late lactation respectively.

The mean ± SE LPB ELISA OD values in positive milk samples were 0.41 ± 0.02, 0.41 ± 0.02 and 0.40 ± 0.03 during early, mid and late lactation respectively and the mean ± SE LPB ELISA OD values of positive samples differed significantly from negative samples (Table 13 and Fig. 10).

The per cent prevalence of SCM in goats based on LPB ELISA as diagnostic test was found to be highest during early lactation followed by mid lactation and least in late lactation.

4.2.5. Comparison of parity wise and lactation stage wise prevalence of SCM in goats

The parity wise and lactation stage wise prevalence of SCM in goats based on SCC, EC, NAGase activity and LPB ELISA as diagnostic tests were presented in table 14 to 15 and depicted in fig. 11 to 12.

4.3. Haematological and biochemical studies

The mean ± SE haematological and biochemical values of healthy and SCM affected goats were presented in tables 16 to 17 and fig. 13 to 16.

4.3.1. Total leucocyte count (TLC)

The mean ± SE values of TLC in healthy goats and goats affected with SCM were 8.28 ± 0.83 and 11.31 ± 0.89 ($\times 10^3/\mu L$) respectively and there was a statistically significant difference ($P \leq 0.05$) indicating that SCM significantly increases TLC in goats (Table 16 and Fig. 13).

4.3.2. Differential leucocyte count (DLC)

The mean ± SE values of granulocytes in healthy goats and goats affected with SCM were 42.25 ± 2.96 and 57.63 ± 5.45 per cent respectively and there was a statistically significant difference ($P \leq 0.05$) indicating that SCM significantly increases granulocytes in goats.

The mean ± SE values of lymphocytes in healthy goats and goats affected with SCM were 51.88 ± 3.35 and 37.25 ± 5.43 per cent respectively and there was a statistically significant difference ($P \leq 0.05$) indicating that SCM significantly decreases lymphocytes in goats.

The mean ± SE values of monocytes in healthy goats and goats affected with SCM were 5.88 ± 0.69 and 5.13 ± 0.64 per cent respectively and statistically there was no significant difference ($P \geq 0.05$) between the healthy and affected goats (Table 16 and Fig. 13).

4.3.3. Alanine transaminase (ALT)

The mean ± SE values of ALT in healthy goats and goats affected with SCM were 29.38 ± 9.26 and 33.71 ± 7.97 U/L respectively and statistically there was no significant difference ($P \geq 0.05$) between the healthy and affected goats (Table 17 and Fig. 14).

4.3.4. Aspartate transaminase (AST)

The mean ± SE values of AST in healthy goats and goats affected with SCM were 26.08 ± 7.15 and 38.88 ± 4.94 U/L respectively and statistically there was no significant difference ($P \geq 0.05$) between the healthy and affected goats (Table 17 and Fig. 14).

4.3.5. Gamma glutamyl transferase (GGT)

The mean ± SE values of GGT in healthy goats and goats affected with SCM were 37.62 ± 7.58 and 68.10 ± 11.32 U/L respectively and there was a statistically significant difference ($P \leq 0.05$) indicating that SCM significantly increases GGT in goats (Table 17 and Fig. 14).

4.3.6. Total protein

The mean ± SE values of plasma total protein in healthy goats and goats affected with SCM were 6.67 ± 0.31 and 7.14 ± 0.22 g/dL respectively and statistically there was no significant difference ($P \geq 0.05$) between the healthy and affected goats (Table 17 and Fig. 15).

4.3.7. Albumin

The mean ± SE values of albumin in healthy goats and goats affected with SCM were 3.21 ± 0.21 and 3.19 ± 0.15 g/dL respectively and statistically there was no significant difference ($P \geq 0.05$) between the healthy and affected goats (Table 17 and Fig. 15).

4.3.8. Globulin

The mean ± SE values of globulin in healthy goats and goats affected with SCM were 3.46 ± 0.24 and 3.95 ± 0.16 g/dL respectively and statistically there was no significant difference ($P \geq 0.05$) between the healthy and affected goats (Table 17 and Fig. 15).

4.3.9. Fibrinogen

The mean ± SE values of fibrinogen in healthy goats and goats affected with SCM were 242.50 ± 31.85 and 518.75 ± 61.40 mg/dL respectively and there was a statistically significant difference ($P \leq 0.01$) indicating that SCM significantly increases fibrinogen in goats (Table 17 and Fig. 16).

4.4. Therapeutic study

Forty goats which were positive for SCM based on SCC and LPB ELISA were randomly allocated into five groups namely Group I (Control), Group II (Enrofloxacin), Group III (Enrofloxacin + Vitamin E and Selenium combination), Group IV (Enrofloxacin + Trisodium citrate) and Group V (Enrofloxacin + organic Selenium) with

each group comprising of eight animals. Results of the therapeutic study are tabulated in Tables 18 to 19 and Fig. 17 to 18.

4.4.1. Somatic cell count

The mean ± SE of SCC of each group on different days of post treatment is given in Table 18 and Fig. 17.

Group I (control group): The mean ± SE of SCC (lakh/ml) on zero, 7^{th}, 14^{th}, 21^{st} and 30^{th} day post treatment were 14.31 ± 2.25, 13.78 ± 2.23, 14.22 ± 2.59, 13.16 ± 2.10 and 15.09 ± 2.6 respectively and no statistical significant was noticed and the mean ± SE values of SCC were found to be above cut off value of 5 lakh/ml indicating that goats in this group were positive for SCM throughout the experimental period.

Group II (Enrofloxacin alone): The mean ± SE of SCC (lakh/ml) on zero, 7^{th}, 14^{th}, 21^{st} and 30^{th} day post treatment were 16.69 ± 2.34, 4.88 ± 0.72, 6.00 ± 0.65, 6.94 ± 1.38 and 7.84 ± 2.04 respectively and significant difference was noticed from the base value viz., zero day. When SCC of more than 5 lakh/ml was considered positive for SCM in goats, SCC less than 5 lakh/ml was observed only on 7^{th} day post treatment and on 14^{th}, 21^{st} and 30^{th} day post treatment mean ± SE of SCC more than 5 lakh/ml with an increasing trend till end of experiment.

Group III (Enrofloxacin + Vitamin E and Selenium combination): The mean ± SE of SCC (lakh/ml) on zero, 7^{th}, 14^{th}, 21^{st} and 30^{th} day post treatment were15.44 ± 2.63, 4.09 ± 0.46, 3.25 ± 0.62, 3.69 ± 0.42 and 2.97 ± 0.58 respectively and significant difference was noticed from the base value viz., zero day. When SCC of more than 5

lakh/ml was considered positive for SCM in goats, SCC less than 5 lakh/ml was observed from 7th day post treatment till the end of the experimental period.

Group IV (Enrofloxacin + Trisodium citrate): The mean ± SE of SCC (lakh/ml) on zero, 7th, 14th, 21st and 30th day post treatment were 18.59 ± 2.12, 5.31 ± 0.67, 6.13 ± 0.61, 4.94 ± 0.40 and 5.47 ± 0.39 respectively and significant difference was noticed from the base value viz., zero day. When SCC of more than 5 lakh/ml was considered positive for SCM in goats, SCC less than 5 lakh/ml was observed only on 21st day post treatment, then it increased with the subsequent collection.

Group V (Enrofloxacin + organic Selenium): The mean ± SE of SCC (lakh/ml) on zero, 7th, 14th, 21st and 30th day post treatment were 13.81 ± 1.85, 4.56 ± 0.37, 4.41 ± 0.37, 4.09 ± 0.43 and 4.03 ± 0.43 respectively and significant difference was noticed from the base value viz., zero day. When SCC of more than 5 lakh/ml was considered positive for SCM in goats, SCC less than 5 lakh/ml was observed from 7th day post treatment till the end of the experimental period.

On comparison of mean ± SE of SCC between the groups, there was no statistically significant difference on Zero day ($P \geq 0.05$). However, the mean ± SE of SCC of Groups II, III, IV and V differed significantly ($P \leq 0.05$) from Group I (control) on 7th, 14th, 21st and 30th day post treatment and were statistically lower in Groups II, III, IV and V. At the same time there was no statistically significant difference ($P \geq 0.05$) between Groups II, III, IV and V during this period.

When SCC of more than 5 lakh/ml was considered positive for SCM in goats, it was observed that Enrofloxacin alone (Group II) administered resulted in SCC less than 5 lakh/ml only on 7^{th} day post treatment and on 14^{th}, 21^{st} and 30^{th} day post treatment mean ± SE SCC more than 5 lakh/ml with an increasing trend till end of study. When Enrofloxacin + Vitamin E and Selenium combination (Group III) was administered, SCC was lower than 5 lakh/ml on 7^{th}, 14^{th}, 21^{st} and 30^{th} day post treatment. When Enrofloxacin + Trisodium citrate (Group IV) was administered, it resulted in SCC less than 5 lakh/ml only on 21^{st} day post treatment. Enrofloxacin + organic Selenium (Group V) treatment resulted in SCC lower than 5 lakh/ml on 7^{th}, 14^{th}, 21^{st} and 30^{th} day post treatment.

This indicated that Enrofloxacin + Vitamin E and Selenium combination and Enrofloxacin + organic Selenium resulted in lowered SCC throughout the study period and proved to be the best treatment regimen in this study. Vitamin E and Selenium combination and organic Selenium administration as an additional therapeutic agent resulted in a prolonged reduction of SCC as compared to antibacterial therapy alone. Therapeutic regimen with Enrofloxacin + Trisodium citrate and Enrofloxacin alone reduced the mean ± SE of SCC to less than 5 lakh/ml only on 21^{st} and 7^{th} day post treatment respectively.

4.4.2. LPB ELISA

The mean ± SE of LPB ELISA OD values of each group on different days of post treatment are given in Table 19 and Fig. 18.

Group I (control group): The mean ± SE of LPB ELISA OD values on zero, 7th, 14th, 21st and 30th day post treatment were 0.48 ± 0.02, 0.51 ± 0.01, 0.46 ± 0.04, 0.43 ± 0.04 and 0.45 ± 0.04 respectively and no statistical significant difference was noticed and the mean ± SE of LPB ELISA OD values were found to be below cut off value of 0.55 indicating that goats in this group were positive for SCM throughout the study period.

Group II (Enrofloxacin alone): The mean ± SE of LPB ELISA OD values on zero, 7th, 14th, 21st and 30th day post treatment were 0.50 ± 0.01, 0.59 ± 0.01, 0.61 ± 0.02, 0.57 ± 0.02 and 0.54 ± 0.03 respectively and significant difference was noticed from the base value viz., zero day. When LPB ELISA OD value of less than 0.55 was considered positive for SCM in goats, it was observed that LPB ELISA OD values were more than 0.55 on 7th, 14th and 21st day post treatment and later decreased on 30th day post treatment.

Group III (Enrofloxacin + Vitamin E and Selenium combination): The The mean ± SE of LPB ELISA OD values on zero, 7th, 14th, 21st and 30th day post treatment were 0.43 ± 0.04, 0.60 ± 0.01, 0.62 ± 0.01, 0.59 ± 0.01 and 0.61 ± 0.01 respectively and significant difference was noticed from the base value viz., zero day. When LPB ELISA OD value of less than 0.55 was considered positive for SCM in goats, it was observed that LPB ELISA OD values were more than 0.55 from 7th day post treatment till the end of the study period.

Group IV (Enrofloxacin + Trisodium citrate): The mean ± SE of LPB ELISA OD values on zero, 7th, 14th, 21st and 30th day post treatment were 0.46 ± 0.04, 0.60 ± 0.01, 0.59 ± 0.01, 0.62 ± 0.01 and 0.58 ± 0.02 respectively and significant difference was

noticed from the base value viz., zero day. When LPB ELISA OD values of less than 0.55 was considered positive for SCM in goats, it was observed that LPB ELISA OD values were more than 0.55 from 7^{th} day post treatment till the end of the study period.

Group V (Enrofloxacin + organic Selenium): The mean ± SE of LPB ELISA OD values on zero, 7^{th}, 14^{th}, 21^{st} and 30^{th} day post treatment were 0.42 ± 0.04, 0.63 ± 0.01, 0.62 ± 0.02, 0.64 ± 0.01 and 0.66 ± 0.01 respectively and significant difference was noticed from the base value viz., zero day. When LPB ELISA OD value of less than 0.55 was considered positive for SCM in goats, it was observed that LPB ELISA OD values were more than 0.55 from 7^{th} day post treatment till the end of the study period.

The mean ± SE of LPB ELISA OD values on zero day did not exhibit any statistically significant difference ($P \geq 0.05$) when different groups were compared. However, the mean ± SE of LPB ELISA OD values of Groups II, III, IV and V on 7^{th}, 14^{th}, 21^{st} and 30^{th} day post treatment were significantly higher ($P \leq 0.05$) as compared to Group I (control). Further the mean ± SE OD values in Groups II, III, IV, and V did not exhibit statistically significant difference ($P \geq 0.05$) on 7^{th}, 14^{th} and 21^{st} day post treatment, but there was significant difference ($P \leq 0.05$) between Group II and V only on 30^{th} day post treatment.

When LPB ELISA OD value of less than 0.55 is considered as positive for SCM in goats, it was observed that Enrofloxacin alone (Group II) administered resulted in LPB ELISA OD value more than 0.55 on 7^{th}, 14^{th} and 21^{st} day post treatment. Further, all other treatment groups viz., Enrofloxacin + Vitamin E and Selenium combination (Group III), Enrofloxacin + Trisodium citrate (Group IV) and Enrofloxacin + organic Selenium

(Group V), resulted in LPB ELISA OD value more than 0.55 on 7^{th}, 14^{th}, 21^{st} and 30^{th} day post treatment.

This indicated that therapeutic effect of Enrofloxacin + organic Selenium was highest followed by Enrofloxacin + Vitamin E and Selenium combination, Enrofloxacin + Trisodium citrate and Enrofloxacin alone, in descending order. Organic Selenium, Vitamin E and Selenium combination and Trisodium citrate administration as an additional therapeutic agent were more beneficial as compared to antibacterial therapy alone.

When only Enrofloxacin was used as therapeutic agent, the mean ± SE of LPB ELISA OD value was found to be more than 0.55 only on 7^{th}, 14^{th} and 21^{st} day post treatment, thus indicating the duration of Enrofloxacin therapeutic effect reduced by 30^{th} day post treatment. However, when Vitamin E and Selenium combination, Trisodium citrate and organic Selenium was supplemented along with Enrofloxacin, mean ± SE of LPB ELISA OD values remained above cutoff value of 0.55 indicating beneficial effect of Vitamin E and Selenium combination, Trisodium citrate and organic Selenium in treatment of SCM in goats.

Table 1. Mean ± SE of SCC, EC, NAGase activity and LPB ELISA OD values of milk samples

Screening Test	Overall	SCM Positive	SCM Negative
SCC (lakh/ml)	10.15 ± 0.55	15.28 ± 0.61 [a]	3.21 ± 0.10 [b]
EC (mS/cm)	7.22 ± 0.17	9.12 ± 0.14 [a]	5.12 ± 0.09 [b]
NAGase (µmoles/min/ml)	16.91 ± 0.73	26.26 ± 0.81 [a]	8.95 ± 0.26 [b]
LPB ELISA (OD values)	0.54 ± 0.01	0.41 ± 0.01 [a]	0.60 ± 0.01 [b]

Means bearing different superscript in a row differ significantly ($P \leq 0.05$)

Table 2. Per cent positivity of subclinical mastitis in goats detected by different diagnostic tests

Screening test (n=200)	No. of animals positive	No. of animals negative	Per cent positivity
SCC	115	85	57.50
EC	105	95	52.50
NAGase	92	108	46.00
LPB ELISA	70	130	35.00

Table 3. Sensitivity and specificity of different diagnostic tests as compared with LPB ELISA for subclinical mastitis in goats

Sl. No.	Diagnostic test	Sensitivity (%)	Specificity (%)
1	Somatic Cell Count	91.43	60.77
2	Electrical Conductivity Test	87.14	66.15
3	NAGase activity	71.43	67.69

Table 4. Per cent positivity of subclinical mastitis in goats as detected by combination of two/three diagnostic tests

Screening test	No. of animals positive	No. of animals negative	Per cent positivity
SCC & EC	98	102	49.00
SCC & NAGase	87	113	43.50
EC & NAGase	79	121	39.50
SCC, EC & NAGase	79	121	39.50

Table 5. Coefficient of correlation (r) between different diagnostic tests used for diagnosis of subclinical mastitis in goats

Screening test	SCC	EC	NAGase	LPB ELISA
SCC	-	0.66**	0.65**	-0.86**
EC	-	-	0.59**	-0.56**
NAGase	-	-	-	-0.58**

** Significant at $P \leq 0.01$ level

Table 6. Parity wise prevalence of subclinical mastitis in goats based on somatic cell count (lakh/ml)

Parity	Total no. of goats	No. of goats positive	No. of goats negative	% Prevalence
I	34	5 (15.30 ± 3.56)*	29 (2.80 ± 0.16)	14.71
II	31	4 (14.44 ± 5.37)	27 (3.26 ±0.16)	12.90
III	34	16 (10.92 ± 0.90)**	18 (3.40 ± 0.19)	47.06
IV	30	23 (11.92 ± 0.80)**	7 (3.86 ± 0.35)	76.67
V	30	28 (14.21 ± 0.93)**	2 (3.50 ± 0.25)	93.33
VI	27	25 (19.18 ± 1.42)**	2 (4.38 ± 0.38)	92.59
VII	14	14 (21.14 ± 1.58)	-	100.00

Figures in the parenthesis indicate mean ± SE of SCC

* Means in a row differ significantly ($P \leq 0.05$)

** Means in a row differ significantly ($P \leq 0.01$)

Table 7. Lactation stage wise prevalence of subclinical mastitis in goats based on somatic cell count (lakh/ml)

Lactation stage	Total no. of goats	No. of goats positive	No. of goats negative	% Prevalence
Early	80	51 (15.16 ± 0.95**	29 (3.24 ± 0.18)	63.75
Mid	75	48 (14.76 ± 0.89)**	27 (3.36 ± 0.17)	64.00
Late	45	16 (17.19 ± 1.76)**	29 (3.05 ± 0.15)	35.56

Figures in the parenthesis indicate mean ± SE of SCC

** Means in a row differ significantly ($P \leq 0.01$)

Table 8. Parity wise prevalence of subclinical mastitis in goats based on electrical conductivity (mS/cm)

Parity	Total no. of goats	No. of goats positive	No. of goats negative	% Prevalence
I	34	9 (8.52 ± 0.25)**	25 (5.06 ± 0.20)	26.47
II	31	3 (9.30 ± 0.26)**	28 (5.19 ± 0.16)	9.68
III	34	12 (8.81 ± 0.42)**	22 (5.24 ± 0.18)	35.29
IV	30	20 (9.26 ± 0.38)**	10 (4.88 ± 0.26)	66.67
V	30	25 (8.79 ± 0.30)**	5 (5.34 ± 0.45)	83.33
VI	27	22 (9.46 ± 0.28)**	5 (4.82 ± 0.24)	81.48
VII	14	14 (9.59 ± 0.42)	-	100.00

Figures in the parenthesis indicate mean ± SE of EC

** Means in a row differ significantly ($P \leq 0.01$)

Table 9. Lactation stage wise prevalence of subclinical mastitis in goats based on electrical conductivity (mS/cm)

Lactation stage	Total no. of goats	No. of goats positive	No. of goats negative	% Prevalence
Early	80	46 (9.03 ± 0.23)**	34 (5.07 ± 0.15)	57.50
Mid	75	44 (9.17 ± 0.21)**	31 (5.27 ± 0.16)	58.67
Late	45	15 (9.24 ± 0.43)**	30 (5.02 ± 0.15)	33.33

Figures in the parenthesis indicate mean ± SE of EC

** Means in a row differ significantly ($P \leq 0.01$)

Table 10. Parity wise prevalence of subclinical mastitis in goats based on NAGase activity (µmoles/min/ml)

Parity	Total no. of goats	No. of goats positive	No. of goats negative	% Prevalence
I	34	3 (15.83 ± 0.30)**	31 (8.51 ± 0.43)	8.82
II	31	2 (17.39 ± 1.34)	29 (7.70 ± 0.51)	6.45
III	34	9 (20.73 ± 1.95)**	25 (9.52 ± 0.61)	26.47
IV	30	19 (22.67 ± 1.48)**	11 (10.34 ± 0.54)	63.33
V	30	23 (27.22 ± 1.49)**	7 (10.51 ± 0.35)	76.67
VI	27	23 (30.47 ± 1.67)**	4 (10.37 ± 1.27)	85.19
VII	14	13 (29.94 ± 1.57)*	1(12.04)	92.86

Figures in the parenthesis indicate mean ± SE of NAGase activity

* Means in a row differ significantly ($P \leq 0.05$)

** Means in a row differ significantly ($P \leq 0.01$)

Table 11. Lactation stage wise prevalence of subclinical mastitis in goats based on NAGase activity (µmoles/min/ml)

Lactation stage	Total no. of goats	No. of goats positive	No. of goats negative	% Prevalence
Early	80	45 (26.22 ± 1.23)*	35 (9.17 ± 0.48)	56.25
Mid	75	35 (25.64 ± 1.19)*	40 (8.90 ± 0.40)	46.67
Late	45	12 (28.20 ± 2.52)*	33 (8.76 ± 0.49)	26.67

Figures in the parenthesis indicate mean ± SE of NAGase activity

* Means in a row differ significantly ($P \leq 0.05$)

Table 12. Parity wise prevalence of subclinical mastitis in goats based on LPB ELISA OD values

Parity	Total no. of goats	No. of goats positive	No. of goats negative	% Prevalence
I	34	2 (0.43 ± 0.10)	32 (0.62 ± 0.01)	5.88
II	31	4 (0.46 ± 0.05)	27 (0.62 ± 0.01)	12.90
III	34	10 (0.49 ± 0.01)**	24 (0.60 ± 0.01)	29.41
IV	30	7 (0.47 ± 0.03)**	23 (0.59 ± 0.01)	23.33
V	30	16 (0.41 ± 0.02)**	14 (0.58 ± 0.01)	53.33
VI	27	18 (0.36 ± 0.02)**	9 (0.60 ± 0.01)	66.67
VII	14	13 (0.37 ± 0.03)	1 (0.56)	92.86

Figures in the parenthesis indicate mean ± SE of LPB ELISA OD

**Means in a row differ significantly ($P \leq 0.01$)

Table 13. Lactation stage wise prevalence of subclinical mastitis in goats based on LPB ELISA OD values

Lactation stage	Total no. of goats	No. of goats positive	No. of goats negative	% Prevalence
Early	80	33 (0.41 ± 0.02)**	47 (0.60 ± 0.01)	41.25
Mid	75	26 (0.41 ± 0.02)**	49 (0.60 ± 0.01)	34.67
Late	45	11 (0.40 ± 0.03)**	34 (0.61 ± 0.01)	24.44

Figures in the parenthesis indicate mean ± SE of LPB ELISA OD

**Means in a row differ significantly ($P \leq 0.01$)

Table 14. Parity wise prevalence of subclinical mastitis in goats as detected by different diagnostic tests

Parity	% Prevalence			
	SCC	EC	NAGase activity	LPB ELISA
I	14.71	26.47	8.82	5.88
II	12.90	9.68	6.45	12.90
III	47.06	35.29	26.47	29.41
IV	76.67	66.67	63.33	23.33
V	93.33	83.33	76.67	53.33
VI	92.59	81.48	85.19	66.67
VII	100.00	100.00	92.86	92.86

Table 15. Lactation wise prevalence of subclinical mastitis in goats as detected by different diagnostic tests

Lactation stage	% Prevalence			
	SCC	EC	NAGase activity	LPB ELISA
Early	63.75	57.50	56.25	41.25
Mid	64.00	58.67	46.67	34.67
Late	35.56	33.33	26.67	24.44

Table 16. Mean ± SE of haematological parameters in healthy and subclinical mastitis affected goats

Parameters	Healthy goats	Affected goats
TLC ($x10^3/\mu L$)	8.28 ± 0.63	11.31 ± 0.89*
Granulocyte (%)	42.25 ± 2.96	57.63 ± 5.45*
Lymphocyte (%)	51.88 ± 3.35	37.25 ± 5.43*
Monocyte (%)	5.88 ± 0.69	5.13 ± 0.64

*Means in a row differ significantly ($P \leq 0.05$)

Table 17. Mean ± SE of biochemical parameters in healthy and subclinical mastitis affected goats

Parameters	Healthy goats	Affected goats
ALT (U/L)	29.38 ± 9.26	33.71 ± 7.97
AST (U/L)	26.08 ± 7.15	38.88 ± 4.94
GGT (U/L)	37.62 ± 7.58	68.10 ± 11.32*
Total protein (g/dL)	6.67 ± 0.31	7.14 ± 0.22
Albumin (g/dL)	3.21 ± 0.21	3.19 ± 0.15
Globulin (g/dL)	3.46 ± 0.24	3.95 ± 0.16
Fibrinogen (mg/dL)	242.50 ± 31.95	518.75 ± 61.40**

*Means in a row differ significantly ($P \leq 0.05$)

**Means in a row differ significantly ($P \leq 0.01$)

Table 18. Mean ± SE of SCC (lakh/ml) in different treatment groups of subclinical mastitis affected goats

Post treatment days	Group I (Control)	Group II (E)	Group III (E+VES)	Group IV (E+TC)	Group V (E+OS)
Zero	14.31 ± 2.25 ^{ab}	16.69 ± 2.34 ^{ab}	15.44 ± 2.63 ^{ab}	18.59 ± 2.12 ^{a}	13.81 ± 1.85 ^{ab}
7th	13.78 ± 2.23 ^{ab}	**4.88 ± 0.72 ^{c}**	**4.09 ± 0.46 ^{c}**	5.31 ± 0.67 ^{c}	**4.56 ± 0.37 ^{c}**
14th	14.22 ± 2.59 ^{ab}	6.00 ± 0.65 ^{c}	**3.25 ± 0.62 ^{c}**	6.13 ± 0.61 ^{c}	**4.41 ± 0.37 ^{c}**
21st	13.16 ± 2.10 ^{b}	6.94 ± 1.38 ^{c}	**3.69 ± 0.42 ^{c}**	**4.94 ± 0.40 ^{c}**	**4.09 ± 0.43 ^{c}**
30th	15.09 ± 2.6 ^{ab}	7.84 ± 2.04 ^{c}	**2.97 ± 0.58 ^{c}**	5.47 ± 0.39 ^{c}	**4.03 ± 0.43 ^{c}**

Means bearing different superscript differ significantly ($P \leq 0.05$)

Table 19. Mean ± SE of LPB ELISA OD values in different treatment groups of subclinical mastitis affected goats

Post treatment days	Group I (Control)	Group II (E)	Group III (E+VES)	Group IV (E+TC)	Group V (E+OS)
Zero	0.48 ± 0.02 ^{abc}	0.50 ± 0.01 ^{bc}	0.43 ± 0.04 ^{ab}	0.46 ± 0.04 ^{ab}	0.42 ± 0.04 ^{ab}
7th	0.51 ± 0.01 ^{bcd}	**0.59 ± 0.01 ^{efg}**	**0.60 ± 0.01 ^{efg}**	**0.60 ± 0.01 ^{efg}**	**0.63 ± 0.01 ^{efg}**
14th	0.46 ± 0.04 ^{ab}	**0.61 ± 0.02 ^{efg}**	**0.62 ± 0.01 ^{efg}**	**0.59 ± 0.01 ^{efg}**	**0.62 ± 0.02 ^{efg}**
21st	0.43 ± 0.04 ^{ab}	**0.57 ± 0.02 ^{def}**	**0.59 ± 0.01 ^{efg}**	**0.62 ± 0.01 ^{efg}**	**0.64 ± 0.01 ^{fg}**
30th	0.45 ± 0.04 ^{ab}	0.54 ± 0.03 ^{cde}	**0.61 ± 0.01 ^{efg}**	**0.58 ± 0.02 ^{efg}**	**0.66 ± 0.01 ^{g}**

Means bearing different superscript differ significantly ($P \leq 0.05$)

Fig. 1. Mean SCC, EC, NAGase activity and LPB ELISA OD values of positive and negative milk samples of SCM in goats

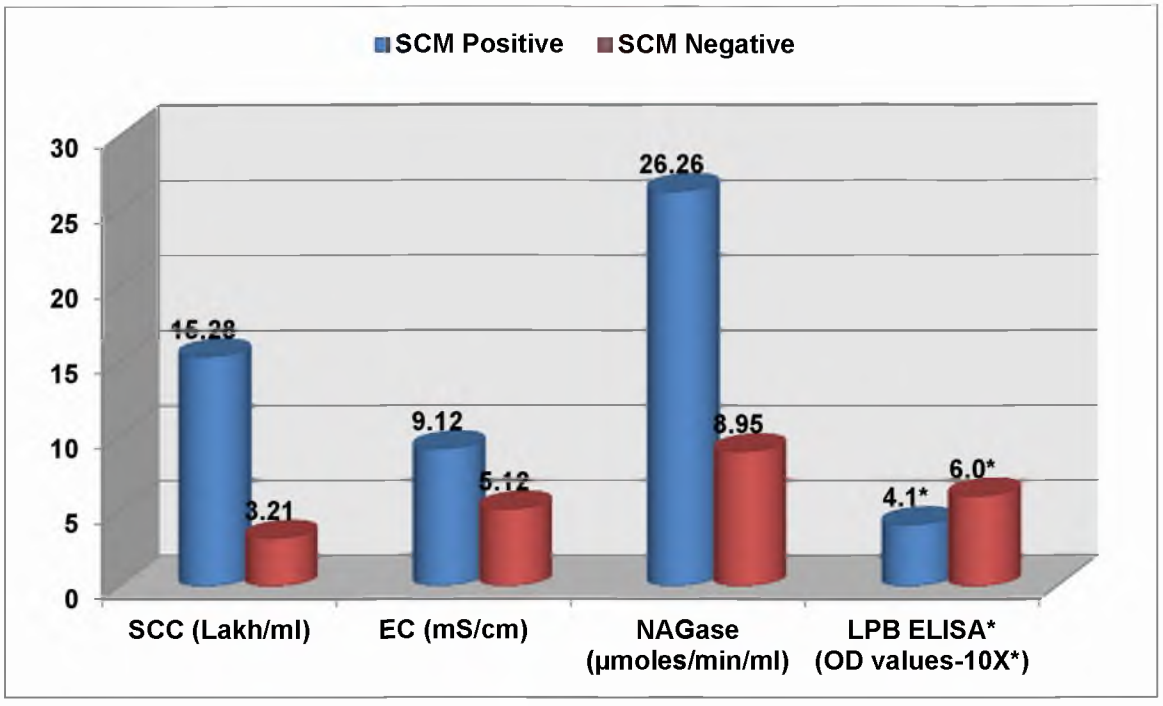

Fig. 2. Per cent positivity of subclinical mastitis in goats as detected by different diagnostic tests

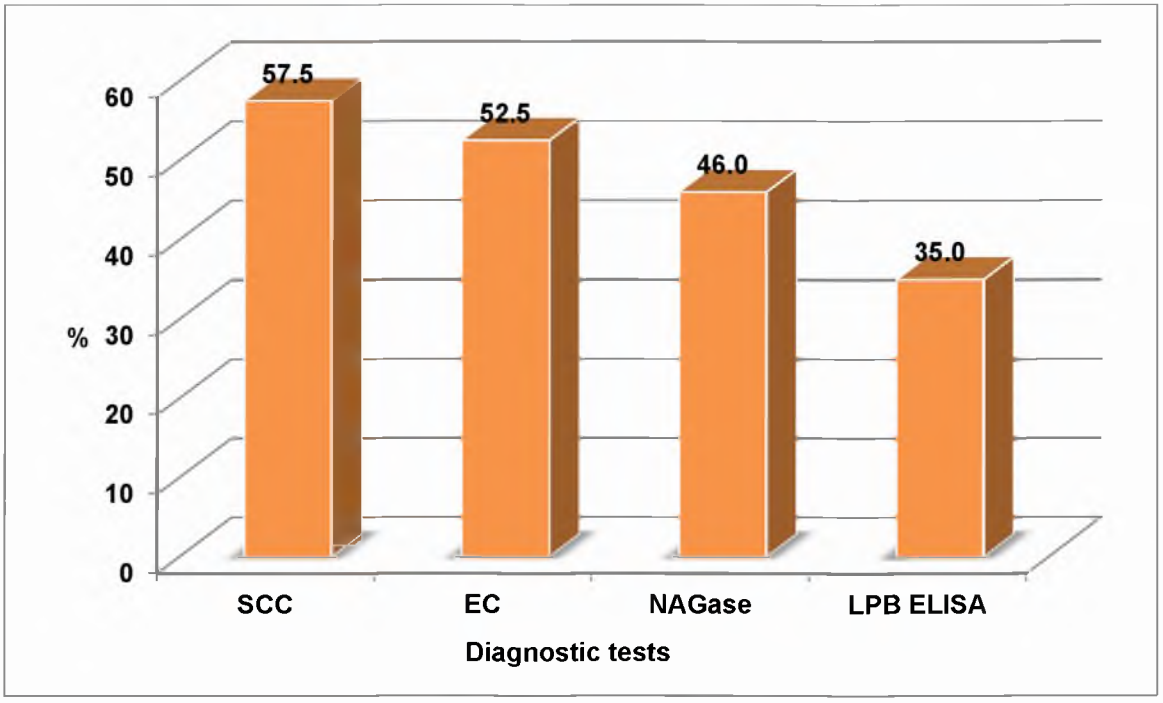

Fig. 3. Parity wise prevalence of subclinical mastitis in goats based on SCC

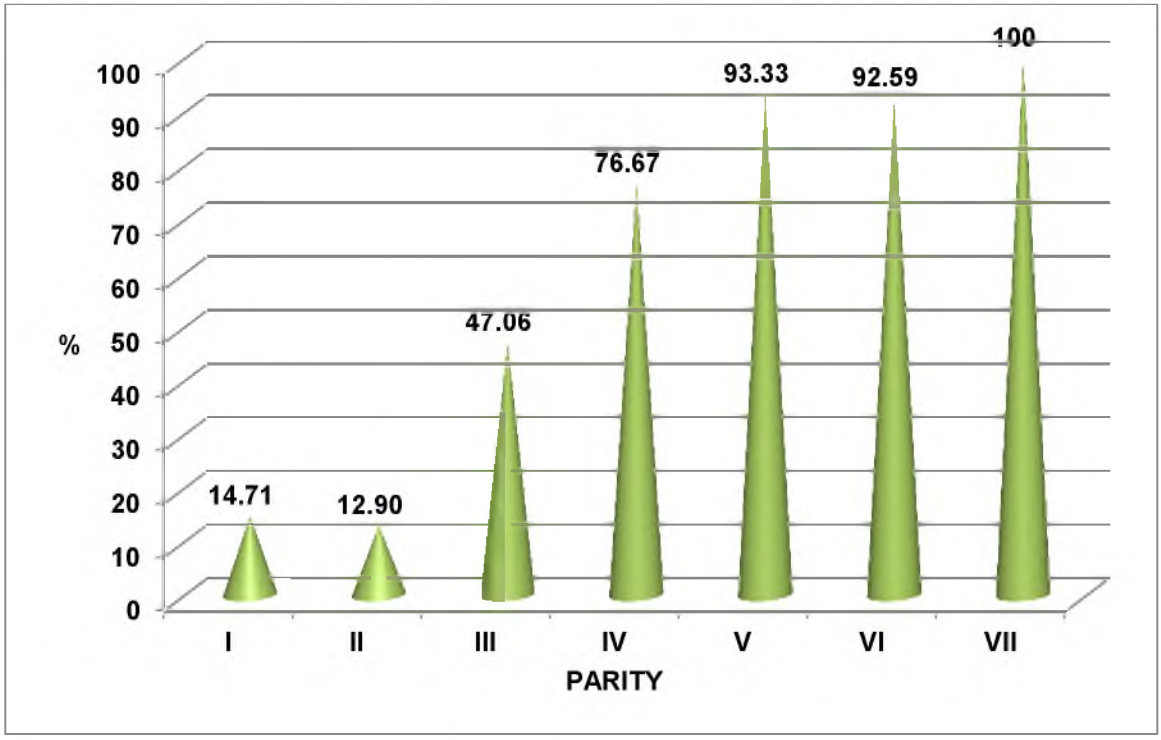

Fig. 4. Lactation stage wise prevalence of subclinical mastitis in goats based on SCC

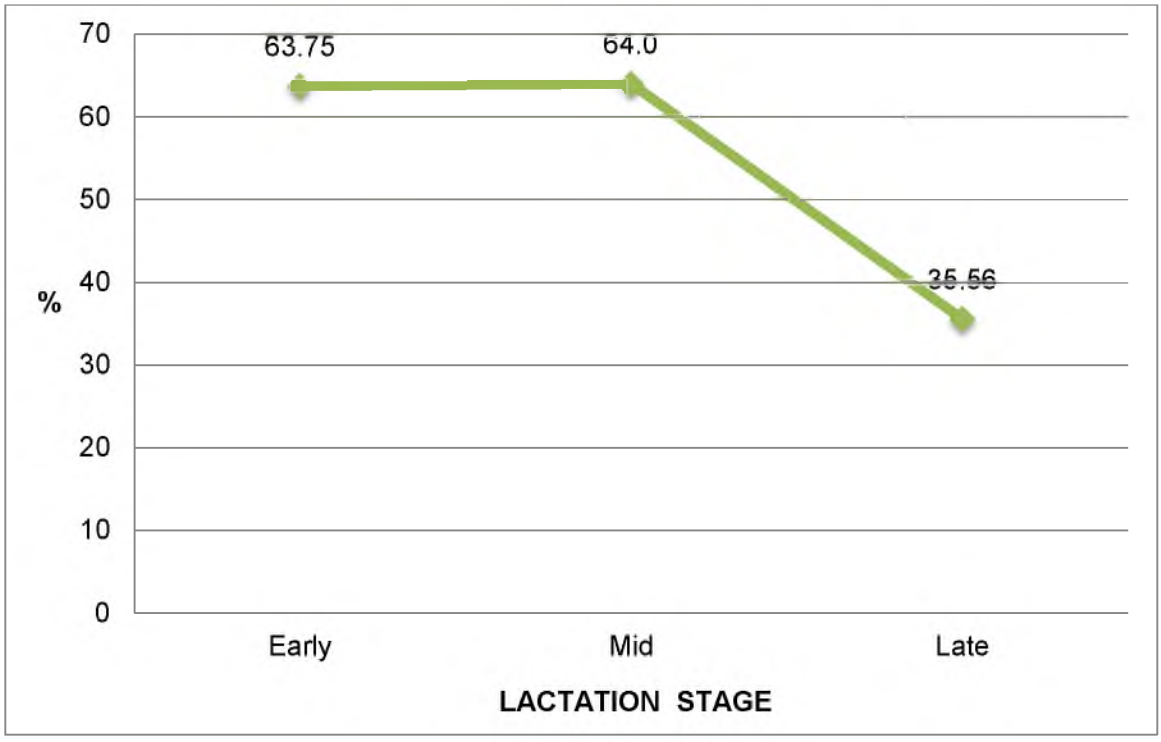

Fig. 5. Parity wise prevalence of subclinical mastitis in goats based on EC

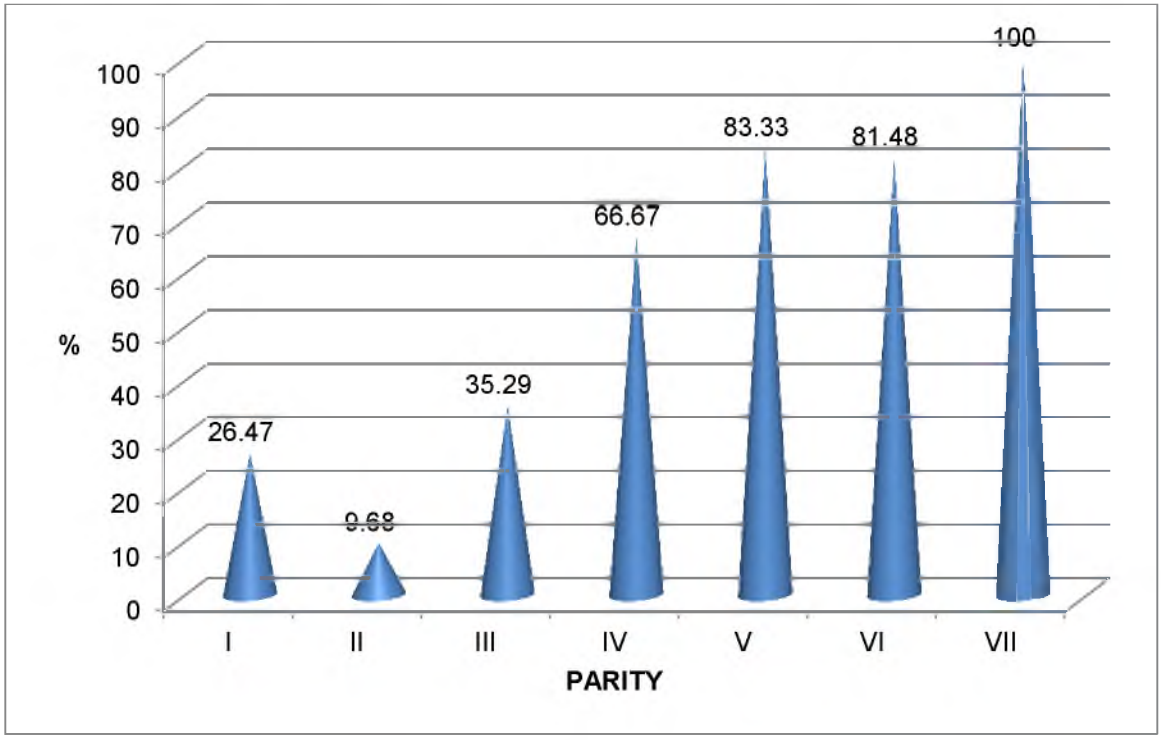

Fig. 6. Lactation stage wise prevalence of subclinical mastitis in goats based on EC

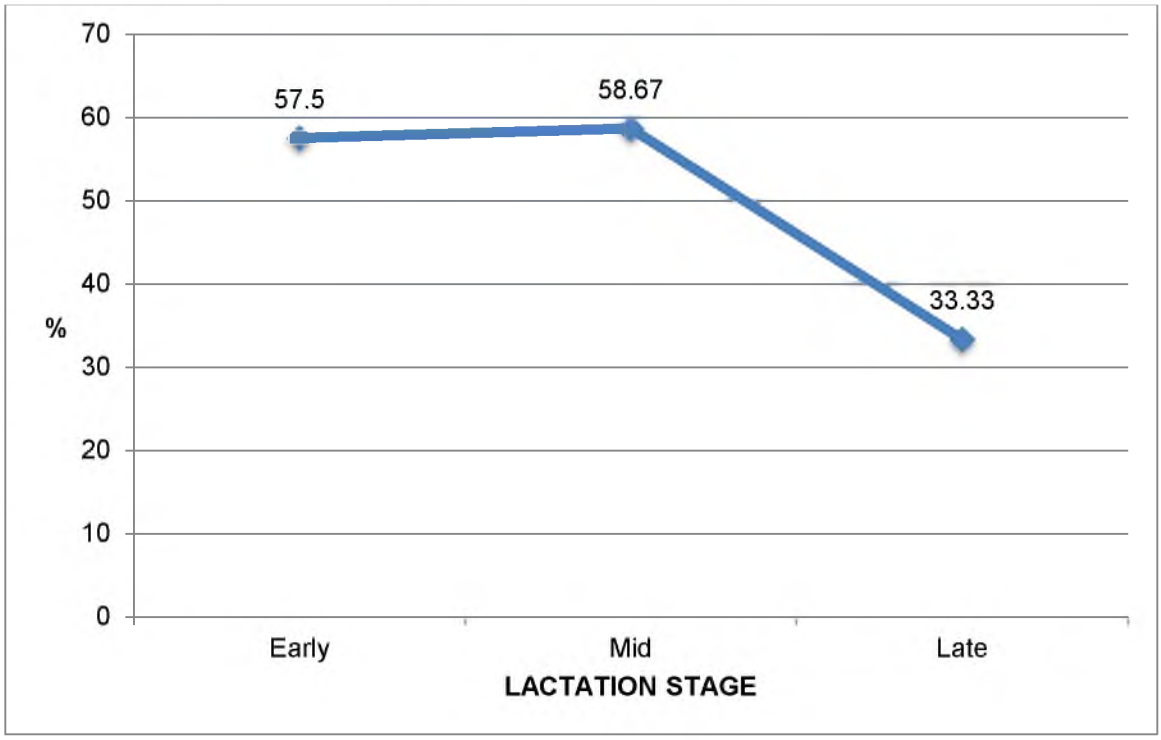

Fig. 7. Parity wise prevalence of subclinical mastitis in goats based on NAGase activity

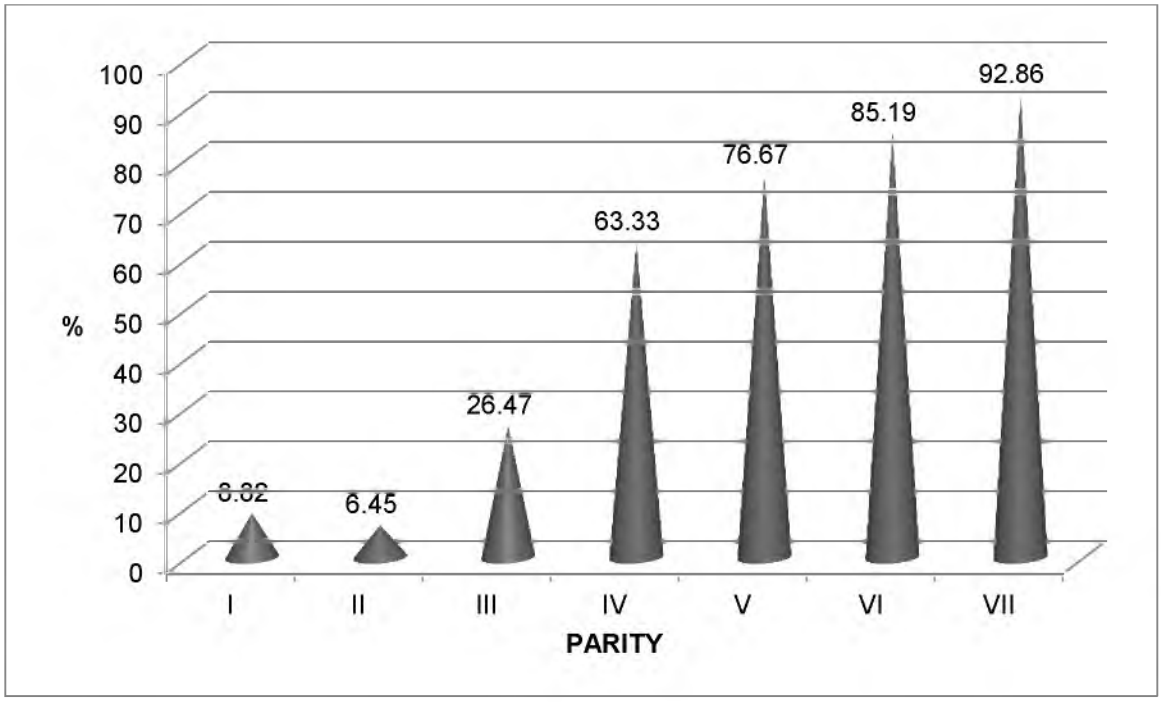

Fig. 8. Lactation stage wise prevalence of subclinical mastitis in goats based on NAGase activity

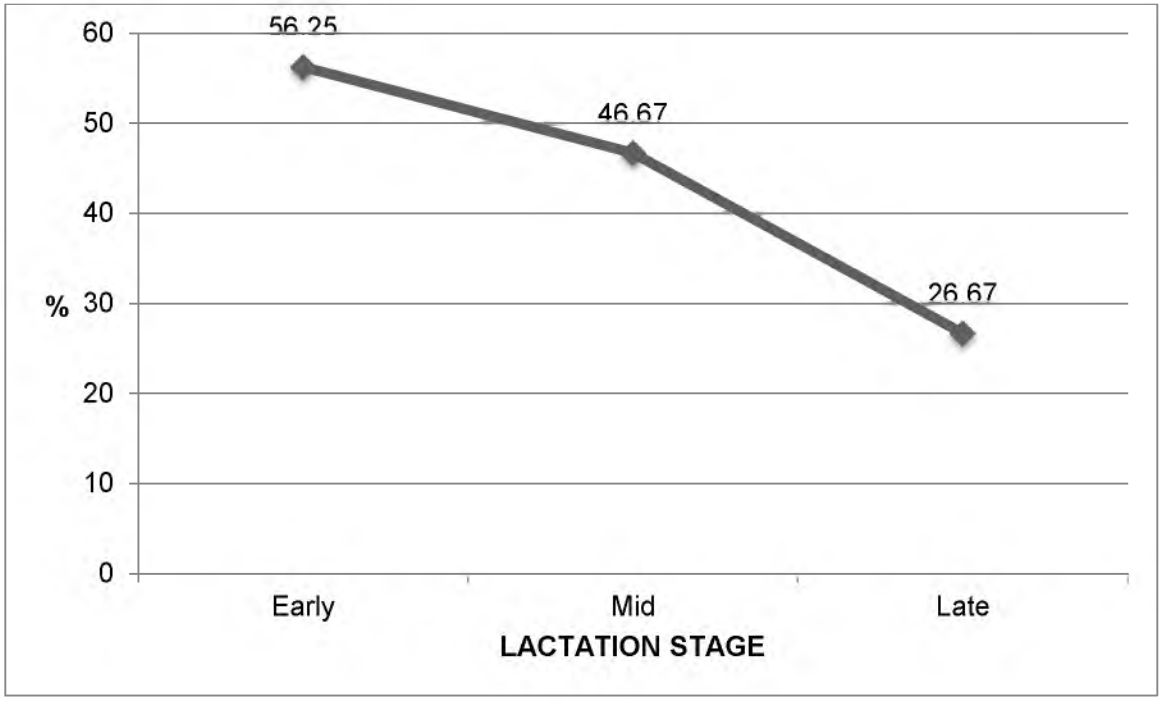

Fig. 9. Parity wise prevalence of subclinical mastitis in goats based on LPB ELISA

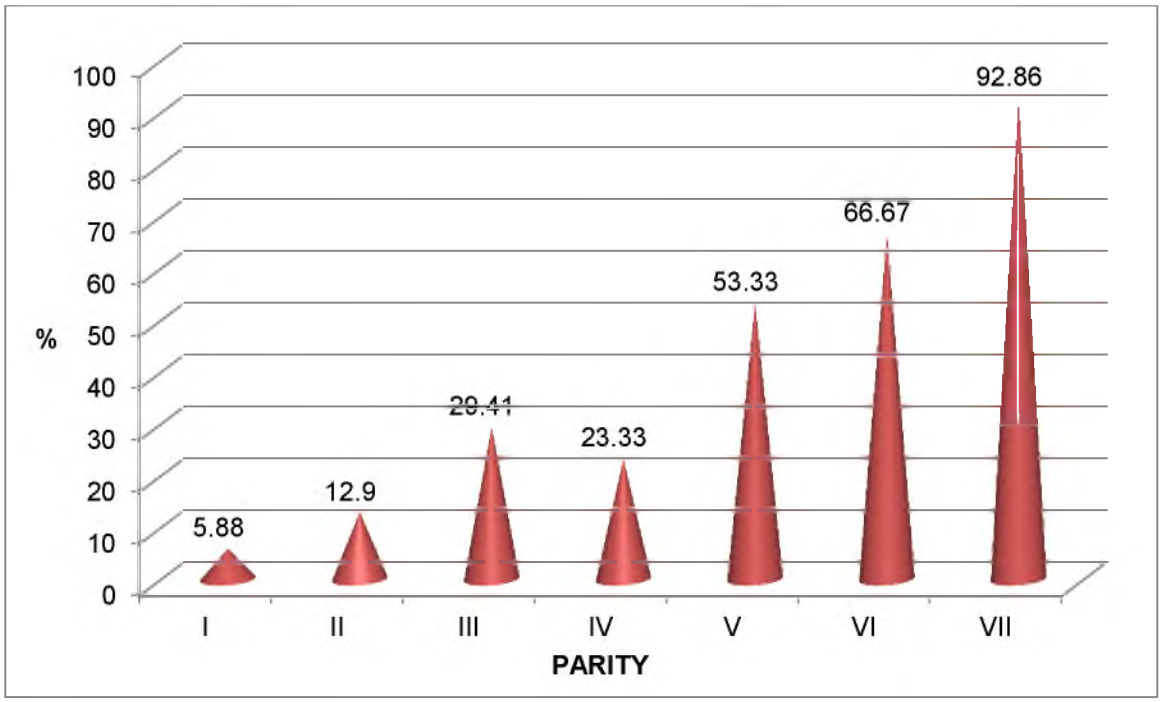

Fig. 10. Lactation stage wise prevalence of subclinical mastitis in goats based on LPB ELISA

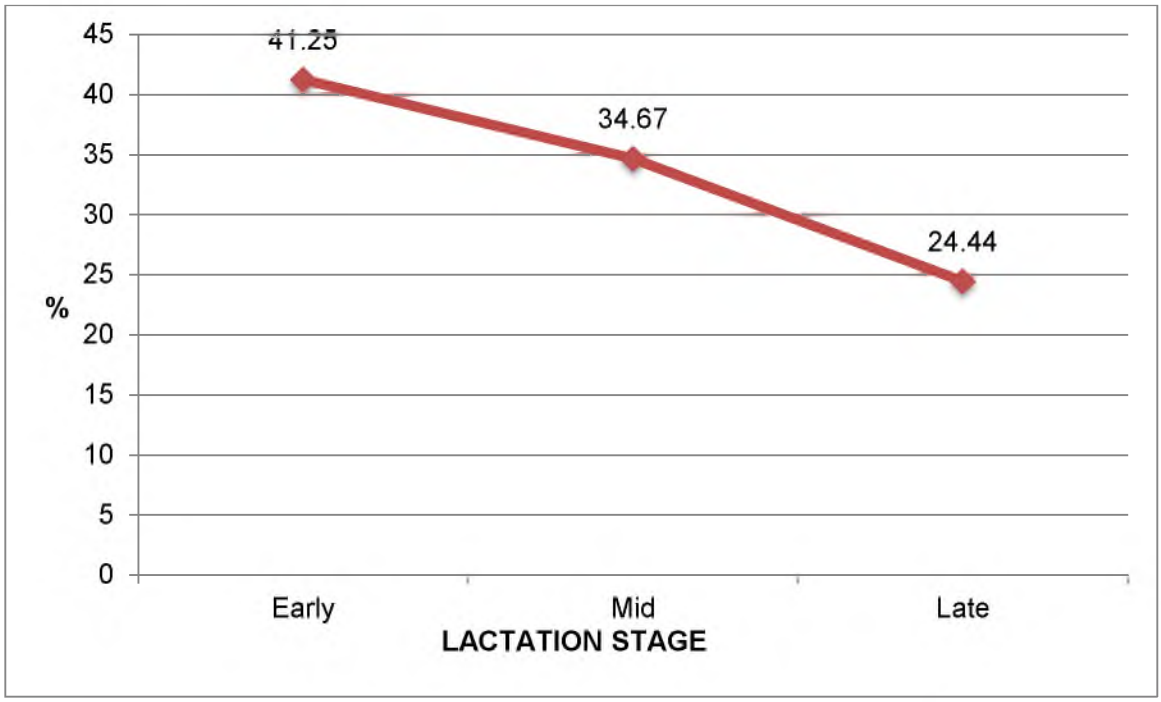

Fig. 11. Parity wise prevalence of subclinical mastitis in goats as detected by different diagnostic tests

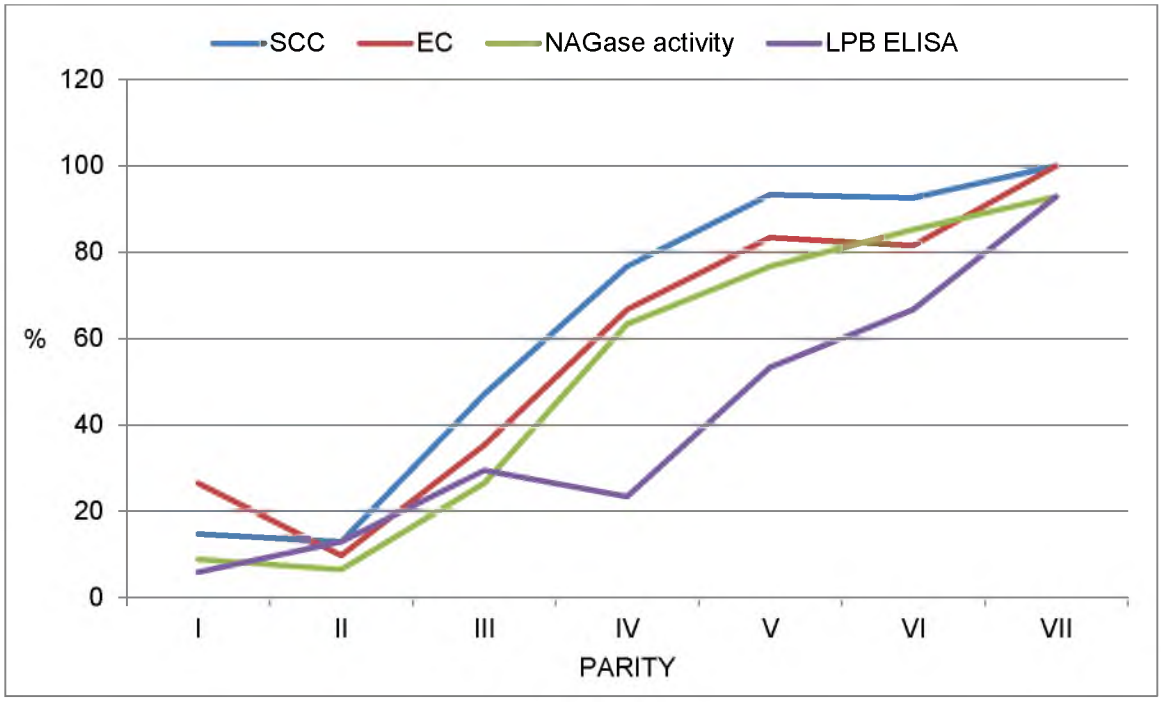

Fig. 12. Lactation stage wise prevalence of subclinical mastitis in goats as detected by different diagnostic tests

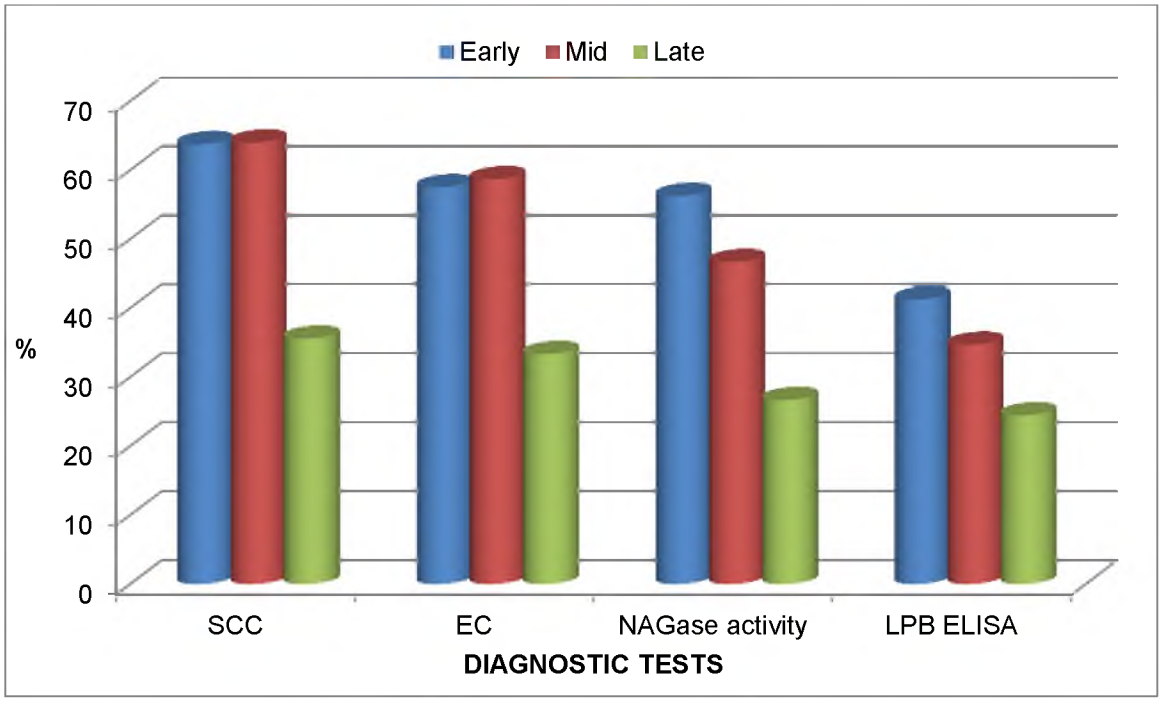

Fig. 13. Mean haematological parameters in healthy and subclinical mastitis affected goats

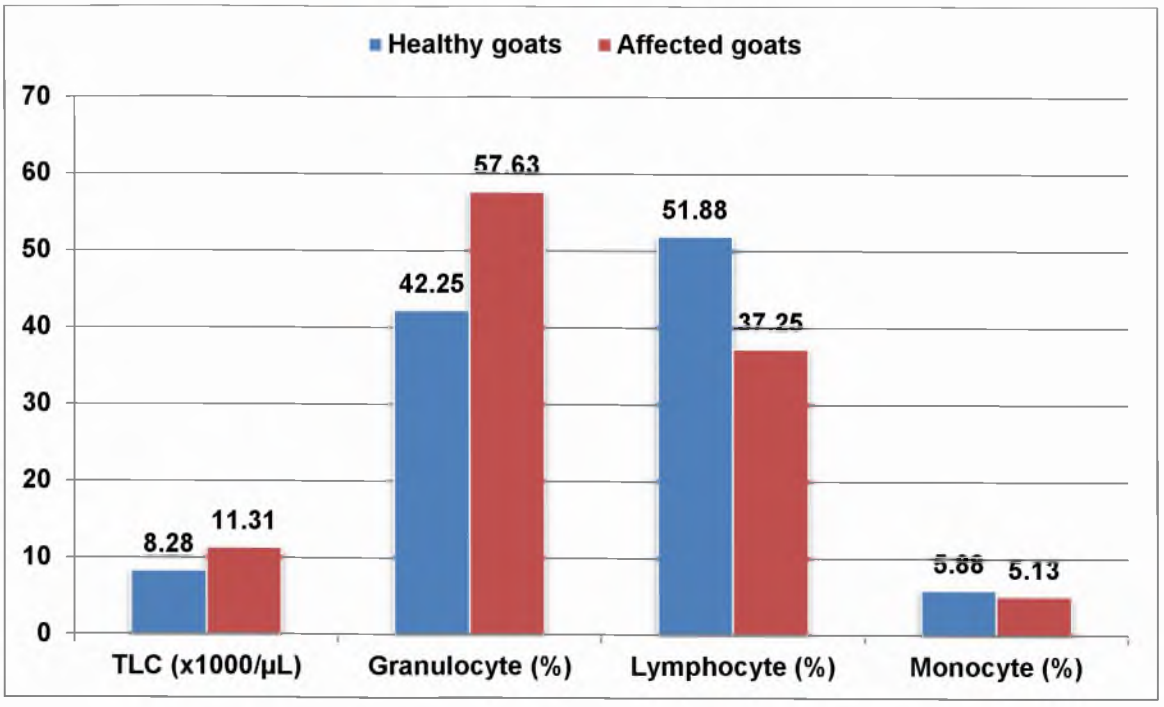

Fig. 14. Mean serum enzymes activity in healthy and subclinical mastitis affected goats

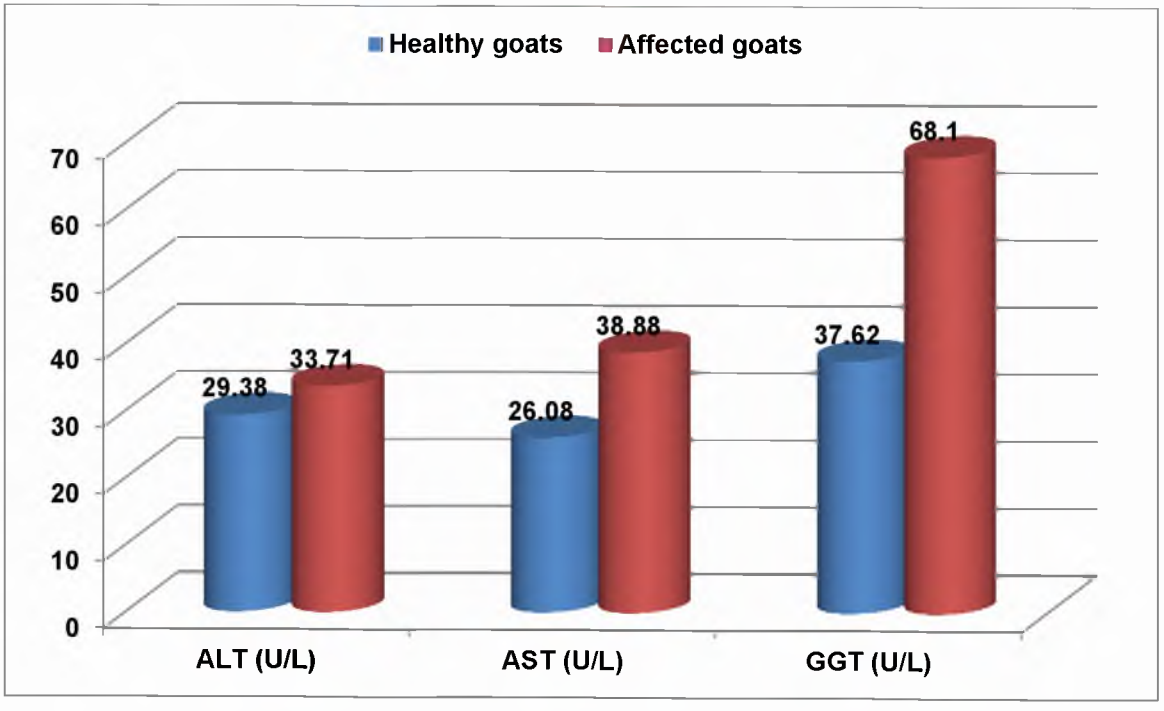

Fig. 15. Mean biochemical parameters in healthy and subclinical mastitis affected goats

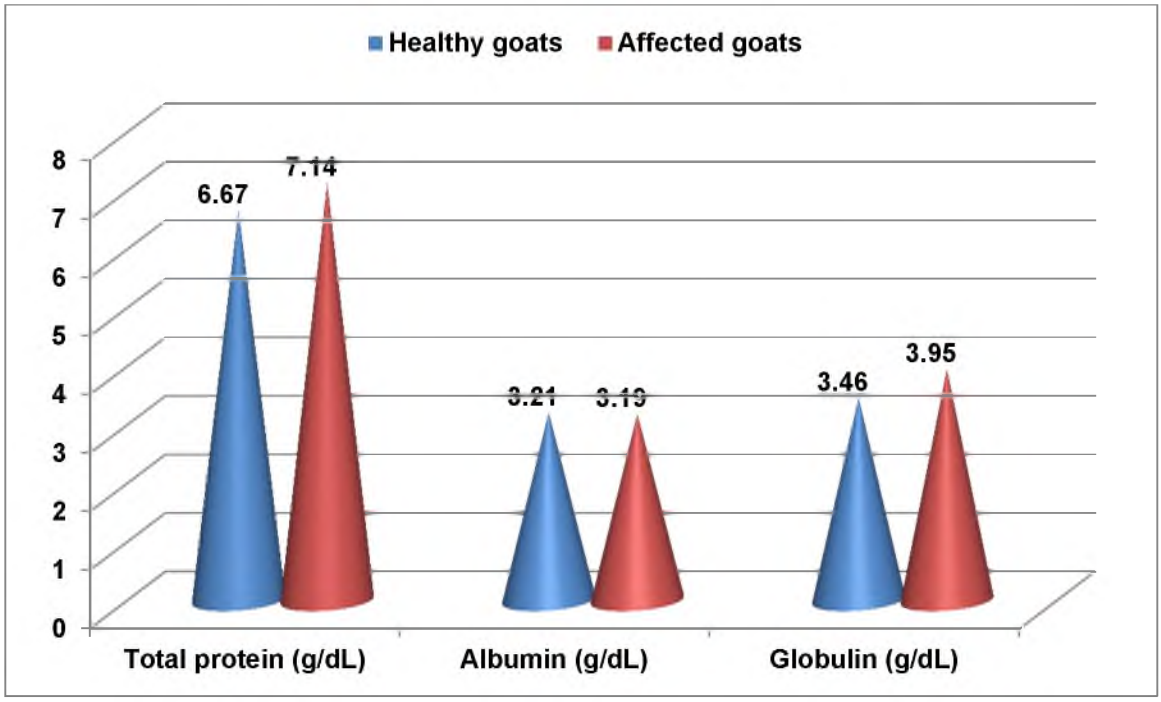

Fig. 16. Mean fibrinogen concentration in healthy and subclinical mastitis affected goats

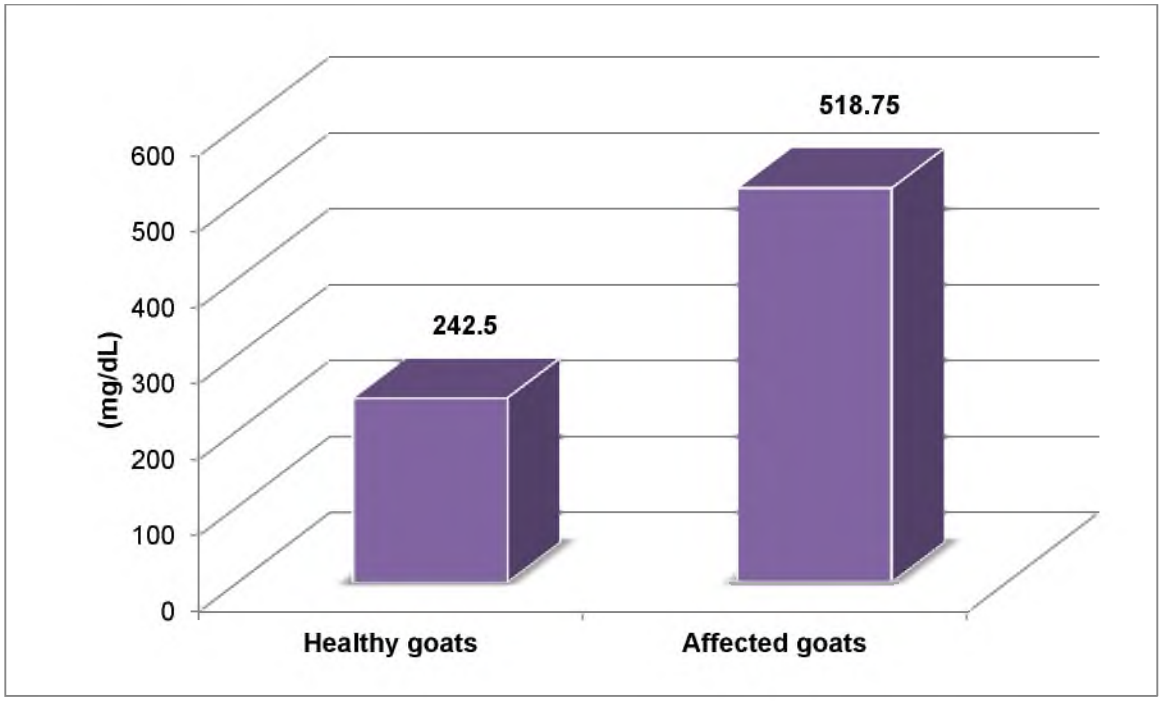

Fig. 17. Mean SCC in different treatment groups of subclinical mastitis in goats

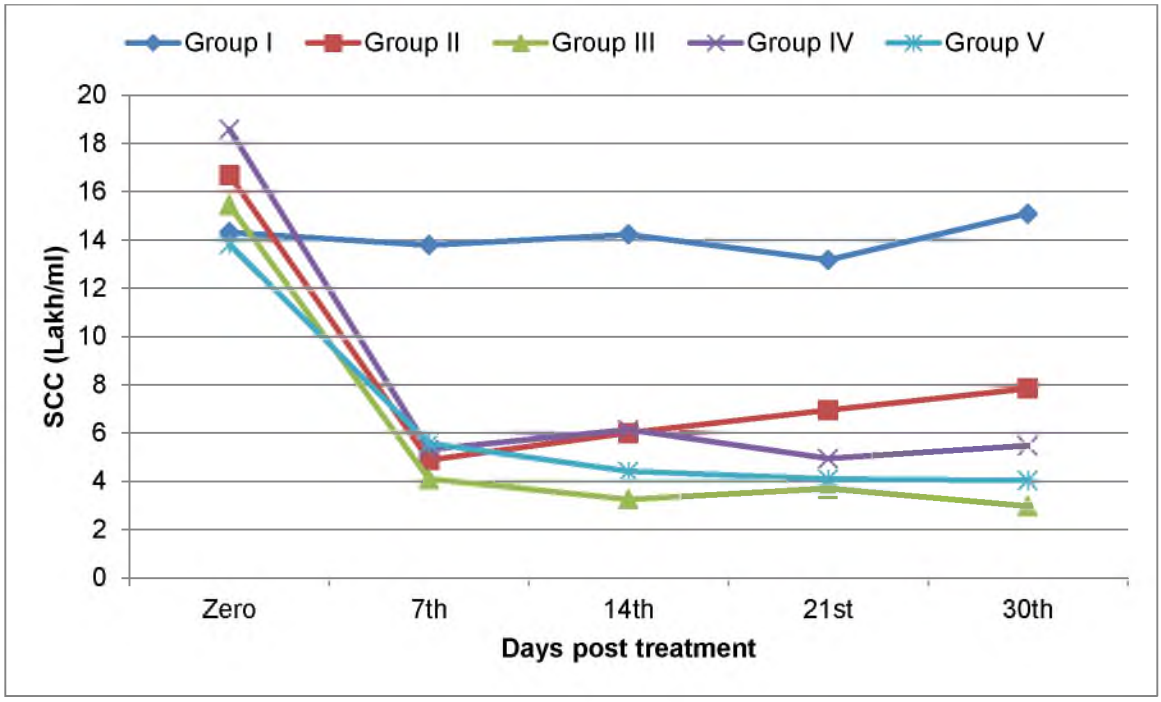

Fig. 18. Mean LPB ELISA OD values in different treatment groups of subclinical mastitis in goats

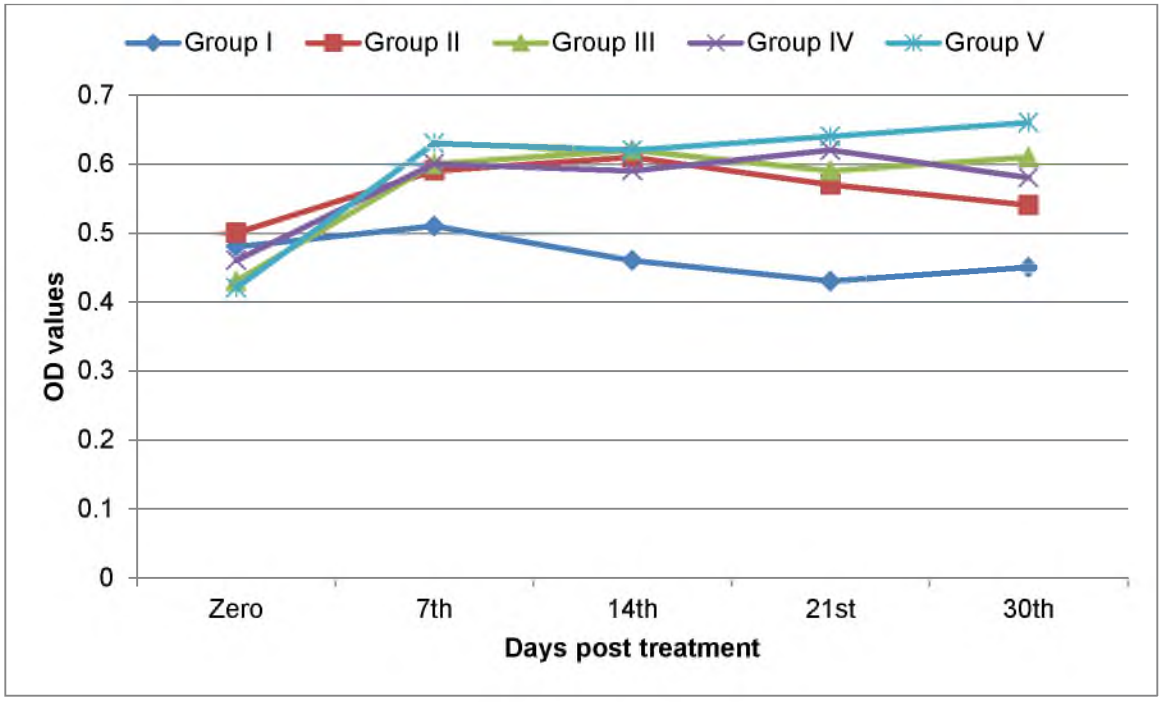

Plate 1. Flock of goats

Plate 2. A Doe with kids

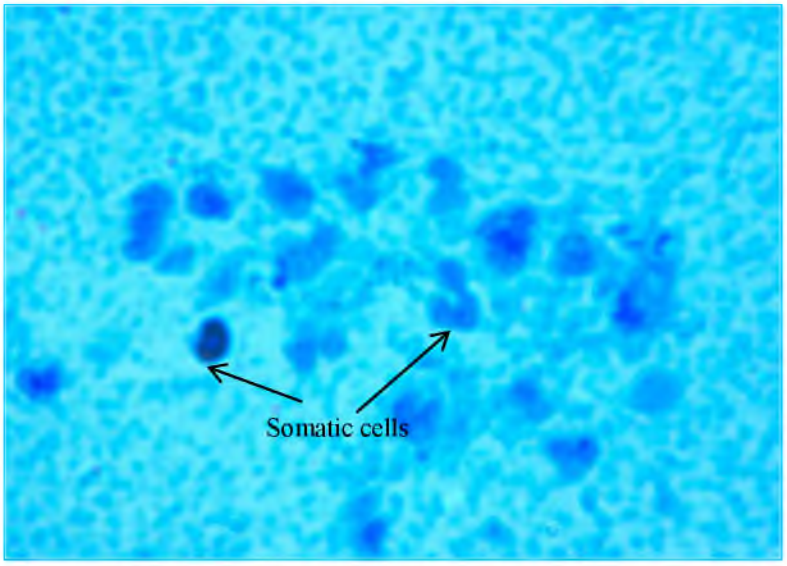

Plate 3. Milk smear stained with modified Newman's stain (100X)

Plate 4. Milk Electrical Conductivity tester (ECO Testr®)

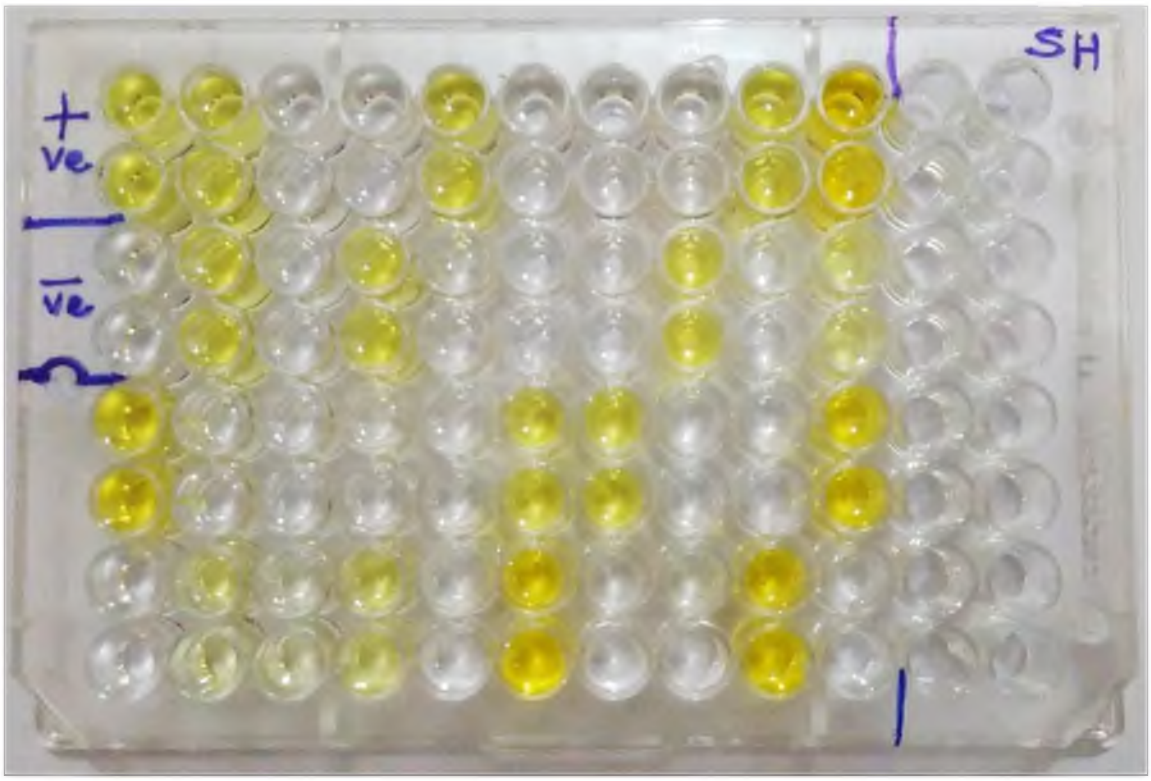

Plate 5. Microtitre plate showing NAGase activity estimation of goat milk samples

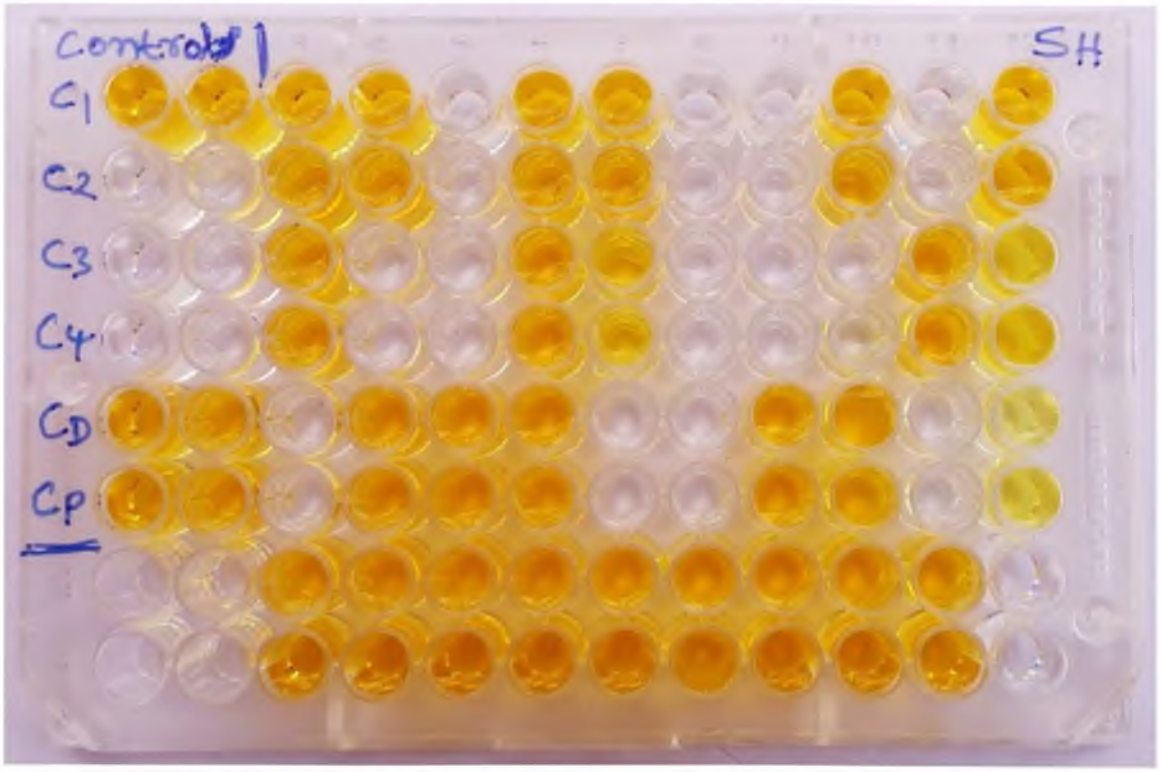

Plate 6. Liquid phase blocking ELISA microtitre plate showing different controls and results of goat milk samples

C1 – PIBB Antigen + Hyper immune sera + Conjugate + Substrate chromogen

C2 – PIBB Antigen + Conjugate + Substrate chromogen

C3 – PIBB Antigen + Substrate chromogen

C4 – Only Substrate chromogen

CD – PIBB Antigen + Defatted milk sample + Hyper immune sera + Conjugate + Substrate chromogen

CP – PIBB Antigen + Phosphate buffer + Hyper immune sera + Conjugate + Substrate chromogen

Column 3 to 12 – Test milk samples in duplicate

DISCUSSION

V. DISCUSSION

The present work was undertaken to detect SCM, prevalence of SCM, to study haematological and biochemical alterations and to evaluate the efficacy of various supportive therapies along with antibacterial for treatment of SCM in goats. The results of the study are discussed below.

5.1. Diagnosis of SCM in goats based on different diagnostic tests

A total of 200 composite milk samples collected from goats in and around Bidar, Karnataka were subjected to Somatic Cell Count (SCC), Electrical Conductivity (EC), N-acetyl Beta D glucosaminidase activity (NAGase) and Liquid Phase Blocking Enzyme Linked Immunosorbent Assay (LPB ELISA).

Out of 200 milk samples 115 samples had SCC more than 5 lakh/ml and mean ± SE of SCC was found to be 15.28 ± 0.61 lakh/ml. Remaining 85 samples had mean ± SE of SCC (3.21 ± 0.10 lakh/ml) less than 5 lakh/ml. This indicated that 57.50 per cent samples were positive for SCM in goats when SCC was employed as diagnostic test (Table 1 and 2).

Out of 200 milk samples 105 samples had EC more than 6.8 mS/cm and mean ± SE of EC was found to be 9.12 ± 0.14 mS/cm. Remaining 95 samples had mean ± SE of EC (5.12 ± 0.09 mS/cm) less than 6.8 mS/cm. This indicated that 52.50 per cent samples were positive for SCM in goats when EC was employed as diagnostic test (Table 1 and 2).

Similarly, out of 200 milk samples 92 samples had NAGase activity more than 14.04 µmoles/min/ml and mean ± SE of NAGase activity was found to be 26.26 ± 0.81 µmoles/min/ml. Remaining 108 samples had mean ± SE of NAGase activity (8.95 ± 0.26 µmoles/min/ml) less than 14.04 µmoles/min/ml. This indicated that 46.00 per cent samples were positive for SCM in goats when NAGase activity was employed as diagnostic test (Table 1 and 2).

Further, out of 200 milk samples 70 samples had LPB ELISA OD value less than 0.55 and mean ± SE of LPB ELISA OD value was found to be 0.41 ± 0.01. Remaining 130 samples had mean ± SE of LPB ELISA OD value (0.60 ± 0.01) more than 0.55. This indicated that 35.00 per cent samples were positive for SCM in goats when LPB ELISA was employed as diagnostic test (Table 1 and 2).

5.1.1. Somatic cell count

The use of the SCC is one of the most established methods for the diagnosis of udder health in cows (Paape *et al.*, 2007). Unfortunately, SCC could not be established yet as a proven marker for SCM in goats. Factors like parity, stage of lactation, estrus and breed contribute to significant changes of SCC in milk of dairy goats. SCC is also affected by the nature of infection with minor or major pathogens.

In the present study, estimating SCC of milk samples was employed as a diagnostic test. Earlier workers namely Lerondelle *et al.* (1992), Contreras *et al.* (1996), Gonzalo *et al.* (2002), Haenlein (2002), Paape *et al.* (2007), Leitner *et al.* (2008) and Prabavathy (2013) have also employed SCC as a parameter to diagnose SCM in goats. It

was recorded that SCC was found to be a reliable diagnostic test in goats (Contreras *et al.*, 1996; Haenlein, 2002; Paape *et al.*, 2007; Leitner *et al.*, 2008 and Prabavathy, 2013).

In the present study, mean SCC of milk samples above 5 lakh/ml was considered as positive for SCM and this is based on the report of Contreras *et al.* (1996). Out of 200 milk samples 115 milk samples (57.50 %) were positive for SCM (Table 2). In the present study, the SCC significantly increased in goats with SCM when compared to healthy / non infected goats. When SCC was employed as a diagnostic test, the sensitivity and specificity of SCC considering LPB ELISA as a standard diagnostic test were 91.43 per cent and 60.77 per cent respectively (Table 3). It was observed that SCC as a diagnostic test had positive correlation with EC (r = 0.66), NAGase activity (r = 0.65) and negative correlation with LPB ELISA (r = -0.86).

In our observation the mean ± SE SCC in SCM milk samples was 15.28 ± 0.61 lakh/ml. This agrees with the findings of Haenlein (2002) who has reported a mean SCC of 1515 $x10^3$ in SCM affected goats. However, other workers *viz.* Lerondelle *et al.* (1992), Gonzalo *et al.* (2002) and Christodoulopoulos *et al.* (2008) have reported higher mean SCC in SCM milk samples. Contreras *et al.* (1996) concluded that the best SCC threshold for defining SCM in goats was 500 x 10^3.

In the present study, SCC and EC as a diagnostic test for SCM in goats showed a significant positive correlation (r = 0.66) (Table 5). The positive correlation observed between SCC and EC agrees with the findings of Diaz *et al.*, (2011) who observed a significant but moderate positive correlation between SCC and EC. Further, Park and Nuti (1985), Park (1990) and Petzer *et al.* (2008) also found low positive correlation

between SCC and EC. The present study result does not agree with the findings of Park (1991) who opined that significant correlation between SCC and EC as known in dairy cows seems not to exist in dairy goats.

Based on the results of the present study, it may be concluded that SCC is one of the diagnostic test for diagnosis of SCM. SCC reflects / represents the inflammatory status of the udder.

Milk secretion in goats is apocrine in nature. Cytoplasmic particles are physiologically shed into milk from the apical portion of secretory cells. Although most of them are anucleated, some of these particles have been observed to contain nuclear fragments and could contribute to a slight increase to the total cell count. Cytoplasmic particles are similar in size to milk somatic cells. Somatic cells are the leucocytes released into the udder tissue to combat infections and also increased during various non-pathological influences. Factors like type of birth, season, parity, stage of lactation, estrus, breed, management or farming systems, different milking practices, time of milking, infective microorganisms and infusion products or intramammary treatment contribute to significant changes of SCC in milk of dairy goats. This effect may explain 48 per cent of SCC variance (Gonzalo *et al.*, 2002). Vaccinations and alimentary stress can induce transient increase in SCC (Lerondelle *et al.*, 1992). Bacterial infections of the mammary gland remain the major cause of variation in SCC. Haenlein (2002) examined the non-pathological influences on somatic cell counts and found that variation can be as much as 90 per cent and also found that cow's milk contributed 5 to 20 per cent neutrophils whereas goat milk contains 45 to 74 per cent neutrophils. For this reason the

SCC of milk represents a sensitive marker to the health of the udder and is considered a useful parameter to evaluate the relationship between intramammary infection and changes in milk characteristics.

Increase in SCC in milk samples from SCM cases could be attributed to immune response to bacterial infection which could be either due to current infection or latent infection as indicated by Leitner *et al.* (2008). The latent infection could be due to colonization of mastitis causing organism in the mammary gland (Guha and Gera, 2001).

Parity wise analysis revealed highest SCC in healthy goats during 6^{th} parity (4.38 ± 0.38) and least in 1^{st} parity (2.80 ± 0.16) which is in agreement with De-Cremoux *et al.*(1995) who found that in healthy goat udders, SCC progressively increases during lactation, rising from 200×10^3 to $>1\times10^6$ cells/ml in goats and attributed that somatic cell counts were higher in multiparous than in primiparous goats, confirming greater gland damage, probably due to their greater exposure to pathogens in previous lactations and the physiologic deterioration of the membranes of epithelial cells in the mammary gland occurring after several lactations.

However, SCC was recorded highest in SCM affected goats during 7^{th} parity and least in 3^{rd} parity. The present findings were contrary to the findings of Rota *et al.* (1993) and Zeng *et al.* (1997) who observed high SCC at the beginning of lactation. Aulrich and Barth (2008) and Zeng and Escobar (1995) stated that parity does not influence the SCC.

The SCC gradually increased from mid to late lactation of healthy milk samples. The similar increase in SCC in late lactation was recorded by Wilson *et al.* (1995), Zeng

and Escobar (1995), Min *et al.* (2005), Moroni *et al.* (2005), Paape *et al.* (2007) and Aulrich and Barth (2008). The increasing levels of SCC may be linked to a decreasing amount of milk produced by goats during late lactation, leading to a higher concentration of pathogens in the milk (Zeng and Escobar, 1995). Increase in SCC in later lactation and in older goats, even in the absence of IMI was reported by Wilson *et al.* (1995).

5.1.2. Electrical conductivity

The EC is the measure of the concentration of Na^+ and Cl^- ions in milk which increased during an intramammary infection not only in cows (Kromker, 2007) but also in goats. Reports on the published data of detection of goat mastitis by EC are very scarce (Arguello, 2011). EC is well established to monitor the udder health in dairy cows but it is not very commonly used in small ruminants (Barth, 2009). As there is lot of variation in EC studies on goats, reported previously that daily measurement of EC by mammary gland by EC test has the advantage of avoiding risk factors such as intramammary infection (IMI) in individual, parity or stage of lactation etc.

In the present study, measuring EC of milk samples was employed as a diagnostic test. Earlier workers namely Hamann and Zecconi (1998), Ying *et al.* (2004), Chen *et al.* (2008), Tangorra *et al.* (2010), Díaz *et al.* (2011) and Prabavathy (2013) have also employed EC as a parameter to diagnose SCM in goats. It was recorded that EC was found to be a reliable diagnostic test in goats (Hamann and Zecconi, 1998; Chen *et al.*, 2008; Díaz *et al.*, 2011; Romero *et al.*, 2012 and Prabavathy, 2013).

In the present study, EC of more than 6.8 mS/cm was considered as positive for detection of SCM in goats and this is based on the results of Chen *et al.* (2008) who

recorded a normal EC of below 6.8 mS/cm in healthy goats and opined that EC value above 6.8 mS/cm indicates mastitis. However, Ying *et al.* (2004) recorded EC of 5.6 mS/cm in healthy animals and Romero *et al.* (2012) obtained best EC mastitis detection characteristics at 5.20 mS/cm threshold. Barth (2009) and Tangorra *et al.* (2010) opined that a reliable absolute threshold to differentiate between infected and non-infected udder glands to be still worked out.

In the present study, it was noticed that mean ± SE EC of SCM samples was 9.12 ± 0.14 mS/cm. Díaz *et al.* (2011) recorded EC of 8.61 ± 1.65 in *Staphylococcal* bilateral mastitis which is in accordance with the present study. Whereas Romero *et al.* (2012) recorded 5.14 mS/cm in milk of infected glands and Lien *et al.* (2005) recorded 6.68 mS/cm from mastitis half, which differed from the present study. This variation may be attributed to difference in the diet and management which in turn may cause variation of milk composition and thus altering EC as rightly opined by Hamann and Zecconi (1998). Increased EC in milk samples indicate udder infection. However, EC variations may also be attributed to difference between individual animals apart from difference in number of parity, month of lactation, farm conditions as these factors also leads to variation in EC value of milk samples as rightly indicated by Hamann and Zecconi (1998).

In mastitis the milk-blood barrier gets damaged leading to change in the permeability of the udder tissue. Hence, there is leakage of various dissociated inorganic salts like chlorides, sodium and potassium salts which are the main contributors of EC in milk and are responsible for over 60 per cent of the EC value. The concentrations of Na^+ and Cl^- and sodium: potassium ratio in milk, increases during an intramammary infection

not only in cows but also in goats (Hamann and Zecconi, 1998). Fernando *et al.* (1982 and 1985) and Díaz *et al.* (2011) observed that variation in chloride concentration might lead to the high variance of EC in cow and goat milk respectively. This change is detectable by measurement of the EC. EC is increased on establishment of infection mainly in bilaterally infected glands, which increases both variables (chlorides and sodium: potassium) and in unilateral infected glands, the EC increase was lower, with only chloride content being increased. In our study the sensitivity and specificity of EC considering LPB ELISA as a standard diagnostic test, were found to be 87.14 and 66.15 per cent respectively. It may be safe to conclude that EC may not be useful as a single diagnostic test and similar opinion is expressed by Pyorala (2003).

The mean ± SE of EC in SCM goats was highest during 7^{th} parity (9.59 ± 0.42) and least in 3^{rd} parity (8.81 ± 0.42) and the values are showing increasing trend form 3^{rd} parity to 7^{th} parity. The present findings are in agreement with Díaz *et al.* (2011). Higher EC were obtained for multiparous goats compared with primiparous ones (Holdaway *et al.*, 1996 and Zecconi *et al.*, 2004) which correlated to higher permeability of the milk-blood barrier in multiparous animals. Changes at the end of lactation in the blood-milk barrier decrease the tightness of the mammary epithelium and increase its permeability and different milk composition.

The mean EC in SCM goats showed increasing trend from early to late lactation. The present findings are in agreement with Díaz *et al.* (2011) who observed steady increase in EC with progression of lactation. Tangorra *et al.* (2010) reported higher EC values in infected glands compared to healthy ones only at 0–60 days in milk period

(11.74 vs 10.71 mS), but no significant differences were obtained in the other lactation stages considered.

5.1.3. N-acetyl-β-D-glucosaminidase activity

NAGase activity is a suitable parameter for the diagnosis of SCM in dairy goats, although the level of NAGase activity in goat milk is nearly 4-fold lower as compared to bovine milk (Sharma *et al.*, 2007). Obara (1985) found a significant increase in NAGase activity in infected mammary gland than in control groups. Hence, estimation of NAGase activity would be the best method for diagnosis of caprine mastitis when compared to SCC and white side test (Vihan, 1989).

In the present study, measuring NAGase activity of milk samples was employed as a diagnostic test. Earlier workers namely Obara (1985), Maisi and Riipinen (1988), Vihan (1989), Maisi (1990b), Leitner *et al.* (2004) and Sharma *et al.* (2007) Prabavathy (2013) have also employed NAGase activity as a parameter to diagnose SCM in goats. It was recorded that NAGase activity was found to be a reliable diagnostic test in goats (Obara, 1985; Maisi and Riipinen, 1988; Vihan, 1989; Maisi, 1990b and Prabavathy, 2013).

NAGase activity of 14.04 µmoles/min/ml (equivalent to OD value 0.27 at 405 nm), was considered as positive for detection of SCM in goats. This cut off value is considered in the present study based on the findings of Kitchen (1976).

In the present study, 200 milk samples were subjected for estimation of NAGase activity and 92 milk samples (46.00 %) exhibited NAGase activity above 14.04

μmoles/min/ml and the mean ± SE of NAGase activity in these milk samples was 26.26 ± 0.81 μmoles/min/ml. This indicated that 46.00 per cent samples were positive for SCM in goats. The mean ± SE NAGase activity in the remaining 108 samples was 8.95 ± 0.26 μmoles/min/ml and considered as negative for SCM in goats (Table 1).

The values in both groups were very high compared to the results of Vihan (1989) who recorded the range of NAGase activity of two herds which varied from 0.012 to 0.41 and 0.028 to 0.036 μmoles/ml in infected halves and negative samples showed 0.0054 ± 0.0001 and 0.0029 ± 0.00007 μmoles/ml. The NAGase activity in infected goat udder is reported to be significantly higher than in control udder and it is suggested that NAGase activity is the best indicator of damage to udder epithelial cell (Obara, 1985).

Both in clinical and subclinical mastitis the increase of polymorphonuclear leukocytes (PMN) and macrophages indicate the body defense against the inflammatory process (Perdigon *et al.,* 1986). During this process NAGase is released in order to degrade damaged tissues. Furthermore, NAGase activity is reported as a suitable parameter for the diagnosis of SCM in dairy goats (Maisi and Riipinen, 1988) and the level of NAGase activity in goat milk is approximately 4-fold lower as compared to bovine milk (Sharma *et al*., 2007).

The sensitivity and specificity of NAGase activity as a diagnostic test considering LPB ELISA as a standard diagnostic test was found to be 71.43 and 67.69 per cent respectively (Table 3). Further, NAGase activity as a diagnostic test correlated positively with SCC (r = 0.65) and EC (r = 0.59) whereas it is negatively or inversely correlated with LPB ELISA (r = -0.58) (Table 5). Timms and Schultz (1985) recorded correlation

between NAGase and SCC as 0.54 which is similar to the present study. They opined that somatic cells contribute less to milk NAGase activity in the goat milk compared to the cow milk with larger within-gland NAGase contribution, resulting from sloughed cytoplasmic particles of the secretory epithelium. Leitner *et al.* (2004) correlated between SCC and NAGase activity and found it to be less ($R<0.7$) and opined that no interaction was found between bacteriological status and flock in its effect on SCC or NAGase activity. They also suggested that within that limited period SCC and NAGase activity should be complemented with bacterial testing to assess IMI.

Estimation of NAGase activity is found to be reliable method for diagnosis of SCM. Further, it was observed that NAGase activity of milk sample positively correlated with SCC and negatively correlated with LPB ELISA and this draws the support from the observations made by Kitchen (1976); Kitchen and Middleton (1976) and Vihan (1989).

In present observation, the mean NAGase activity of SCM affected goats were showing a increasing trend form 1^{st} parity to 6^{th} parity. Leitner *et al.* (2004) recorded lowest levels of NAGase in goats in their first lactation. Stuhr *et al.* (2012) opined that correlation between NAGase and parity was not significant.

In the present study, the mean NAGase activity of SCM affected goats was highest in late lactation followed by early and least in the mid lactation. Similar observation is made by Maisi (1990b), who reported that NAGase activity increased during the first week of lactation and increased significantly after 270 days of lactation.

5.1.4. Liquid phase blocking ELISA

Biomarkers are chemical substances which are released very early during an infection or inflammation. A highly sensitive and specific laboratory test to detect the cytokines which are present in minute quantities and noticed very early in intramammary infection is necessary.

In the present study measuring LPB ELISA OD values of milk samples was employed as a diagnostic test. Earlier workers namely Suryanarayana (2012) and Sripad *et al.* (2013a) have also employed LPB ELISA as a parameter to diagnose SCM in dairy cows. It was recorded that LPB ELISA was found to be a reliable diagnostic test for SCM in dairy cows (Suryanarayana, 2012 and Sripad *et al.*, 2013a).

In the present study 200 milk samples were subjected to LPB ELISA test. LPB ELISA OD value of 0.55 was taken as the cut off value as per the report of Suryanarayana (2012). It was noticed that 70 samples out of 200 had an LPB ELISA OD value of less than 0.55 and the mean ± SE of LPB ELISA OD value was 0.41 ± 0.01 indicating 35.00 per cent samples were positive for SCM in goats. The mean ± SE of LPB ELISA OD value of negative samples was 0.62 ± 0.01 and it differed significantly from positive samples (Table 1).

The per cent positivity of SCM in goats based on LPB ELISA was found to be 35.00. Perusal of literature indicated that Sripad *et al.* (2013a) reported per cent positivity of SCM to be 24.89 in dairy cows.

The mean LPB ELISA OD values is found to be less than 0.55 in SCM milk samples and low OD values as compared to normal milk samples is attributed to the release of cytokines from udder following infection or inflammation. The increased release of cytokines from infected gland could be detected as early as two hours post infection / inflammation and elevated cytokine levels remains elevated during the inflammation / infection. Similar opinion is expressed by Winter and Colditz (2002).

The LPB ELISA is considered as a reliable diagnostic test for the diagnosis of SCM because this test detects major proinflammatory cytokines like Interleukin 8, Pentraxime, Complement 3, Protease peptone 3 and L selectin, which are released during early stage of inflammation. No nonspecific reaction is observed because the antiserum is raised against a recombinant protein which carries major immunogenic epitopes of the above mentioned five cytokines. LPB ELISA works well irrespective of the pathogen involved. Further Interleukin 8 is released immediately after Interleukin 12, which signals the infection (Suryanarayana, 2012).

The SCC, EC and estimation of NAGase activity are the three diagnostic tests employed commonly for diagnosis of SCM. In the present study the specificity of SCC, EC and NAGase activity were 60.77, 66.15 and 67.69 per cent respectively (Table 3). Perusal of available literature failed to provide information regarding specificity of SCC, EC and NAGase activity as diagnostic tests in SCM of goats. However, Sripad (2013b) reported 49.72, 24.02 and 34.63 per cent specificity for SCC, EC and NAGase activity respectively for diagnosis of SCM in dairy cows. This indicated that SCC, EC and

NAGase activity as diagnostic tests have certain drawbacks with respect to specificity. Similar opinion was expressed by Vihan (1989) and Pyorala (2003).

Based on the observations of this study it is safe to conclude that comparatively LPB ELISA was found to be more specific diagnostic test for diagnosis of SCM in goats and similar opinion is expressed by Sripad *et al.* (2013a) in their study on SCM in cows.

5.2. Prevalence of subclinical mastitis

In the present study, the overall prevalence of SCM in goats based on SCC, EC, NAGase activity and LPB ELISA was 57.50, 52.50, 46.00 and 35.00 per cent respectively (Table 2). This observation of overall prevalence is in corroboration with the observations made by earlier workers namely Manser (1986); Leitner *et al.* (2004); Diaz *et al.* (2011); Islam *et al.* (2012) and Zhao *et al.* (2015), who have reported prevalence of SCM in goats to be 15-79, 52.00, 48.60, 44.59 and 45.82 per cent respectively based on different diagnostic tests.

Earlier workers namely Manju *et al.* (2012) and Prabavathy (2013) have recorded a higher overall prevalence of SCM ranging from 72.50 to 96.81 per cent. This observation in the present study is not in agreement with the observations of these workers.

Further, overall prevalence of SCM in goats as observed by Ndegwa *et al.* (2000); Kostelic *et al.* (2009); Gebrewahid *et al.* (2012) and Mishra *et al.* (2013) have recorded lower prevalence than the current observation and ranged from 9.70 to 28.14 per cent.

Further, overall prevalence of SCM in sheep as observed by Cruz *et al.* (1994); Lafi *et al.* (1998) and Al-Majali and Jawabreh (2003) was lower than the current observation and ranged from 18.30 to 36.70 per cent.

Wide variation in the percentage prevalence of SCM observed by previous workers could be due to the difference in the managemental condition and different diagnostic tests employed (Mishra *et al,,* 2014) and could also be due to breed of the animal, immune response of animals and climatic condition (Gebrewahid *et al.,* 2012). Per cent prevalence of SCM varies significantly with variation or interplay of the risk factors. Further, these studies have been conducted in different geographical areas and that can be another reason for recording different percentage prevalence of SCM by some of the earlier workers (Islam *et al.,* 2012)

High prevalence of SCM observed by earlier workers could be due to presence of many subclinical carriers, poor milking hygiene and less prevention awareness of SCM cases. Due to the poor management, the infected goats including clinical and subclinical mastitis were usually not separated from the healthy animals, and this contaminative environment and equipment would cause a new infection might be greatly contributed to high prevalence or any other deficiency in the management as opined by Zhao *et al.* (2015).

Low prevalence of SCM observed by earlier workers may be due to adoption of better managerial practices at the farms, improved techniques of husbandry and inculcation of awareness among dairymen for timely and appropriate treatment of animals and good hygienic management (Tiwari *et al.,* 2000). Adverse climatic

conditions which is unfavourable for the maintenance and transmission of mastitis causing bacteria might reduce the prevalence of SCM in lactating goats (Megersa *et al.*, 2010).

5.2.1. Parity wise prevalence of subclinical mastitis

In the present study the parity wise per cent prevalence of SCM in goats was found to be highest during seventh parity and least prevalence was noticed during first and second parity. Further the per cent prevalence of SCM in goats was found to be increasing trend from second to seventh parity (Table 14 and Fig. 11).

This observation of parity wise prevalence is in agreement with the observations made by earlier workers namely Sanchez *et al.* (1999) and Kostelic *et al.* (2009) who have reported a prevalence of SCM in goats increase with the number of lactation and Beheshti *et al.* (2010) reported higher prevalence of SCM in multiparous ewes.

Contrary to the present findings of parity wise prevalence, Leitner *et al.* (2004) and Gebrewahid *et al.* (2012) reported no association between parity and prevalence of SCM in goats.

Increase in the prevalence of SCM with increase in number of parity could be associated with gradual loss of immune response in the body of the animal, which makes it susceptible to infection and may also be associated with inefficient sphincters. Similar observations were made by Sudhan *et al.* (2005). The increase in prevalence can be explained as a consequence of the long-term mechanical irritation of the teat, the streak canal and the entire udder, due to nursing goat kids and milking (Kostelic *et al.*, 2009)

The low prevalence of SCM among primiparous goats could be because of better polymorphonuclear leucocyte function in them than in multiparous goats. Comparatively better PMN function is directly associated with the higher resistance to the infection in primiparous than their multiparous counterparts (Samanta *et al.*, 2006).

5.2.2. Lactation stage wise prevalence of subclinical mastitis

In the present study, the lactation stage wise per cent prevalence of SCM in goats was found to be highest during early and mid lactation and least in late lactation (Table 15 and Fig. 12).

Contrary to the present findings of lactation stage wise prevalence, Leitner *et al.* (2004) and Gebrewahid *et al.* (2012) reported no association between lactation stage and per cent prevalence of SCM in goats.

Variation in the prevalence of SCM observed by previous workers could be due to breed of the animal, immune response of animals and climatic condition (Bachaya *et al.*, 2005). It is strongly suggested that increased prevalence during early lactation stage is associated with intramammary infection during this period. Similar opinion was expressed by Leitner *et al.* (2004).

5.3. Haematological and biochemical studies

A total of eight goats which were found to be affected with SCM were subjected for haematological and biochemical investigation and were compared with haematological and biochemical values of eight goats which were apparently healthy and negative for SCM.

5.3.1. Total leucocyte count

The mean ± SE TLC in blood samples collected from healthy and SCM affected goats were found to be 8.28 ± 0.63 and 11.31 ± 0.89 ($\times 10^3/\mu L$) respectively. There was a statistically significant difference indicating that SCM significantly increases TLC in goats .This agrees with the findings of Singh *et al.* (1998) who reported elevated TLC in candidial mastitic goats. Further, Ajuwape *et al.* (2005) and Fasulkov *et al.* (2014) also reported increased TLC in mastitis of goats. Zaki *et al.* (2008) reported higher TLC in SCM buffaloes. Singh *et al.* (2014) and Chandrasekaran *et al.* (2015) reported increased TLC in cows affected with mastitis.

Significant increase in TLC in SCM affected goats might be attributed to the stressful effect of infection which might force great number of white blood cells to buildup natural resistance (Zaki *et al.*, 2008 and Chandrasekaran *et al.*, 2015).

Leucocytosis is noticed following infection which may be localized or generalized. Further, leucocytosis is also reported following tissue necrosis of any cause. In the present study, statistically significant increase in TLC was noticed in goats suffering from SCM. This may be attributed to localized infection and certain degree of tissue necrosis (Reda and Hathout, 1951).

5.3.2. Differential leucocyte count

The mean ± SE values of granulocytes in healthy goats and goats affected with SCM were 42.25 ± 2.96 and 57.63 ± 5.45 per cent respectively and there was a

statistically significant difference indicating that SCM significantly increases granulocytes in goats.

The results of present study agrees with the findings of Ajuwape *et al.* (2005) and Fasulkov *et al.* (2014) who reported neutrophilia in mastitis of goats. Zaki *et al.* (2008) reported increase in neutrophils in SCM of buffaloes. Singh *et al.* (2014) and Chandrasekaran *et al.* (2015) reported an increase in neutrophils in cows affected with mastitis. In lactating goats, the first line of udder defense consists of neutrophil leucocytes, which migrate rapidly into the udder in response to inflammation (Theilen *et al.*, 1959).

During inflammatory conditions the rate of movement of neutrophils in blood is greater than their movement in tissues, thus increasing in neutrophil count. Further, as the inflammatory stage established there will be increase in bone marrow proliferation with a subsequent increase in the maturation pool leading to neutrophilia (Reda and Hathout, 1951).

The mean ± SE values of lymphocytes in healthy goats and goats affected with SCM were 51.88 ± 3.35 and 37.25 ± 5.43 per cent respectively and there was a statistically significant difference indicating that SCM significantly decreases lymphocytes in goats.

This is in agreement with the findings of Singh *et al.* (2014) and Chandrasekaran *et al.* (2015) who reported decreased lymphocyte count in cows affected with mastitis.

The findings were contrary to findings of Ajuwape *et al.* (2005) who reported an increase in absolute lymphocyte count in mastitic goats.

The statistically significant decrease in mean lymphocyte per cent in SCM affected goats may be attributed to the compensatory increase in neutrophils in response to inflammation.

The mean ± SE values of monocytes in healthy goats and goats affected with SCM were 5.88 ± 0.69 and 5.13 ± 0.64 per cent respectively and statistically there was no significant difference between the healthy and affected goats.

The present observation corroborates with findings of Singh *et al.* (2014) who reported no significant difference in monocytes of healthy and subclinical affected cows. But, Ajuwape *et al.* (2005) reported increase in absolute monocyte count in mastitic goats.

Monocytosis is reported in all chronic suppurative disease conditions and in later phase of acute inflammation, disease producing tissue debris, granulomatous disease condtion such as Tuberculosis, Brucellosis and systemic mycotic disease (Theilen *et al.*, 1959). The present study is restricted to SCM in goats which do not create much stress, produce signs of tissue debris nor is not of chronic nature. This may be the reason for observation of no significant difference in mean monocyte per cent.

The total and differential leucocyte counts are true indicators of mastitis and will even assist in predicting the prognosis of mastitic goats (Ajuwape *et al.*, 2005).

5.3.3. Alanine transaminase

The mean ± SE values of ALT in healthy goats and goats affected with SCM were 29.38 ± 9.26 and 33.71 ± 7.97 U/L respectively and statistically there was no significant difference between the healthy and affected goats. This indicates that ALT activity may not increase in SCM of goats. This agrees with the findings of Fasulkov *et al.* (2014) who reported that ALT values were unaltered between healthy and mastitis affected goats. Further, Dwivedi *et al.* (2004), Zaki *et al.* (2010), Pandey *et al.* (2012) and Gain *et al.* (2015) also reported no significant alteration in ALT values of healthy and mastits affectd cows. However, Jain *et al.*, (2013) reported that ALT values in mastitc cows were found to be significantly higher than the control animals.

Elevation in the activity of serum ALT is considered to be specific for hepatic injury in dogs and cats. In ruminants the activity of ALT in the liver is low and in liver injury the serum ALT is not remarkably elevated (Kaneko *et al.*, 2008). This may be the reason for observation of no significant difference in the mean ALT activity in healthy and SCM affected goats.

5.3.4. Aspartate transaminase

The mean ± SE values of AST in healthy goats and goats affected with SCM were 26.08 ± 7.15 and 38.88 ± 4.94 U/L respectively and statistically there was no significant difference between the healthy and affected goats. This indicates that AST activity may not increase in SCM of goats. This agrees with the findings of Fasulkov *et al.* (2014) who also reported that AST values were unaltered between healthy and mastitis affected goats. However Zaki *et al.* (2010), Pandey *et al.* (2012), Chandrasekaran *et al.* (2015) and Gain

et al. (2015) reported that AST values in mastitc cows were found to be significantly higher than the healthy animals. The workers concluded that the invasion of pathogenic organism in udder bring about the damage to ducts and secretory epithelium that leads to increased level of AST in serum.

The AST is a potential marker for clinical mastitis that too in early stage of lactation rather than for SCM (Moyes *et al.*, 2009) and this may be the reason for observation of no significant difference in the mean AST activity between healthy and SCM affected goats.

5.3.5. Gamma glutamyl transferase

The mean ± SE values of GGT in healthy goats and goats affected with SCM were 37.62 ± 7.58 and 68.10 ± 11.32 U/L respectively and there was statistically significant difference ($P \leq 0.05$) between the healthy and affected goats. This indicates that GGT activity increases in SCM of goats. Contrary to the present findings lower values were recorded by Matei *et al.* (2010) in cows affected with SCM.

The GGT is found in many tissues but liver is the primary source. GGT is associated with glutathione metabolism. The increased serum GGT with hepatic inflammation was reported by Kaneko *et al.* (2008). Hence, it may be safe to conclude that inflammatory changes in liver caused due to translocation of bacteria to the liver tissue might have resulted in statistically significant increase in the mean GGT values in SCM affected goats

5.3.6. Total protein

The mean ± SE values of plasma total protein in healthy goats and goats affected with SCM were 6.67 ± 0.31 and 7.14 ± 0.22 g/dL respectively and statistically there was no significant difference ($P \geq 0.05$) between the healthy and affected goats. This indicates that plasma total protein values may not increase in SCM of goats. This agrees with the findings of Fasulkov *et al.* (2014) who also reported that total protein values were unaltered between healthy and mastitis affected goats. Further the present findings are in agreement with Chandrasekaran *et al.* (2015) and Gain *et al.* (2015), who also reported no significant alteration in total protein values of healthy and mastits cows. However, significant higher values were reported by Singh *et al.* (1998) in Candidial mastitis in goats, Pandey *et al.* (2012), Singh *et al.* (2014) and Chandrasekaran *et al.* (2015) in clinical mastitis affected cows. Contrary to the present findings lower values were recorded by Zaki *et al.* (2008) in buffaloes affected with SCM.

5.3.7. Albumin

The mean ± SE values of albumin in healthy goats and goats affected with SCM were 3.21 ± 0.21 and 3.19 ± 0.15 g/dL respectively and statistically there was no significant difference between the healthy and affected goats. This indicates that albumin values may not increase in SCM of goats. This agrees with the findings of Fasulkov *et al.* (2014) who reported that albumin values were unaltered between healthy and mastitis affected goats. Further, present findings are in agreement with Pandey *et al.* (2012) who also reported no significant alteration in albumin values of healthy and subclinical mastits affectd cows. Contrary to the present findings, lower values were recorded by Singh *et al.*

(2014) and Chandrasekaran *et al.* (2015) in mastitis affected cows, who concluded that hypoalbuminaemia enhances protein catabolism as happens in various infections and trauma.

Decreased albumin concentration is noticed in conditions like malnutrition, diseases of chronic nature, increased protein catabolism, leakage in damaged tissue, secondary to increase in globulin concentration (Kaneko, 2008). In the present study no such conditions and hence no significant difference in mean albumin concentration.

5.3.8. Globulin

The mean ± SE values of globulin in healthy goats and goats affected with SCM were 3.46 ± 0.24 and 3.95 ± 0.16 g/dL respectively and statistically there was no significant difference between the healthy and affected goats. This indicates that globulin values may not increase in SCM of goats. This agrees with the findings of Jain *et al.* (2013) who also reported that globulin values were unaltered in mastitis affected cows. The present findings are contrary to the findings of Pandey *et al.* (2012), Singh *et al.* (2014) and Chandrasekaran *et al.* (2015) who recorded higher globulin values in mastitis affected cows. Increased globulin levels in clinical mastitis cases might be attributed to the activation of immune response following infection of the mammary gland (Matei *et al.*, 2010) and it will reflects the response of reticulo-endothelial system to antigens.

5.3.9. Fibrinogen

The mean ± SE values of fibrinogen in healthy goats and goats affected with SCM were 242.50 ± 31.85 and 518.75 ± 61.40 mg/dL respectively and there was statistically

significant difference between the healthy and affected goats. This agrees with the findings of Fasulkov *et al.* (2014) who also reported significantly higher fibrinogen values in mastitis affected goats. Further, the present findings are in agreement with Tabrizi *et al.* (2008) and Singh *et al.* (2014), who also recorded higher fibrinogen values in SCM among cows. But the present findings were contrary to El-Deeb (2013), who reported significantly lower levels of fibrinogen in gangrenous mastitis affected goats.

Fibrinogen is a soluble plasma protein produced in the microsomes of hepatic parenchymal cells (Forman and Barnhart, 1964). Increased fibrinogen will be seen in inflammation caused by bacteria, trauma and chemicals. Tissue destruction also increases fibrinogen level within 24 hours of injury. Fibrinogen is considered as a more sensitive indicator of an inflammatory process than the total leucocyte count (Schalm *et al.,* 1970). Elevated fibrinogen level in cattle suffering from mastitis is reported by McSherry *et al.* (1970).

Fibrinogen is the coagulation factor I, acute phase protein. Among the positive acute phase proteins, fibrinogen belongs to the group of moderate acute phase protein, whose concentration increases 2 to 10 times during the response to inflammation (Eckersall, 2000). It is produced more rapidly than degraded during the inflammation. It specifically binds to CD11/CD18 integrins on the cell surface of migrated phagocytes, thereby triggering a cascade of intracellular cytotoxicity and delay of apoptosis (Sitrin *et al.,* 1998 and Ruble *et al.,* 2001).

It is safe to conclude that plasma fibrinogen estimation may be used to detect SCM in goats in conjugation with other routine diagnostic tools.

5.4. Therapeutic study

Forty goats which were positive for SCM based on SCC and LPB ELISA OD value were randomly allocated into five groups namely Group I (Control), Group II (Enrofloxacin), Group III (Enrofloxacin + Vitamin E and Selenium combination), Group IV (Enrofloxacin + Trisodium citrate) and Group V (Enrofloxacin + organic Selenium) with each group comprising of eight animals.

5.4.1. Somatic cell count

The mean SCC of Group II (Enrofloxacin alone), Group III (Enrofloxacin + Vitamin E and Selenium combination), Group IV (Enrofloxacin + Trisodium citrate) and Group V (Enrofloxacin + organic Selenium) differed significantly from Group I (control) on 7^{th}, 14^{th}, 21^{st} and 30^{th} day post treatment and were statistically lower in Groups II, III, IV and V. At the same time statistically there was no significant difference between Groups II, III, IV and V during this period.

In Group II, post treatment mean SCC decreased from a base value of 16.69 to a lowest value of 4.88 lakh/ml on 7^{th} day post treatment. In Group III, there was a drop in the post treatment mean SCC from a base value of 15.44 lakh/ml and the lowest mean SCC of 2.97 lakh/ml was recorded on 30^{th} day post treatment. In Group IV, there was a drop in the post treatment mean SCC from a base value of 18.59 lakh/ml and the lowest mean SCC of 4.94 lakh/ml was recorded on 21^{st} day post treatment. In Group V, there was a definite drop in the post treatment mean SCC from a base value of 13.81 lakh/ml and the lowest mean SCC of 4.03 lakh/ml was recorded on 30^{th} day post treatment.

The mean SCC on different days of post treatment within each treatment group revealed significant decrease from the base value viz., zero day, in all the four treatment groups, whereas the control group mean SCC did not reveal any significant difference. This indicated that all the four treatments when compared to the control group, resulted in significant reduction of mean SCC from 7^{th} day post treatment to till the end of the experiment i.e., 30^{th} day.

When SCC of more than 5 lakh/ml was considered positive for SCM in goats, the result of this study exhibited that treatment with Enrofloxacin + Vitamin E and Selenium combination (Group III) and Enrofloxacin + organic Selenium (Group V) reduced SCC to less than 5 lakh/ml from 7^{th} day post treatment and continued to be so till the end of experiment. However, in treatment with Enrofloxacin alone (Group II) and Enrofloxacin + Trisodium citrate (Group IV), SCC was lower than 5 lakh/ml only on 7^{th} and 21^{st} day post treatment respectively. This indicated that Enrofloxacin + Vitamin E and Selenium combination and Enrofloxacin + organic Selenium were found to be the best treatment regimen in treating SCM in goats.

5.4.2. LPB ELISA

The mean LPB ELISA OD values on zero day did not exhibit any statistically significant difference when different groups were compared. However, the mean LPB ELISA OD values of Group II (Enrofloxacin alone), Group III (Enrofloxacin + Vitamin E and Selenium), Group IV (Enrofloxacin + Trisodium citrate) and Group V (Enrofloxacin + organic Selenium), on 7^{th}, 14^{th}, 21^{st} and 30^{th} day post treatment were significantly

higher as compared to Group I (control) and no significant difference was noticed when mean OD values of Group II, Group III. Group IV and Group V were compared.

In Group II, post treatment mean LPB ELISA OD values increased from a base OD value of 0.50 to highest OD value of 0.61 on 14^{th} day. In Group III, there was a definite increase in the post treatment mean LPB ELISA OD values from a base OD value of 0.43 to a highest OD value of 0.62 on 14^{th} day post treatment. In Group IV, post treatment mean LPB ELISA OD values increased from a base OD value of 0.46 to a highest OD value of 0.62 on 21^{st} day post treatment. In Group V, there was a definite increase in the post treatment mean LPB ELISA OD values from a base OD value of 0.42 to a highest OD value of 0.66 on 30^{th} day post treatment.

The mean LPB ELISA OD values within Group II revealed significant increase in OD values on 7^{th}, 14^{th} and 21^{st} day post treatment from the base value i.e., zero day. In Groups II, IV and V mean LPB ELISA OD values on 7^{th}, 14^{th}, 21^{st} and 30^{th} day post treatment were significantly higher than the basal mean OD value viz., zero day.

When LPB ELISA OD value of 0.55 and less was considered as positive for SCM in goats, the result of this study exhibited that the treatment groups viz., Enrofloxacin + Vitamin E and Selenium combination (Group III), Enrofloxacin + Trisodium citrate (Group IV) and Enrofloxacin + organic Selenium (Group V) resulted in LPB ELISA OD value more than 0.55 from 7^{th} day post treatment and continued to be so till the end of experiment i.e., 30^{th} day post treatment. However in treatment with Enrofloxacin alone (Group II) LPB ELISA OD value was more than 0.55 only on 7^{th}, 14^{th} and 21^{st} day post treatment. This indicated that Enrofloxacin + organic Selenium, Enrofloxacin + Vitamin

E and Selenium combination and Enrofloxacin + Trisodium citrate in decreasing order were found to be the best treatment regimen in treating SCM in goats.

The observation of reduced mean SCC following treatment during lactation period is in agreement with the observation made by earlier workers namely Storper *et al.* (1981), Ziv and Storper (1985) and Owens *et al.* (1997).

Treatment of SCM with Enrofloxacin alone resulted in significant reduction in mean SCC as well as significant increase in LPB ELISA OD values. This indicates Enrofloxacin may be the antibacterial of choice in the treatment of SCM in goats.

This observation is in agreement with the findings of Singh (2000), Kader *et al.* (2002), Ramprabu and Rajeshwar (2006), Saluja *et al.* (2005), Awasthi and Upadhyay (2006), Sahoo *et al.* (2009), Suresh *et al.* (2010) and Rajeev *et al.* (2010). Marin *et al.* (2010) and Prabavathy (2013) reported the *invitro* sensitivity pattern of Enrofloxacin was highly sensitive to isolates of caprine mastitis, thus providing additional support to conclude that Enrofloxacin is an antibacterial of choice in treatment of SCM in goats.

Treatment of SCM with Enrofloxacin + Trisodium citrate resulted in significant reduction in mean SCC as well as significant increase in LPB ELISA OD values. Trisodium citrate alters the milk pH making it unfavourable for growth of bacteria (Jena and Gupta, 2016) and this may be reason for reduced SCC in Trisodium citrate treated group.

When Vitamin E and Selenium combination and organic Selenium were administered individually along with parenteral Enrofloxacin, these two treatment

regimens resulted in lowered mean SCC and increased LPB ELISA OD value and their effect was noticed for prolonged period as compared to group treated with Enrofloxacin alone. This indicated that Enrofloxacin in combination with Vitamin E and Selenium combination or organic Selenium is beneficial in the treatment of SCM in goats.

The beneficial therapeutic effect of Vitamin E and Selenium combination and organic Selenium observed in the present study derives support from the findings of Chetan Kumar *et al.* (2014) and Sripad *et al.* (2016) who have reported that Vitamin E and Selenium is beneficial in treatment of SCM in cows. The beneficial effects of administration of Vitamin E and Selenium combination in treatment of SCM could be attributed to the antioxidant property, increased glutathione peroxidise activity, increased conversion of T4 to active T3 form of Thyroxine as indicated by Mahan (2001).

Further, administration of Vitamin E and Selenium combination and organic Selenium resulted in reduction of mean SCC up to 30^{th} day post treatment as compared to 21^{st} day post treatment in goats treated with Trisodium citrate, indicating Vitamin E and Selenium combination and organic Selenium has better therapeutic effect in the treatment of SCM in goats as compared to Trisodium citrate.

Vitamin E and Selenium combination and organic Selenium supplementation along with antibacterial resulted in reduction in SCC and increase in LPB ELISA OD values. Vitamin E and Selenium reported to improve the bactericidal activity of neutrophils (NRC, 1989 and Hogan *et al.,* 1990) and has a role in enhancing the resistance of mammary gland to infection (Weiss *et al.,* 1990). Vitamin E and Selenium also play a role in boosting the immune response thus, improving general health status of

animals (Finley *et al.*, 2001 and Klein *et al.*, 2003). In addition, Vitamin E and Selenium induces self-cure of SCM and decreases the prevalence of SCM by recruiting phagocytes to the infected milk compartment of the udder and induces an unspecified bactericidal activity in milk lactoserum (Ali Vehmas *et al.*, 1997). Vitamin E and Selenium also restrict the growth of mastitis causing pathogens, lowers SCC in milk and results in high milk quality (Jan *et al.*, 2005).

In view of these properties, Vitamin E and Selenium combination and organic Selenium as a supportive therapy seems to be beneficial in the treatment of SCM in goats.

Based on the results of the present study, it is safe to conclude that treatment regimens of Enrofloxacin + Vitamin E and Selenium combination, Enrofloxacin + organic Selenium, Enrofloxacin + Trisodium citrate and Enrofloxacin alone can be the preferred therapeutic regimens in that order for the treatment of SCM in goats.

Conclusion:

- Somatic Cell Count, Electrical Conductivity, N-acetyl-β-D-glucosaminidase activity and Liquid Phase Blocking ELISA tests could be employed as diagnostic tests for the diagnosis of Sub Clinical Mastitis in goats.
- SCC test has highest sensitivity followed by EC and NAGase activity. NAGase activity has highest specificity followed by EC and SCC, by considering LPB ELISA as standard diagnostic test to detect SCM in goats.

- SCC positively correlated with EC and NAGase activity, EC positively correlated with NAGase but LPB ELISA was negatively correlated with SCC, EC and NAGase activity.
- LPB ELISA was most specific diagnostic test to detect SCM in goats as compared to SCC, EC and NAGase activity.
- Combination of two or three diagnostic tests could be employed for diagnosis of SCM in goats and this would have an advantage of high specificity.
- Overall prevalence of SCM in goats based on SCC, EC, NAGase activity and LPB ELISA was 57.50, 52.50, 46.00 and 35.00 per cent respectively.
- Parity wise per cent prevalence of SCM in goats increased with increase in the parity and was highest during seventh parity compared to primiparous goats.
- Lactation stage wise per cent prevalence of SCM in goats was highest during early and mid lactation and least in late lactation.
- SCM in goats resulted in statistically significant increase in total leucocyte count and granulocytes. Further, a statistically significant decrease in lymphocytes was noticed.
- Results of biochemical study indicated statistically significant increase in gamma glutamyl transferase and fibrinogen level in SCM affected goats
- Results indicated that organic Selenium, Vitamin E and Selenium combination or Trisodium citrate administration as an additional therapeutic agent was more beneficial as compared to Enrofloxacin alone in treatment of SCM in goats.

- The treatment regimens of Enrofloxacin + Vitamin E and Selenium combination, Enrofloxacin + organic Selenium, Enrofloxacin + Trisodium citrate and Enrofloxacin alone can be the preferred therapeutic regimens in that order for the treatment of SCM in goats.

SUMMARY

VI. SUMMARY

The present work was undertaken to detect subclinical mastitis, prevalence of SCM, to study the haematological and biochemical alterations and to evaluate the efficacy of various supportive therapies along with antibacterial for treatment of SCM in goats. A total of two hundred composite milk samples collected from goats in and around Bidar, Karnataka were subjected to Somatic cell count, Electrical conductivity, N-acetyl-β-D-glucosaminidase activity and Liquid phase blocking enzyme linked immunosorbent assay. The mean ± SE values of SCM positive milk samples by SCC, EC, NAGase activity and LPB ELISA were 15.28 ± 0.61 lakh/ml, 9.12 ± 0.14 mS/cm, 26.26 ± 0.81 µmoles/min/ml and 0.41 ± 0.01 (OD value) respectively. Similarly, the mean ± SE values of SCM negative milk samples by SCC, EC, NAGase activity and LPB ELISA were 3.21 ± 0.10 lakh/ml, 5.12 ± 0.09 mS/cm, 8.95 ± 0.26 µmoles/min/ml and 0.60 ± 0.01 (OD value) respectively.

The diagnostic tests were evaluated individually, the per cent positivity of SCM in goats based on SCC, EC, NAGase activity and LPB ELISA tests were 57.50, 52.50, 46.00 and 35.00 per cent respectively. LPB ELISA was found to be more specific diagnostic test for detection of SCM in goats. Sensitivity and specificity of SCC, EC and NAGase activity were calculated by considering LPB ELISA as standard diagnostic test to detect SCM in goats. Sensitivity and specificity were 91.43 and 60.77 (SCC), 87.14 and 66.15 (EC) 71.43 and 67.69 (NAGase) per cent respectively.

Based on the results of these diagnostic tests it was concluded that SCC, EC, NAGase activity and LPB ELISA tests could be employed as diagnostic tests for the diagnosis of SCM in goats.

Among these four diagnostic tests, LPB ELISA has many advantages over other three diagnostic tests such as it detect major proinflammatory cytokines like Interleukin-8 which are released during early stage of inflammation followed by pentraxime-3, L-selectin, protease peptone-3 and complement component-3. Further, no nonspecific reaction was observed in this cytokine based test because the antiserum is raised against a recombinant protein which carries major immunogenic epitopes of the above mentioned cytokines and LPB ELISA works well as a diagnostic test irrespective of the pathogen involved.

Though, SCC, EC and NAGase activity were the three diagnostic tests employed commonly for diagnosis of SCM, often they have certain drawbacks such as wide variation and poor reliability. Further in the present study, the specificity of SCC, EC and NAGase activity were 60.77, 66.15 and 67.69 per cent respectively. Based on these facts LPB ELISA could be considered as most specific diagnostic test to detect SCM in goats as compared to SCC, EC and NAGase activity.

Test of correlation between the four tests used for diagnosis of SCM in goats was carried out and it was observed that SCC had positive correlation with EC ($r = 0.66$) and NAGase activity ($r = 0.65$), EC had positive correlation with NAGase activity ($r = 0.59$) but only LPB ELISA had negative correlation with SCC ($r = -0.86$), EC ($r = -0.56$) and NAGase ($r = -0.58$). The coefficient of correlations were highly significant ($P \leq 0.01$).

In the present study, the overall prevalence of SCM in goats based on SCC, EC, NAGase activity and LPB ELISA was 57.50, 52.50, 46.00 and 35.00 per cent respectively. The parity wise per cent prevalence of SCM in goats was found to be highest during seventh parity and least prevalence was noticed during first and second parity. Further the per cent prevalence of SCM in goats was found to be of increasing trend from second to seventh parity. The lactation stage wise per cent prevalence of SCM in goats was found to be highest during early and mid lactation and least in late lactation.

A total of eight goats which were found to be affected with SCM were subjected for haematological and biochemical study and were compared with haematological and biochemical values of eight goats which were apparently healthy and negative for SCM. SCM in goats resulted in statistically significant increase in total leucocyte count and granulocytes. Further, a statistically significant decrease in lymphocytes was noticed. Results of biochemical study indicated a statistically significant increase in gamma glutamyl transferase and fibrinogen level in SCM affected goats. Statistically there was no significant difference in alanine transaminase, aspartate transaminase, total protein, albumin and globulin between healthy and SCM affected goats. It is safe to conclude that plasma fibrinogen estimation may be used to detect SCM in goats in conjugation with other routine diagnostic tests.

Forty goats which were positive for SCM based on SCC and LPB ELISA, were randomly allocated into five groups namely Group I (Control), Group II (Enrofloxacin), Group III (Enrofloxacin + Vitamin E and Selenium), Group IV (Enrofloxacin + Trisodium citrate) and Group V (Enrofloxacin + organic Selenium) with each group

comprising of eight animals, were utilized for therapeutic study. Milk samples from all the forty goats belonging to different experimental groups were collected on zero, 7^{th}, 14^{th}, 21^{st} and 30^{th} day post treatment and were subjected to SCC and LPB ELISA.

Treatment of SCM with Enrofloxacin alone resulted in significant reduction in mean SCC (less than 5 lakh/ml) as well as significant increase in LPB ELISA OD values (more than 0.55 OD). This indicates that Enrofloxacin can be an antibacterial of choice in the treatment of SCM in goats. Treatment of SCM with Enrofloxacin + Trisodium citrate resulted in significant reduction in mean SCC as well as significant increase in LPB ELISA OD values. Trisodium citrate alters the milk pH making it unfavourable for growth of bacteria and this may be reason for reduced SCC in Trisodium citrate treated group. Further, administration of Vitamin E and Selenium combination and organic Selenium resulted in reduction of mean SCC up to 30^{th} day post treatment as compared to 21^{st} day post treatment in goats treated with Trisodium citrate, indicating Vitamin E and Selenium combination as well as organic Selenium has better therapeutic effect in the treatment of SCM in goats as compared to Trisodium citrate.

Based on the results of this study, it is safe to conclude that treatment regimens of Enrofloxacin + Vitamin E and Selenium combination, Enrofloxacin + organic Selenium, Enrofloxacin + Trisodium citrate and Enrofloxacin alone can be the preferred therapeutic regimens in that order for the treatment of SCM in goats.

BIBLIOGRAPHY

VII. BIBLIOGRAPHY

AJUWAPE, A.T.P., ROBERTS, A.A., SOLARIN, O.O., and ADETOSOYE, A.I., 2005. Bacteriological and haematological studies of clinical mastitis in goats in Ibadan, OYO State, Nigeria. *Small Rumin. Res.*, **60**: 307–310

ALI-VEHMAS, T., VIKERPUUR, M., FANG, W. and SANDHOLM, M., 1997. Giving Selenium supplements to dairy cows strengthens the inflammatory response to intramammary infection and induces a growth-suppressing effect on mastitis pathogens in whey. *J. Vet. Med.*, **44**(9-10): 559-571

AL-MAJALI, A.M. and JAWABREH, S., 2003. Period prevalence and etiology of subclinical mastitis in Awassi sheep in southern Jordan. *Small Rumin. Res.*, **47**: 243-248

ARGUELLO, A., 2011. Trends in goat research: a review. *J. Appl. Anim. Res.*, **39**(4): 429-434

AULRICH, K. and BARTH, K., 2008. Intramammary infections caused by coagulase-negative staphylococci and the effect on somatic cell counts in dairy goats. *Landbauforschung*, **58**(1-2): 59-64

AWASTHI, P. and UPADHYAY, A.K., 2006. Screening of subclinical mastitis using cow side test and California mastitis test. *Indian Vet. J.*, **83**(3): 275-276

BACHAYA, H.A., ZAFAR, I., ABDUL, J., ABBAS, R.Z. and RIASAT, A., 2005. Subclinical bovine mastitis in Attock District of Punjab (Pakistan). *Int. J. Agri. Bio.*, **7**(6): 1034-1035

BAGNICKA, E., STRAZAKOWSKA, N., JOZWOK, A., KABA, J. and KRZYZEWSI, J., 2007. Is somatic cell count in goat's milk a reliable indicator of udder health? *Wiadomosci Zootechniczne*, **45**(1/2): 35-41

BARTH, K., 2009. Eutergesundheitsüberwachung bei Milchschafen und Milchziegen - welche Methoden sind geeignet. *Landbauforschung,* **332**: 89-95

BEHESHTI, R., SHAIEGHI, J., ESHRATKAH, B., GHALEHKANDI, G.J. and MAHERISIS, N., 2010. Prevalence and etiology of subclinical mastitis in Ewes of the Tabriz Region, Iran. *Global Veterinaria.,* **4**(3): 299-302

BERGONIER, D., DE CREMOUX, R., RUPP, R., LAGRIFFOUL, G. and BERTHELOT, X., 2003. Mastitis of dairy small ruminants. *Vet. Res.*, **34**: 689-716

BOGDAN, L., GROZA, I., CIUPE, S., MORAR, I., POP, A.R., PESTEAN, C. and CENARIU, M., 2005. Comparative researches concerning the diagnosis and treatment of subclinical mastitis in cows. *Lucrai-Stiinifice-Medicina-Veterinara,Universitatea-de-Stiinte-Agricole-si-Medicina-Veterinara-"Ion-Ionescu-de-la- Brad"-Iasi,* **48**(7): 390-396

BOSCOS, C., STEFANAKIS, A., ALEXOPOULOS, C. and SAMARTZI, F., 1996. Prevalence of subclinical mastitis and influence of breed, parity, stage of lactation and mammary bacteriological status on Coulter Counter Counts and California Mastitis Test in the milk of Saanen and autochthonous Greek goats. *Small Rumin. Res.*, **21**: 139-147

BRAR, R.S., SANDHU, H.S. and SINGH, A., 2000. Veterinary clinical diagnosis by laboratory methods, *1st edn.*, Kalyani publishers, New Delhi, pp: 89-91

CHAGUNDA, M. G., LARSEN, T., BJERRING, M. and INGVARTSEN, K. L., 2006. L-lactate dehydrogenase and N-acetyl-beta-D-glucosaminidase activities in bovine milk as indicators of non-specific mastitis. *J. Dairy Res.*, **73**: 431-440

CHAHAR, A., 2007. Studies on comparative evaluation of various screening tests for detection of sub clinical mastitis in cows. *Intas-Polivet.,* **8**(1): 208-211

CHANDRASEKARAN, D., KAVITHA, S., SUBAPRIYA, S., NAMBI, A.P., THIRUNAVUKKARASU, P.S. and VARIAMUTHU, S., 2015. Haemato-biochemical alterations of resistant mastitis in dairy cows. *Indian Vet. J.*, **92**(12): 11–13

CHEN S., ANKUO, S., JINSHYAN, W., YENU, W. and JENNCHANG, L., 2008. An auto-detecting system for mastitis in dairy goats. *J. Taiwan Liv. Res.*, **41**(3): 163-171

CHETAN-KUMAR, G.K. and BHAT, M.N., 2014. Studies on efficacy of meloxicam, Vitamin E and Selenium in conjuction with antibiotics in the treatment of bovine mastitis. *Indian J. Anim. Res.*, **48**(5): 513-515

CHRISTODOULOPOULOS, G., N. SOLOMAKOS, P. D., KATSOULOS, A., MINAS, and KRITAS, S.K., 2008. Influence of oestrus on the heat stability and other characteristics of milk from dairy goats. *J. Dairy Res.*, **75**: 64–68

CHUL, K. Y., HAYEON, P., YUNG, L. Y., JEONG, L. C., GUKHYUN, S. and CHAIYONG, L., 2008. Comparison of 5 staining methods for somatic cells in dairy goat milk samples. *J. Vet. Clinics.*, **25**(4): 274-279

COLLEE, J. G., MARMION, B. P., FRASER, A. G. and SIMMONS, A., 1996. Mackie and McCartney Practical medical microbiology, *14th edn.* Longman Singapore publishers (Pte) Ltd., *pp*: 445, 851-852

CONTRERAS, A., CORRALES, J. C., SIERRA, D. and MARCO, J., 1995. Prevalence and aetiology of non-clinical intramammary infection in Murciano-Granadina goats. *Small Rumin. Res.*, **17**: 71–78

CONTRERAS, A., MIRANDA, R.E., SANCHEZ, A., DE LA FE, C., SIERRA, D., LUENGO, C. and CORRALES, J.C., 2008. Presence of mycoplasma species and somatic cell counts in bulk-tank goat milk. *Small Rumin. Res.*, **75**: 247–251

CONTRERAS, A., PAAPE, M.J., and MILLER, R.H., 1999. Prevalence of subclinical intramammary infection caused by *Staphylococcus epidermidis* in a commercial dairy goat herd. *Small Rumin. Res.*, **31**: 203-208

CONTRERAS, A., SIERRA, A. D., CON ALES, B. J .C. SANCHEZ, A. and MARCO, J., 1996. Physiological threshold of somatic cell count and California Mastitis Test for diagnosis of caprine subclinical mastitis. *Small Rumin. Res.*, **21**: 259-264

CRUZ, M.D.L., SERRANO, E., MONTORO, V., MARCO, J., ROMEO, M., BASELGA, R., ALBIZU, I. and AMORENA. B., 1994. Etiology and prevalence of subclinical mastitis in the Manchega sheep at mid-late lactation. *Small Rumin. Res.*, **14**: 175-180

DAS, M. and SINGH, M., 2000. Variation in blood leucocytes, somatic cell count, yield and composition of milk of crossbred goats. *Small Rumin. Res.*, **35**: 169-174

DE CRÉMOUX, R., POUTREL, B., BERNY, F., 1995. Use of somatic cell counts for presumptive diagnosis of intramammary infections in goats. In: *The 3rd International Mastitis Seminar*, Proceedings II, pp*:* 90

DE, U.K. and MUKHERJEE, R., 2009. Prevalence of Mastitis in Croossbred Cows. *Indian Vet.* J., **86**: 858-859

DHILLON, K.S., SINGH, S., VARINDRA. and SINGH, T.J., 2000. Restoration of buffalo milk constituents during mastitis treatment with tri-sodium citrate. *Ind. J. Dairy. Sci.*, **53:** 323-325

DIANA, S. and ROTARU, O., 2006. Somatic cells and milk health. *Seria Medicina Veterinaria.*, **63**: 385-390

DIAZ, J. R., ROMERO, G., MUELAS, R., SENDRA, E., PANTOJA, J. C. F. and PAREDES, C., 2011. Analysis of the influence of variation factors on electrical conductivity of milk in Murciano Granadina goats. *J. Dairy Sci.*, **94**: 3885–3894

DOGRUER, G., SARIBAY, M.K., ERGUN, Y., ASLANTAS, O., DEMIR, C. and ATES, C.T., 2010. Treatment of subclinical mastitis in Damascus goats during lactation. *Small Rumin. Res.*, **90**: 153-155

DRAKE, E. A., PAAPE, M. J., DICARLO, A. L., LEINO, L. and KAPTURE, J., 1992. Evaluation of bulk tank goat milk samples. Proceedings of the 31st Annual Meeting of National Mastitis Council, Arlington, VA, pp: 236

DWIVEDI, H.P., ASATI, M., JAIN, V., KUMAR, M. and UPADHYAY, A.K., 2004. Comparative study of blood profile in mastitic cow. *XXII Annual convention of ISVM and National Symposium*, Izatnagar, Uttar Pradesh.

ECKERSALL, P.D., 2000. Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. *Revue. Med. Vet.*, **151**: 577-584

EL-DEEB, W.M., 2013. Clinicobiochemical investigations of gangrenous mastitis in does: immunological responses and oxidative stress biomarkers. *J. Zhejiang Univ-Sci B (Biomed & Biotechnol)*, **14**: 33-39

EMANUELSON, U., OLSSON, T., HOLMBERG, O., HAGELTORN, M., MATTILA, T., NELSON, N. and ASTROM, G., 1987. Comparison of some screening test for detecting mastitis. *J. Dairy Sci.*, **70:** 880-887

FAO, 2010. Country Pasture / Forage Resource Profiles – Brazil. Food Animal Organization http://www.fao.org/ag/AGP/AGPC/doc/counprof/Brazil brazil.htm

FASULKOV, I., KARADAEV, M., VASILEV, N., URUMOVA, V., and MIRCHEVA, T., 2014. Determination of plasma fibrinogen and haptoglobin, hematological and blood biochemical changes in Bulgarian local goats with experimentally induced *Staphylococcus aureus* mastitis. *Turk. J. Vet. Anim. Sci.*, **38**: 439-444

FERNANDO, R. S., RINDSIG, R. B. and SPAHR. S. L., 1982. Electrical conductivity of milk for detection of mastitis. *J. Dairy Sci.*, **65**: 659–664

FERNANDO, R. S., SPAHR, S. and JASTER. E. H., 1985. Comparison of electrical conductivity of milk with other indirect methods for detection of subclinical mastitis. *J. Dairy Sci.*, **68**: 449–456

FINLEY, J.W., IP, C., LISK, D.J., DAVIS, K.J., HINTZE. and WHANGER, P.D., 2001. Cancer protective properties of high Selenium broccoli. *J. Agric. Food Chem.,* **49:** 2679-2683

FORMAN, W.B. and BARNHART, M.I., 1964. Cellular site for fibrinogen synthesis. *J. Am. Vet. Med. Assoc.,* **187**: 128-132

GAIN, A., MUKHERJEE, J., CHATTERJEE, S., BATABYAL, S. and GUHA, C., 2015. Alteration in the activity of blood and milk leukocytes together with the serum enzyme profile during sub-clinical mastitis in cross-bred cows. *Indian J. Anim. Sci.,* **85**(8): 856–860

GAMBO, H. and ETCHIKE, C.A., 2001. Detection of subclinical mastitis in lactating Gudali zebus in North Cameroon. *Revue-d'Elevage-et-de-Medecine-Veterinaire-des-Pays-Tropicaux,* **54**(1): 5-10

GEBREWAHID, T.T., ABERA, B.H. and MENGHISTU, H.T., 2012. Prevalence and etiology of subclinical mastitis in small ruminants of Tigray regional state, North Ethiopia. *Vet. World,* **5**(2): 103-109

GERARDI, G., BERNARDINI, D., ELIA, C.A., FERRARI, V., IOB, L. and SEGATO, S., 2009. Use of serum amyloid A and milk amyloid A in the diagnosis of subclinical mastitis in dairy cows. *J. Dairy. Res.,* **76**(4): 411-417

GOMES, V., LIBERA, A.M.M.P.D., PAIVA, M., MADUREIRA, K. M. and ARAUJO, W.P., 2006. Effect of the stage of lactation on somatic cell counts in healthy goats (Caprae hircus) breed in Brazil. *Small Rumin. Res.*, **64**(1/2): 30-34

GONZALO, C., ARIZNABARRETA, A., CARRIEDO, J. A. and SAN PRIMITIVO, F., 2002. Mammary pathogens and their relationship to somatic cell count and milk yield losses in dairy ewes. *J. Dairy Sci.*, **85**:1460–1467

GRONLUND, U., SANDGREN, C.H. and WALLER, K.P., 2005. Haptoglobin and serum amyloid A in milk from dairy cows with chronic sub-clinical mastitis. *Vet. Res.*, **36:** 191-198

GROVE, T.M. and JONES, G.M., 1992. Use of an enzyme-linked immunosorbent assay to monitor the control of *Staphylococcus aureus* mastitis. *J. Dairy Sci.*, **75**(2): 423-434

GUHA, A. and GERA, S., 2001. Etio-prevalence of subclinical mastitis in Holstein X Haryana crossbred cattle. *EAMR.*, **1:** 75-78

GUPTA, V. K., ASHOK KUMAR., SHARMA, S. D. and KUMAR, A., 1999. Incidence of clinical caprine mastitis in farm conditions. *Indian J. Anim. Sci.*, **69**(12): 1032-1034

GUPTA, V.K., SHARM, S.D. and KUMAR, A., 2002. *In vitro* and *in vivo* therapeutic studies on sub clinical mastitis in goats. *Indian. J. Anim. Sci.*, **72**(2): 125-127

HAENLEIN, G. F. W., 2002. Relationship of somatic cell counts in goat milk to mastitis and productivity. *Small Rumin. Res.*, **45**: 163–178

HAENLEIN, G. F. W., 2004. Goat milk in human nutrition. *Small Rumin. Res.*, **51**: 155–163

HALL, S. M. and RYCROFT, A. N., 2007. Causative organisms and somatic cell counts in subclinical intramammary infections in milking goats in the UK. *Vet. Record*, **160**(1): 19-22

HAMANN, J. and ZECCONI, A., 1998. Evaluation of the electrical conductivity of milk as a mastitis indicator. *Bull. IDF.*, **334**: 5–22

HERBELINE, C., POUTREL, B., GILBERT, F.B. and RAINARD, P., 1997. Immune Recruitment and Bactericidal Activity of Neutrophils in milk of cows vaccinated with Staphylococcal a- Toxin. *J. Dairy Sci.,* **80**(9): 2025-2034

HISS, S., MUELLER, U., NEU-ZAHREN. and SAUERWEIN, H., 2007. Haptoglobin and lactate dehydrogenase measurements in milk for the identification of subclinically diseased udder quarters. *Veterinarni Medicina.,* **52**(6): 245-252

HOGAN, J.S., SMITH, K.L., WEISS, W.P., TODHUNTER, D.A. and SHOCKEY, W.L., 1990. Relationships among Vitamin E, Selenium and bovine blood neutrophils. *J. Dairy Sci.,* **73:** 2372

HOLDAWAY, R. J., HOLMES, C. W. and STEFFERT, I. J., 1996. A comparison of indirect methods for diagnosis of subclinical intramammary infection in lactating dairy cows. *Aust. J. Dairy Technol.,* **51**:64-82

ISLAM, A., SAMAD, A. and RAHMAN, A.K.A.A., 2012. Prevalence of subclinical caprine mastitis in Bangladesh based on parallel interpretation of three screening tests. *Int. J. Anim. Vet. Adv.*, **4**(3): 225-228

JAIN, J., KARNANI, M., KHAN, A. and SHARMA, S., 2013. Comparative investigation of various biochemical parameters of cattle suffering from mastitis in semi arid Rajasthan. *J. Immunol. Immunopathol.*, **15**(1): 137

JAN, S., TIEN, H., BJORN, AKESSON and JACOB, H. N., 2005. Dietary supplementation with organic Selenium (Sel-Plex®) alters oxidation in raw and pasteurized milk. *Alltech's 21st Annual Symposium,* pp: 249-257

JENA, P.K. and GUPTA, A.R., 2016. Successful management of mastitis in goat using antibacterial, antioxidant and milk buffer. *34th Annual convention of ISVM and National Symposium, Ludhiana.* pp: 33

JING, X., HAN, Y., CAO, D., MOU, S., LIU, H., YAO, J., ZHAO, L., ZHAO, Y., SHANG, C. and CHEN, D., 2012. Kinetics of interleukin-17 and interleukin-17 associated cytokines in sera and milk in dairy goat mastitis experimentally induced with *E. coli*. *J. Anim. Vet. Adv.*, **11**(5): 597-602

JUAREZ, M. and RAMOS, M., 1986. Physico-chemical characteristics of goat milk as distinct from those of cow milk. In International Dairy Federation (Ed.), Proceedings of the IDF seminar production and utilization of ewe's and goat's milk, Bulletin No. 202 Athens, Greece, pp: 54-67

KADER, M.A., SAMAD, M.A., SAHA, S. and TALEB, M.A., 2002. Prevalence and aetiology of subclinical mastitis with antibiotic sensitivity to isolated organisms among milch cows in Bangladesh. *Indian J. Dairy. Sci.,* **55**(4): 218-223

KALOREY, D.R., SHANMUGAM, Y., KURKURE, N.V., CHOUSALKAR, K.R. and BARBUDDHE, S.B., 2007. PCR-based detection of genes encoding virulence determinants in *Staphylococcus aureus* from bovine subclinical mastitis cases. *J. Vet. Sci.,* **8:** 151-154

KANEKO, J.J., HARVEY, J.W. and BRUSS, M.L., 2008. Clinical Biochemistry of Domestic Animals. *Saunders, 6th edn.* Philadelphia, USA.

KATSOULOS, P.D., CHRISTODOULOPOULOS, G., MINAS, A., KARATZIA, M.A., POURLIOTIS, K. and KRITAS, S.K., 2010. The role of lactate dehydrogenase, alkaline phosphatase and aspartate aminotransferase in the diagnosis of subclinical intramammary infections in dairy sheep and goats. *J. Dairy Res.*, **77**(1): 107-111

KEISLER, D. H., ANDREWS, M. L. and MOFFATT, R. J., 1992. Subclinical mastitis in ewes and its effect on lamb performance. *J. Anim. Sci.,* **70**, 1677–1681

KITCHEN, B. J. and MIDDLETON, G., 1976. Enzymic methods for the estmation of the somatic cell count in bovine milk. *Australian Vet. J.,* pp: 491-494

KITCHEN, B.J., 1976. Enzymatic methods for the estimation of the somatic cell count in bovine milk. *J. Dairy Res.*, **43**: 251-258

KLEIN, E.A., THOMPSON, S.M., LIPPMAN, P.J., GOODMAN, D., ALBANES, P.R., TAYLOR. and COLTMAN., 2003. The Selenium and Vitamin E cancer prevention trial. *Urolog. Semin. Orig. Invest.*, **21:** 59-65

KOSTELIC, A., CERGOLJ, M., TARIBA, B., RUPIC, V., BENIC, M., GANTNER, V. and STOKOVIC, I., 2009. Prevalence and aetiology of subclinical mastitis in goats. *Ital. J. Anim. Sci.*, **8**(3): 134-136

KROMKER, V., 2007. Kurzes Lehrbuch Milchkunde und Milchhygiene. *Stuttgart : Parey*, pp: 232

KUMAR, S., KUMAR, B., KUMAR, R., KUMAR, S., KHATKAR, S.K. and KANAWJIA, S.K., 2012. Nutritional features of goat milk – A review. *Indian J. Dairy Sci.*, **65**(4): 266-273

KYOZAIRE, J. K., 2003. Microbiological quality of goat milk obtained under different production systems. Master's Dissertation, 79 *p.*, University of Pretoria.

LAFI, S.Q., AL-MAJALI, A.M., ROUSAN, M.D. and ALAWNEH, J.M., 1998. Epidemiological studies of clinical and subclinical ovine mastitis in Awassi sheep in northern Jordan. *Prev. Vet. Med.*, **33**(1): 171-181

LANGONI, H., ARAUJO, W.N.D.E., SILVA, A.V.D.A., SOUZA, L.C.D.E., DE ARAUJO, W.N., DA SILVA, A.V., DE-SOUZA, L.C., 2000. Treatment of bovine mastitis with amoxicillin and enrofloxacin, alone or in combination. *Arquivos-do-Instituto-Biologico-Sao-Paulo.*, **67**(2): 177-180

LAURINAVICIUTE, V., SIUGZDAITE, J. and URBSIENE, D., 2003. Diagnosis of subclinical mastitis in goats and isolation of pathogens. *Veterinarija ir Zootechnika*. **24**: 30-33

LEITNER, G., MERIN, U., SILANIKOVE, N., EZRA, E., CHAFFER, M., GOLLOP, N., WINKLER, M., GLICKMAN, A. and SARAN, A., 2004. Effect of subclinical intramammary infection on somatic cell counts, NAGase activity and gross composition of goats' milk. *J. Dairy Res.,* **71**(3): 311-315

LEITNER, G., SILANIKOVE, N. and MERIN, U., 2008. Estimate of milk and curd yield loss of sheep and goats with intramammary infection and its relation to somatic cell count. *Small Rumin. Res.*, **74**:221-225

LERONDELLE, C., RICHARD Y. and ISSARTIAL, J., 1992. Factors affecting somatic cell counts in goat milk. *Small Rumin. Res.*, **8**: 129–139

LIEN, C. C., WAN, Y. N. and CHEN, H. N., 2005. Performance evaluation of an online EC measurement system for dairy cow mastitis inspection. *Int. Agric. Eng. J.*, **14**: 89–99

LIEVAART, J., BARKEMA, H.W., HOGEVEEN, H. and KREMER, W., 2009. Reliability of the bulk milk somatic cell count as an indication of average herd somatic cell count. *J. Dairy Res.,* **76**(4): 490-496

LIVESTOCK CENSUS, 2012. 19th Livestock Census, Department of Animal Husbandry, Dairying and Fisheries, GOI, New Delhi.

MAHAN, D.C., 2001. Selenium Metabolism in animals : What role does Selenium yeast have?. *Alltech's 17th Annual Symposium*, pp: 257 -266

MAISI, P. and RIIPINEN, I., 1988. Pathogenicity of different species of staphylococci in caprine udder. *Brit. Vet. J.*, **147**: 126-132

MAISI, P., 1990a. Milk NAGase, CMT and Antitrypsin as indicators of caprine sub-clinical mastitis infections. *Small Rumin. Res.*, **3**: 493-501

MAISI, P., 1990b. Analysis of physiological changes in caprine milk with CMT, NAGase and Antitrypsin. *Small Rumin Res.*, **3**: 485-492

MALBE, M., KLAASSEN, M., FANG, W., MYLLYS, V., VIKERPUUR, M., NYHOLM, K., SANKARI, S., SUORANTA, K. and SANDHOLM, M., 1995. Comparisons of selenite and Selenium yeast feed supplements on Se-incorporation, mastitis and leukocyte function in Se-deficient dairy cows. *J. Vet. Med. Ser.*, **42**(2): 111-121

MALHER, X., SEEGERS, H. and BEAUDEAU, F., 2001. Culling and mortality in large dairy goat herds managed under intensive conditions in western France. *Liv. Prod. Sci.*, **71**: 75-86

MANJU, A., MAITI, S. K., SINGH, N. B. and SURESH, S. K., 2012. Comparative efficacy of indirect tests for detection of sub clinical mastitis (SCM) in goats. *J. Ani. Res.*, **2**(3)

MANSELL, P.D. and SEGUYA, A., 2003. The use of a hand-held conductivity meter for the diagnosis of subclinical mastitis in dairy cows during late lactation. *N Z Vet. J.*, **51:** 21-25

MANSER, P.A., 1986. Prevalence, causes and laboratory diagnosis of subclinical mastitis in the goat. *Vet. Record,* **118**: 552-554

MARIN, P., ESCUDERO, E., FERNANDEZ-VARON, E., CARCELES, C.M., CORRALES, J.C., GOMEZ-MARTIN, A. and MARTINEZ, I., 2010. Fluoroquinolone susceptibility of *Staphylococcus aureus* strains isolated from caprine clinical mastitis in southeast Spain. *J. Dairy Sci.*, **93**: 5243-5245

MATEI, S.T., GROZA, I., ANDREI, S., BOGDAN, L., CIUPE, S. and PETREAN, A., 2010. Serum metabolic parameters in healthy and subclinical mastitis cows. *Bulletin UASVM, Vet. Med.*, **67**(1): 110-114

MAURER, J. and SCHAEREN, W., 2007. Udder health and cell count in goats. *Forum Kleinwiederkauer/Petits-Ruminants*. **11**: 6-11

MAURER, J., SCHAEREN, W. and SOLLBERGER, H., 2004. Gehalte und Eigenschaften von Ziegenmilch. Lipide in Fleisch, Milch und Ei - Herausforderung für die Tierernährung, Zürich: *Schr Inst Nutztierwiss.* **25**: 205-208

McDOUGALL, S., SUPRE, K., VLIEGHER, S.D., HAESEBROUCK, F., HUSSEIN, H., CLAUSEN, L. and PROSSER, C., 2010. Diagnosis and treatment of subclinical mastitis in early lactation in dairy goats. *J. Dairy Sci.*, **93**: 4710-4721

McSHERRY, B.J., HORNEY, F.D. and DE-GROOT, J.J., 1970. Plasma fibrinogen levels in normal and sick cows. *Can. J. Comp. Med.,* **34**: 191-197

MEGERSA, B., TADESSE, C., ABUNNA, F., REGASSA, A., MEKIBIB, B. and DEBELA, E., 2010. Occurrence of mastitis and associated risk factors in lactating goats under pastoral management in Borana, Southern Ethiopia. *Trop. Anim. Health Prod.*, **42:** 1249-1255

MELE, M., SECCHIARI, P., SERRA, A., FERRUZZI, G., PAOLETTI, F. and BIAGIONI, M., 2001. Application of the 'tracking signal' method to the monitoring of udder health and oestrus in dairy cows. *Livestock Prod. Sci.,* **72**(3): 279-284

MENZIES, I. P. and RAMANOON, Z. S., 2001. Mastitis of sheep and goats. *Vet. Clin. N. Am. Food Anim. Pract.*, **17**: 333–358

MHASE, P. P., HARNE, S. D. and SURYAWANSHI, P. R., 2007. Aerobic bacterial flora of subclinical goat mastitis in Vidarbha region of Maharashtra. *J. Bombay Vet. Col.,* **15**(1/2): 50-51

MIN, B. R., HART, S. P., SAHLU, T. and SATTER, L. D., 2005. The effect of diets on milk production and composition and on lactation curves in pastured dairy goats. *J. Dairy Sci.*, **88**: 2604-2615

MISHRA, A.K., SHALINI, SHARMA, N., KUMAR, A. and SHIVASHARANAPPA, N., 2014. Prevalence of subclinical mastitis in different breeds of goats. *Vet. Practitioner*, **15**(1): 140-141

MISHRA, A.K., SHARMA, N., KUMAR, N., SHIVASHARANAPPA, N., GURURAJ, K., ABHISHEK, PAUL, S. and CHATURVEDI, V., 2013. Prevalence and Etiology of Subclinical Mastitis in Barbari and Jamunapari Goats. *J. Immunol. Immunopath.*, **15**(1):142-142

MOHAN, M. M., SIJI, P. C., TRESAMOL, P. V. and SASEENDRANATH, M. R., 2004. Management of clinical mastitis in goats. *Indian J. Vet. Med.*, **24**(2): 123-124

MORONI, P., PISONI, G., ANTONINI, M., RUFFO, G., CARLI, S., VARISCO, G. and BOETTCHER, P., 2005. Subclinical mastitis and antimicrobial susceptibility of *Staphylococcus caprae* and *Staphylococcus epidermidis* isolated from two Italian goat herds. *J. Dairy Sci.*, **88**: 1694-1704

MOYES, K.M., LARSEN, T., FRIGGENS, N.C., DRACKLEY, J.K. and INGVARTSEN, K.L., 2009. Identification of potential markers in blood for the development of subclinical and clinical mastitis in dairy cattle at parturition and during early lactation. *J. Dairy* Sci., **92**(11): 5419-5428

NASCHIF J.I.A., NADER, F. A. and OLIVEIRA, J.A., 2006. Sensitivity and specificity of California mastitis test (CMT) and cow milk electrical conductivity throughout lactation. *Ars. Veterinaria.*, **22**(2): 135-137

NDEGWA, E.N., MULEI, C.M. and MUNYUA, S.J.M., 2000. The prevalence of subclinical mastitis in dairy goats in Kenya. *J. S. Afr. Vet. Ass.*, **71**(1): 25-27

NDIWENI, N., FINCH, J.M., 1996. Effects of in vitro supplementation with a-tocopherol and Selenium on bovine neutrophil functions: implications for resistance to mastitis. *Vet. Immunol. Immunopathol.* **51**: 67-78

NICKERSON, S.C. and OWENS, W.E., 1993. *Staphylococcus aureus* mastitis. Reasons for treatment failures and therapeutic approaches for control. *Agri. Pract.* **14**: 18-23

NIELSEN, H.C., EMANUELSON, U., BERGLUND, B. and STRANDBER, G. E., 2009. Relationship between somatic cell count and milk yield in different stages of lactation. *J. Dairy. Sci.,* **92**(7): 3124-3133

NRC., 1989. Nutrient requirements of Dairy Cattle, *6th revised edn.,* National Academy Press. Washington, DC, pp: 268

OBARA, Y., 1985. Diagnosis of bovine mastitis by determination of lysosomal enzymeactivity in milk. *J. Anim. Res.*, **18**: 298-304

OLIVEIRA, C. J. B., HISRICH, E. R., MOURA, J. F. P., GIVISIEZ, P. E. N., COSTA, R.G. and GEBREYES, W.A., 2011. On farm risk factors associated with goat milk quality in Northeast Brazil. *Small Rumin. Res.*, **98**: 64-69

OWENS, W.E., RAY, C.H., WATTS, J.L. and YANCEY, R.J., 1997. Comparison of success of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis. *J. Dairy. Sci.,* **80:** 313-317

PAAPE, M. J., POUTREL, B., CONTRERAS, A., MARCO, J. C., CAPUCO, A. V., 2001. Milk somatic cells and lactation in small ruminants. *J. Dairy Sci.,* **84**: 237-244

PAAPE, M. J., WIGGANS, G. R., BANNERMAN, D. D., THOMAS, D. L., SANDERS, A. H., CONTRERAS, A., MORONI, P. and MILLER, R. H., 2007. Monitoring goat and sheep milk somatic cell counts. *Small Rumin. Res.*, **68**: 114-125

PAAPE, M.J. and CAPUCO, A.V., 1997. Cellular defense mechanisms in the udder and lactation of goats. *J. Anim. Sci.*, **75**: 556–565

PAES, P., LOPES, S., LOPES, R.S., KOHAYAGAWA, A., TAKAHIRA, R.K. and LANGONI, H., 2003. Effect of administration of vitamin E on mammary health and milk cell counts of first parturition goats experimentally challenged with *Staphylococcus aureus*. *Arq. Bras. Vet. Med. Zootec.*, **55**(1): 1-8

PANDEY, V., ADITI, PRATIKSHA, GUPTA, S.K., SHARMA, N. and SHARMA, D., 2012. Impact of subclinical mastitis on blood biochemistry of dairy cows. *Indian. J. Anim. Sci.,* **82**(5): 477–478

PARK, Y.W. and NUTI, L.C., 1985. Electrical conductivity of goat milk in evaluating subclinical mastitis. *Proceedings of the Third Asian-Australasian Association of Animal Production Animal Science Congress,* Seoul, Republic of South Korea, **2**: 1272–1274

PARK, Y.W., 1990. Nutrient profiles of commercial goat milk cheeses manufactured in the United States. *J. Dairy Sci.,* **73**: 3059–3067

PARK, Y.W., 1991. Interrelationships between somatic cell counts, electrical conductivity bacteria counts, per cent fat and protein in goat milk. *Small Rumin. Res.*, **5**: 367-375

PASCHOAL, J. J., ZANETTI, M.A. and CUNHA, J.A., 2006. Milk somatic cell count of Selenium and vitamin E supplemented cows. *Braz. J. of Vet. Res. and Anim. Sci.,* **43**(6): 717-722

PEER, F.U. and BHATTACHARYYA, H.K., 2007. Studies on caprine gangrenous mastitis. *Indian J. Small. Rumin.,* **13**(1): 92-94

PERDIGON, G., MEDICI, M., CECILIA, M., DEMACIAS MEN., HAEDO, R., OLIVER, G. and HOLGADO, A., 1986. Significance of the presence of bovine-milk beta-glucuronidase in mastitis detection. *J. Dairy Sci.*, **69:** 27-31

PETZER, I. M., DONKIN, E. F., PREEZ, E. D., KARZIS, J., VAN DER, T. J., SCHANS, WATERMEYER, J. C. and VAN REENEN, R., 2008. Value of tests for evaluating udder health in dairy goats: somatic cell counts, California Milk Cell Test and electrical conductivity. *Onderstepoort J. Vet. Res.*, **75**: 279–287

POUTREL, B. and LERONDELLE, C., 1983. Cell content of goat milk: California mastitis test, Coulter counter, and Fossomatic for predicting half infection. *J. Dairy Sci.*, **66:** 2575-2579

PRABAVATHY, A.A., 2013. Studies on incidence of mastitis and composition of mastitic milk of goats. PhD Thesis submitted to Karnataka Veterinary Animal and Fisheries Sciences University, Bidar.

PRASAD, H., TEWARI, H. A. and SENGAR, O. P. S., 2005. Milk yield and composition of the Beetal breed and their crosses with Jamunapari, Barbari and Black Bengal breeds of goat. *Small Rumin. Res.*, **58**: 195-199

PYORALA, S., 2003. Indicators of inflammation in the diagnosis of mastitis. *Vet. Res.*, **34:** 565-578

PYORALA, S., 2009. Treatement of mastitis during lactation. *Irish Vet. J.*, **62:** 42-44

RADOSTITIS, O.M., GAY, C.C., BLOOD, D.C. and HINCHCLIFF, K.W., 2000. Veterinary Medicine: A textbook of the disease of Cattle, Sheep, Pigs, Goats and Horses. *9th edn.*, W B Saunders Co. Philadelphia.

RAJEEV, N.K., ISLOOR, S., DAS, P.K. and RATHNAMMA, D., 2010. A dendrogram based analysis of antibiogram of *S.aurues* and *E.coli* isolates from bovine mastitis. *Indian. Vet. J.*, **87**(2): 107-110

RAM-NARESH., DWIVEDI, S. K., SWARUP, D., PATRA, R. C. and NARESH, R., 2002. Evaluation of ascorbic acid treatment in clinical and subclinical mastitis of Indian dairy cows. *Asian Australas. J. Anim. Sci.*, **15**(6): 905-911

RAMPRABHU, R. and RAJESWAR, J.J., 2006. Comparative efficacy of different indirect tests in the diagnosis of subclinical mastitis in dairy cows. *Indian Vet. J.*, **83**(8): 903-904

RAYNAL-LJUTOVAC, K., PIRISI, A., DE CREMOUX, R. and GONZALO, C., 2007. Somatic cells of goat and sheep milk: analytical, sanitary, productive and technological aspects. *Small Rumin. Res.*, **68**:126-144

REDA, H. and HATHOUT, A.F., 1951. The haematologic examination of the blood of sheep under physiologic and pathologic condtions. *J. Am. Vet. Med. Assoc.*, **118**: 253-255

REKIB, A. and VIHAN, V. S., 1997. Economic losses in goat production diseases. Proceedings of III National Seminar on Small Ruminant Diseases. CIRG, Mukhdoom, Mathura.

ROMERO, A., PANTOJA, J. C. F., SENDRA, E., PERIS, C. and DIAZ, J. R., 2012. Analysis of the electrical conductivity in milking fractions as a mean for detecting and characterizing mastitis in goats. *Small Rumin. Res.*, **107**: 157–163

ROTA, A. M., GONZALO, C., RODRIGUEZ, P. L., ROJAS, A. I., MARTIN, L. and TOVAR, J. J., 1993. Effects of stage of lactation and parity on somatic cell counts in milk of Verata goats and algebraic models of their lactation curves. *Small Rumin. Res.* **12**: 211–219

RUBLE, C., FERNANDEZ, G.C., DRAN, G., BOMPADRE, M.B., ISTURIZ, M.A. and PARMO, M.S., 2001. Fibrinogen promotes neutrophil activation and delays apoptosis. *J. Immun.*, **166**: 2002-2010

RUPP, R. and BOICHARD, D., 2001. Phenotypic and genetic relationships between milk somatic cell counts and clinical mastitis in the French Holstein cattle population. *Productions Animales.*, **14**(3):193-200

SAFI, S., KHOSHVAGHTI, A., JAFARZADEH, S.R., BOLOURCHI, M. and NOWROUZIAN, I., 2009. Acute phase proteins in the diagnosis of bovine subclinical mastitis. *Vet.Clin. Pathol.*, **38**(4): 471-476

SAHOO, S.S., SAHOO, N. and PARIDA, G.S., 2009. Antibiogram of bacterial isolates from bovine subclinical mastitis. *Indian Vet. J.*, **86**(12): 1298-1299

SALUJA, P.S., GUPTA, S.L., KAPUR, M.P. and SHARMA, A., 2005. Antibiogram of bacterial isolates of bovine intramammary origin. *Indian Vet. J.*, **82**(3): 323-324

SAMANTA, A., PRASAD, S. and GHOSH, C.P., 2006. Incidence of subclinical mastitis in Karan Swiss and Karan Fries crossbred cows. *Indian J. Dairy. Sci.*, **59**(1): 1318

SAMORE, A.B., CANAVESI, F. and OLZI, E., 2001. Somatic cell count as indicator of mastitis resistance in selection. Emergenza BSE; il ruolodelbuiatranellefilieraalimentare: il bovinodall'allevamento alla tavola. Congressonazionalealghero 11-13 maggio 2001, Italy. *Atti-della-Societa-Italiana-di-Buiatria.*, **33:** 77-84

SANCHEZ, A., CONTRERAS, A. and CORRALES, J.C., 1999. Parity as a risk factor for caprine subclinical intramammary infection. *Small Rum. Res.*, **31**: 197-201

SANCHEZ, J., MONTES, P., JIMENEZ, A and ANDRES, S., 2007. Prevention of clinical mastitis with barium selenate in dairy goats from a Selenium-deficient area. *J. Dairy Sci.*, **90**: 2350-2354

SAR, T.K., SAMANTA, I., PATRA, P.H. and MANDAL, T.K., 2012. Effect of combined therapy of intravenous ceftriaxone and oral polyherbal drug on milk enzyme activity in healthy and mastitic goats. *Applied Biol. Res.*, **14**(1): 95-99

SCHAEREN, W. and MAURER, J., 2006. Prevalence of subclinical udder infections and individual somatic cell counts in three dairy goat herds during a full lactation. *Schweizer Archiv fur Tierheilkunde*, **148**(12): 641-648

SCHALM, O. W., CARROLL, E. J. and JAIN, N. C., 1971. Bovine mastitis. Philadelphia, Lea and Febiger.

SCHALM, O.W., SMITH, R. and KANEKO, J.J., 1970. Plasma protein: Fibrinogen ratios in routine clinical material from cats, dogs, horses and cattle – III. *Calif. Vet.*, **24**: 6-7; 9-10

SHARMA, S. K., TANWAR, R. K., GAHLOT, A. K. and JOSHI, M., 2007. Somatic cell counts in relation to caprine sub clinical mastitis. *Indian J. Vet. Med.*, **27**(2): 149-150

SHETTAR, V.B., 2011. Genetic characterization of Bidri goats. PhD Thesis submitted to Karnataka Veterinary Animal and Fisheries Sciences University, Bidar.

SINGH, P.N., SOOD, P.P., GUPTA, S.K .J, and BANGA, H.S., 1998. Experimental candidal mastitis in goats: Clinical, haematological, biochemical and sequential pathological studies. *Mycopathologia,* **140**: 89–97

SINGH, R., BANSAL, B. K., UPPAL, S. K., MALIK, D. S. and SINGH, R., 2000. Diagnosis of subclinical mastitis: a comparative study of different tests. *Indian J. of Anim. Res.*, **34**(2): 120-123

SINGH, R., BHARDWAJ, R.K., AZAD, M.S. and BEIGH, S.A., 2014. Effect of mastitis on haemato-biochemical and plasma mineral profile in crossbred cattle. *Indian J. Anim. Res.*, **48**(1): 63-66

SINGH, S.V., 2000. Studies on udder health profiles with special reference to acute phase proteins and supplementation therapy. MVSc thesis submitted to G.B. Pant Univ. Agri. and Tech., Pantnagar.

SITRIN, R.G., PAN, P.M. and SRIKANTH, S., 1998. Fibrinogen activates NF-Kappa B transcription factors in mononuclear phagocytes. *J. Immun.*, **161**: 1462-1476

SMITH, G.W., CONSTABLE, P.D. and MORIN, D.E., 2001. Ability of hematologic and serum biochemical variables to differentiate gram-negative and gram-positive mastitis in dairy cows. *J. Vet. Intern. Med.*, **15**: 394-400

SPAKAUSKAS, SEARGENT, J.M., LESLIE, K.E., SHIRLEY, J.E., PULKRABEK, J.L. and LIM, G.M., 2001. Sensitivity and specificity of somatic cell count and California mastitis test for identifying intramammary infection in early lactation. *J. Dairy. Sci.*, **84:** 2018-2024

SREEJA, S., BINEESH, P.P., VIJAYAKUMAR, K. and SASEENDRANATH, M.R., 2013. Comparative study of in vivo and in vitro efficacy of ceftriaxone in goat mastitis. *Indian J. Anim. Res.*, **47**(1): 75-78

SREERAMACHANDRAMURTHY., 2001. Studies on prevalence, treatment and control of bovine mastitis. MVSc thesis submitted to UAS, Bangalore.

SRIPAD, K., 2013. Detection of subclinical mastitis and evaluation of different treatment regimens in bovines. PhD Thesis submitted to Karnataka Veterinary Animal and Fisheries Sciences University, Bidar.

SRIPAD, K., UPENDRA, H.A. and SURYANARAYANA, V.V.S., 2013a. Prevalence of bovine subclinical mastitis in organized farms in and around Bangalore based on liquid phase blocking ELISA. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.*, **34**(2): 39-43

SRIPAD, K., UPENDRA, H.A. and YATHIRAJ, S., 2016. Efficacy of organic and inorganic Selenium in treatment of bovine subclinical mastitis. *IOSR J. Agri. Vet. Sci.*, **9**(4): 31-35

SRIPAD, K., UPENDRA, H.A., ISLOOR, S. and YATHIRAJ, S., 2013b. Prevalence of subclinical mastitis in organized farms in and around Bangalore. *Frontier J. Vet. Anim. Sci.*, **2**(2): 109-112

STORPER, M., ZIV, G. and SARAN, A., 1981. Evaluation of several milk sampling methods for the diagnosis of *Staphylococcus aureus* and *Streptococcus agalactiae* mastitis. *Refuah Vet.*, **38**:149-153

STUHR, T., AULRICH, K., BARTH, K., KNAPPSTEIN, K. and LARSEN, T., 2012. Influence of udder infection status on milk enzyme activities and somatic cell count throughout early lactation in goats. *Small Rumin. Res.*, **111**: 139-146

SUDHAN, N.A., SINGH, R. and SOODAN, J.S., 2005. Studies on prevalence, etiology and diagnosis of subclinical mastitis among crossbred cows. *Indian J. Anim. Res.*, **39**(2): 127-130

SURESH, R.V., SRINIVASAN, S.R., GOWRI, B. and KRISHNA, M.M., 2010. Clinical efficacy of long acting enrofloxacin in bovine subclinical mastitis - a report of six cases. *Intas Polivet*, **11**(1): 31-33

SURYANARAYANA, V.V.S., 2012. NAIP Project Report on 'Bovine Mastitis: Unraveling molecular details of host-microbe interaction and development of molecular diagnostic methods', IVRI, Bangalore.

TABRIZI, A.D., BATAVANI, R.A., REZAEI, S.A. and AHMADI, M., 2008. Fibrinogen and ceruloplasmin in plasma and mikl from dairy cows with subclinical and clinical mastitis. *Pak. J. Biol. Sci.*, **11**(4): 571-576

TANGORRA, F. M., ZANINELLI, M., COSTA. A., AGAZZI. A. and SAVOINI. G., 2010. Milk electrical conductivity and mastitis status in dairy goats: Results from a pilot study. *Small Rumin. Res.*, **90**: 109–113

THEILEN, G.H., SCHALM, O.W., STRAUB, O.C. and HUGHES, J.P., 1959. Bovine haematology – I: Leucocyte response to acute bovine mastits. *J. Am. Vet. Med. Assoc.*, **135**: 481-485

TIMMS, L. L. and SCHULTZ, L. H., 1985. N-Acetyl-β-D-glucosaminidase activity and somatic cells in goat milk. *J. Dairy Sci.*, **68**:3363-3366

TIWARI, A., SISODIA, R.S., SHARMA, R.K., MISRAULIA, K.S. and GARG, U.K., 2000. Incidence of sub-clinical mastitis in cows of Malwa region of Madhya Pradesh. *Indian J. Dairy Sci.,* **53**(4): 328-331

VASIU, C., BOGOLIN, I., CRACIUN, M. and BOLFA, P., 2007. Researches on the correlation between somatic cells number and mammary gland infection in goats. *Agricole si Medicina Veterinara*, **51**(2): 666-671

VIHAN, V.S., 1989. Determination of NAGase Activity in Milk for Diagnosis of Subclinical Caprine Masitits. *Small Rumin. Res.*, **2**: 359-366

VYAVAHARE, S.H., BONDE, S.W., MANGLE, N.S., BALLAMWAR, V.A. and WAGHMARE, S.R., 2009. Alteration in lactate dehydrogenase activity in cow milk related to udder health status. *Indian J. Field Vet.,* **5**(2): 68-70

WEISS, W.P., MORGAN, K.L., SMITH. and HOBLER., 1990. Relationship among Selenium, Vitamin E and mammary gland health in commercial dairy herds. *J. Dairy Sci.,* **73:** 381

WILSON, D. J., STEWART, K. N. and SEARS, P. M., 1995. Effects of stage of lactation, production, parity and season on somatic-cell counts in infected and uninfected dairy goats. *Small Rumin. Res.*, **16**: 165-169

WINTER, P. and COLDITZ, I.G., 2002. Immunological responses of the lactating ovine udder following experimental challenge with *Staphylococcus epidermidis*. *Vet. Immunol. Immunopathol.,* **89**(1-2): 57-65

YING, C.H., YANG, C.H.B. and HSU, J.T., 2004. Relationship of somatic cell count, physical, chemical and enzymatic properties to the bacterial standard plate count in different breeds of dairy goats. *Asian-Australas. J. Anim. Sci*. **17**: 554–559

YING, C.W, WANG, H.T and HSU, J.T., 2002. Relationship of somatic cell count, physical, chemical and enzymatic properties to the bacterial standard plate count in dairy goat milk. *Livest. Prod. Sci.,* **74**: 63-77

YING, Y.D., CAO SUIZHONG., YU SHUMIN., YAO XUEPING., LIU CHANGSONG. and DU LIXIN., 2009a. Research on lactate dehydrogenase activity in cow's milk as an indicator of subclinical mastitis. *Chinese J. Vet. Sci.,* **29**(6): 761-764

YING, Y.D., YAO, X., CAO SUIZHONG, S., YU, SHUMIN., LIU, CHANGSONG. and DU, LIXIN., 2009b. Enzymes in cow milk as diagnostic indicator of subclinical mastitis. *J. Northwest A.F. Univ. Nat. Sci.,* **37**(10): 43-46

ZAFALON, L.F., NADER FILHO, A., OLIVEIRA, J.V. and RESENDE, F.D., 2007. Comparison between California mastitis test and somatic cells count as auxiliary methods for diagnosis of the bovine subclinical mastitis by *Staphylococcus aureus* and *Corynebacterium spp. Boletim de Industria Animal.,* **62**(1): 63-69

ZAFALON, L.F., NADER, F.A., OLIVEIRA, J. V. and RESENDE, F.D., 2005. Electrical conductivity and chloride concentration of milk as auxiliary diagnostic methods in bovine subclinical mastitis. *Pesquisa-Veterinaria-Brasileira,* **25**(3): 159-163

ZAKI, M.S., SHARAF, N.E., MOSTAFA, S.O., FAWZI, O.M. and BATRAWY, N.E., 2008. Effect of subclinical mastitis on some biochemical and clinicopathological parameters in buffalo. *American-Eurasian J. Agric. Environ. Sci.*. **3**(2): 200-204

ZECCONI, A., PICCININI, R., GIOVANNINI, G., CASIRANI, G. and PANZERI. R., 2004. Clinical mastitis detection by on-line measurements of milk yield, electrical conductivity and milking duration in commercial dairy farms. *Milchwissenschaft*, **59**: 240–244

ZENG, S. S. and ESCOBAR, E. N., 1995. Effect of parity and milk-production on somatic-cell count, standard plate-count and composition of goat milk. *Small Rumin. Res.*, **17**: 269-274

ZENG, S. S., ESCOBAR, E. N. and POPHAMO, T., 1997. Daily variation in somatic cell count, composition and production of Alpine goat milk. *Small Rumin. Res.*, **26**: 253-260

ZHAO, Y., LIU, H., ZHAO, X., GAO, Y., ZHANG, M. and CHEN, D., 2015. Prevalence and pathogens of subclinical mastitis in dairy goats in China. *Trop. Anim. Health Prod.*, **47**: 429-435

ZIV, G. and STORPER. M., 1985. Intramuscular treatment of subclinical staphylococcal mastitis in lactating cows with Penicillin G, Methicillin and their esters. *J. Vet. Pharmacol. Ther.*, **8**: 276-283

ABSTRACT

VIII. ABSTRACT

The present study was undertaken with the objectives of detecting subclinical mastitis in goats based on different diagnostic tests, to study the prevalence of SCM, to study certain haematological and biochemical alterations and to evaluate different treatment regimens in treatment of SCM in goats.

A total of 200 milk samples were collected from goats in and around Bidar, Karnataka and screened for SCM by Somatic cell count, Electrical conductivity, N-Acetyl-β-D-Glucosaminidase activity and Liquid phase blocking ELISA. The percentage prevalence of SCM based on SCC, EC, NAGase activity and LPB ELISA was 57.50, 52.50, 46.00 and 35.00 per cent respectively. SCC was positively correlated with EC and NAGase activity, EC was positively correlated with NAGase activity, LPB ELISA was negatively correlated with SCC, EC and NAGase activity. The parity wise per cent prevalence of SCM in goats was increased with increase in the parity and was highest during seventh parity compared to primiparous goats. Lactation stage wise per cent prevalence of SCM in goats was highest during early and mid lactation and least in late lactation. Results of haematology indicated that increase in total leucocyte count and granulocyte count and decrease in lymphocyte count in SCM affected goats. Results of biochemical study indicated that increase in gamma glutamyl transferase and fibrinogen level in SCM affected goats. Results of the study indicated that organic Selenium, Vitamin E and Selenium combination and Trisodium citrate administration as therapeutic additives were more beneficial as compared to Enrofloxacin alone in treatment of SCM in goats.

Key words: Subclinical mastitis, Goats, Diagnosis, Therapy.

APPENDIX

IX. APPENDIX

1. *p*-Nitrophenyl N-aetyl-β-D-glucosaminide – 3.3 mM

p-nitrophenyl N-aetyl-β-D-glucosaminide - 1.12959 g

Citrate Buffer (0.33 mM, pH – 4.6)

2. Citrate Buffer 0.33 mM (pH – 4.6)

Solution A - 25.5 ml

Solution B - 24.5 ml

Distilled water - 50 ml

a) Solution A (0.33 M Citric acid)

Citric acid - 63.4029 g

Distilled water - 1000 ml

b) Solution B (0.33 M Sodium citrate)

Sodium citrate - 97.053 g

Distilled water - 1000 ml

3. Glycine – 1M containing 1% Sodium doxycholate (pH 10.0)

Glycine - 75.07 g

Distilled water - 1000 ml

Add 10 g of Sodium deoxycholate to the Glycine solution.

Adjust the pH to 10.00 with Sodium hydroxide

4. Coating Buffer (Carbonate-bicarbonate Buffer, pH 9.6)

Working Solution – should be freshly prepared each time just before use

Solution A - 7.0 ml

Solution B - 17.0 ml

Distilled water - 76.0 ml

Solution A :

Sodium carbonate (anhydrous) - 2.12 g

Distilled water - 100 ml

Solution B :

Sodium bicarbonate - 1.68 g

Distilled water - 100 ml

5. Phosphate Buffer

Disodium hydrogen phosphate - 1.44 g

Potassium dihydrogen phosphate - 0.2 g

Sodium chloride - 8.0 g

Potassium chloride - 0.2 g

Distilled water - 1000 ml

6. Washing Buffer (PBST)

Phosphate Buffer - 1000 ml

Tween 20 - 500 µl

7. Blocking Buffer (PBST with 3% Skimmed milk powder)

Phosphate Buffer - 1000 ml

Tween 20 - 500 µl

Skimmed milk powder - 30 g

8. Substrate Solution

o-phenyl diamine dihydrochloride - 1 tablet of 5 mg

Distilled water – 12.5 ml

Add 4 µl of 3% Hydrogen peroxide for every ml of substrate solution just before adding to microtitre plate wells.