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Comparative therapeutic efficacy of various teat dip solutions in caprine mastitis

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Abstract
Mastitis is an inflammatory condition of mammary glands characterized by changes in physical characteristics of udder or milk. Its diagnosis is based on clinical signs and increased SCC. The higher SCC indicated the higher possibility of pathogen contamination. In the present study a total of 284 lactating does belonging to the government farms and private goat keepers in and around Jabalpur were screened using MCMT as a diagnostic test for clinical mastitis. The mean pH value recorded as 6.82±0.07 in T_5 group (Inj. Enrofloxacin + H.F.D. + Aloe vera) was nearer to the normal mean value (6.54±0.00) of Somatic Cell Count obtained (18.51±3.77) indicated significant reduction in somatic cell count (SCC). Further the lactose content (4.36^B±0.06) and fat content (4.23^B±0.06) were reported to be increase significantly in T_5 group, whereas mean value of protein (3.41^B±0.01) and SNF (11.25^B±0.03) revealed gradual increase in all the groups under therapeutic trial, but a significant increase was observed in T_5 group. So, on the basis of decreased pH and SCC values and a significant increase in milk parameters viz, lactose %, fat%, protein% and SNF% reported in T_5. Further, in treatment the maximum cure rate was also reported in T_5 group showed overall superiority in terms of the efficacy of the teat dip used under T_5 group as compared to the other teat dips used in T_1 (Inj-Enrofloxacin), T_2 (Inj-Enrofloxacin + Aloe vera), T_3 (Inj-Enrofloxacin + HFT) and T_4 (Inj-Enrofloxacin + 0.4% Chlorhexidine Gluconate).

Keywords: Mastitis, MCMT, somatic cell count, teat dip

Introduction
Mastitis is well known pathological condition of mammary tissues. It refers to the inflammation of mammary glands and is often encountered in dairy goats after parturition. Clinical mastitis is a major cause of loss in milk production to goat husbandry, eventually incurring economic losses to the poor farmers. However, in small ruminants since the milk production is always presumed to be secondary. Hence, mastitis never received prompt attention and remained neglected. The present study was undertaken to assess the efficacy of different teat dips in treating clinical mastitis. For this purpose lactating goats belonging to different private goat keepers and livestock farm Adhartal and Amanala in and around Jabalpur were screened for mastitis. The results of the curative therapies were judged by retesting the milk samples of treated animals at various days by SCC, milk pH, Lactose%, Fat%, Protein% and SNF% and also involved the curative rate under various groups post-treatment.

Material and Methods
Collection of milk samples
The udder of each goat was thoroughly washed with potassium permangnate solution (1:1000), wiped with clean cloth and allowed to dry before collection of the milk samples. The mid stream milk samples was collected from each half of the lactating goats.

Testing of milk samples
Modified California Mastitis Test (MCMT)
The MCMT was performed as per the method described by Shukla (1980) [1]. The reagent was prepared by adding 2ml stock solution B (Bromocresol purple reagent) to make volume 100ml by adding remaining volume of stock solution A (Sodium lauryl sulfate reagent).

MCMT Grading
MCMT Grading Equal quantity of milk and MCMT reagent was added in a mastitis paddle,

giving gentle swirling motion in a horizontal plane within a minimum agitation did mixing of the contents. In negative cases, the mixture remains liquefied. Grading of the test of positive samples was done according to the intensity of viscous and gel formation, reflecting the degree of inflammation and leukocyte count. It was scored as trace, 1+, 2+ and 3+ reaction if depending on the amount of gel formation.

Somatic Cell Count

The procedure for SCC was adopted as per method by Shukla (1980) [1]. The Ieukocyte count in the mastitic milk was made to assess the degree of infection in the repective halves. The CMT positive milk (showing flakes or change in consistency) samples were collected in sterile small glass vials. The name or number of goats from which the sample was collected was labeled on vials. The milk sample collected in vials was transported on ice to the laboratory at College of Veterinary Science and Animal Husbandry, Jabalpur for further examination.

Preparation of milk smear

The smear of milk for SCC was prepared within one hour of it's collection to minimize disintegration of leukocyte. Each milk sample was uniformly mixed by gentle shaking of the vials and the milk (0.01 ml) was spread with sterilized bacteriological loop, over one cm rectangular area on a clean microslide. The milk smear from the test sample was stained by modified Newman‘s stain. A total of 30 fields were counted under oil immersion lens and average number of cells per field was worked out. The average number of cells was multiplied by the multiplication factor of the microscope i.e. 497512 to obtain the number of cells per ml of the milk.

Microscopic Factor Determination

The diameter of the field of 10 x eye piece was predetermined by using stage micrometer. The lowest division of micrometer scale was 0.01 mm. Accordingly, the diameter was measured and obtained as 0.016 cm. The area of the microscopic field was determined by the formula. The calculation was made as under:

Diameter = 0.016 cm
Radius = 0.008 cm
Area = 3.14 x (0.008)2
= 0.000201
Microscopic factor = 100 x (1/Area)
= 497512 x (1/0.000201)
= 497512.43 or 497512

The same calibrated research microscope was used throughout the course of study (Shukla, 1980) [1].

Milk pH

Milk pH was estimated by digital pH meter standardized by non buffer solution thereafter, the pH reading of the normal and mastitic milk sample was recorded on day 0 (pre-treatment) and on day 5 and 10 (post-treatment).

Analysis of milk samples

Analysis of milk sample was done by milk analyser to estimate Protein, Lactose, Fat and Solid not fat (SNF) and reading of the normal and mastitic milk sample was recorded on day 0 (pre-treatment) and on day 5 and 10 (post-treatment). Under the present study the score +3 was taken for the therapeutic regimen. The +3 score is resultant as a result of infiltration of leukocytes in milk leading to gel formation and viscosity depending upon the intensity of infection as reported by Shukla, (1980) [1]. The infected does were divided into 5 groups, each comprising of 6 animals for this study while 6 healthy does were kept as control Table 1.

Table 1: Specific therapies for various groups of does under study

Group	No. of does	Drugs
T_c	6	Healthy Control
T_1	6	Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD for 5 days.
T_2	6	Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + Teat Dip Aloe Vera for 5 days.
T_3	6	Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + HFT Dip 20% for 5 days.
T_4	6	Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + Teat Dip 0.4%chlorhexidine gluconate for 5 days.
T_5	6	Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + Teat Dip Aloe Vera + HFT Dip

The results of the curative therapy were judged by retesting the milk samples after completion of the therapy on (day 5th and day 10th).

Modified California Mastitis Test (MCMT)

In present study, a total of 284 does were screened out of which 30 (10.56%) does were found to be positive as formation of gel or viscous mass on CMT paddle with MCMT score +3. Under the present study the clinical symptoms like pyrexia, reduction in milk yield, udder became tender, swollen, enlarged, painful inflamed, redness sudden onset anorexia are the visible signs recorded in does affected with clinical mastitis. However, there is reduction in milk yield, discoloration of milk, presence of clots, flakes with yellowish fluid or blood watery in consistency streaks of blood as also reported by Smith and Rogunisky (1977) [2], Devendra and Mcleroy (1982) [3], Thomas *et al.*, (1988) [4], Muhammad *et al.*, (1995) [5], and (Radostits,2000) [6]. On the post treatment the physical properties as: colour, consistency and odour of the milk was found to be apparently normal.

Result and Discussion

Somatic Cell Count (SCC)

Shangguan (2008) [7] defined SCC in milk as the combinations of the WBCs known as leukocytes which includes macrophages (66 to 68%) lymphocytes and neutrophils in milk and relatively small amount of epithelial cells accumulated to a greater amount producing an abnormal SCC in milk. Hence, SCC plays an important role in innate mammary gland immune defense system. The SCC increases as per the stage of lactation due to more number of handling for milking thereby more chances of infection as Scott *et al.*, (2002) [8], advocated SCC as better predictor of bacteriological status than the CMT score in goats and sheep. During the present study results obtained showed a drastic decrease in Somatic Cell Count (18.51±3.77) was recorded in T_5 group followed by T_3 (20.17±3.70), T_2 (21.28±3.77), T_4 (24.04±3.71) and T_1 (25.43±3.46) groups Table 2.

Table 2: Mean and standard errors of Somatic Cell Count (10^5 cells/ml) in various treatment groups at different intervals

Treatment	Interval (days)			Overall
	0	5	10	
Control	9.95^a±1.28	9.95^a±1.28	9.95^a±1.28	9.95^b±0.70
T1	43.94^a±2.99	17.41^b±2.13	14.93^b±2.22	25.43^a±3.46
T2	41.45^a±3.56	11.61^b±2.10	10.78^b±2.37	21.28^a±3.77
T3	40.63^a±2.00	10.78^b±2.37	9.12^b±2.00	20.17^a±3.70
T4	43.94^a±3.94	14.92^b±1.82	13.27^b±1.66	24.04^a±3.71
T5	39.80^a±1.82	9.12^b±1.53	6.63^b±1.66	18.51^{ab}±3.77

Note: Means with same superscript within a row for different days do not differ significantly from each other (p≤0.01). Likewise means of groups within the overall column with same superscript do not differ from each other (p≤0.01).

These values were little bit higher than the normal values as also reported by Okada (1960) [9], Deutz *et al.,* (1990) [10] and Khodke *et al.,* (2009) [11] as 7.5, 8.80 and 6.84 ×1 lakh cells/ml respectively. On post treatment the decrease number of SCC was obtained as a result of treatment effect leads to decrease inflammation due to decrease in number of PMN cells. On the other hand, the increased values of SCC reported pre treatment in all the groups can be attributed to increase PMN/neutrophils (Kitchen, 1981) [12], as a result of various enzymes into the body fluid was from damaged tissues or inflammed cells and liberation of parenchyma cells of udder resulted in increased SCC of milk (Kitchen *et al.,* 1970) [13]. As compared to control group 9.95 lakh cells /ml of milk; Similar observations have been reported by Vihan and Rubino (1996) [14] as 70 lakh cells/ml of milk in infected does as compared to uninfected goats with 3.2 lakh cells / ml of milk.

Milk pH

Milk pH could serve as the best indicator to assess the condition of udder health in dairy animals, and mentioned that highly significant difference in milk pH was due to severity in mastitis. The findings of the present study revealed that mean value of pH was increased in all the groups on day 1st with control group comparatively but on post treatment these values were decreased in all the groups significantly. The reduction in the pH was maximum in T5 group as 7.22±0.04, 6.69±0.05 and 6.56±0.04 on day 0, 5 and 10 post treatments respectively Table 3. The observations of Khodke *et al.,* (2009) [11], are nearby our findings as 6.40, as the normal ph values reported by them respectively. The probable reason for increased pH may be due to severity of mastitis that may attribute to lowered acidity as found in mastitic milk (Horvarth *et al.,* 1980) [15]. This lowered acidity is due to the reduction in lactose content, as the lactic acid formation is minimum in mastitc milk. The decreased pH value is because of reduction in the alkalinity due to inflammation thereby increased Na^+ and Cl^- ions in the milk following damage to mammary epithelium and also as a result of treatment as also observed by Ali and Hasan, (1998) [16], as 6.73 and 6.77 respectively. The study on viability of pH in milk was conducted by Agnihotri and Rajkumar (2007) [17], and observations were b/w 6.53 to 6.34 and concluded the reason being effect of the breed, stage of lactation, health of animal and bacterial invasion in raw milk. Whereas, Imran *et al.,* (2008) [18], reported the pH 6.93. Further, Juarez and Ramos (1986) [19] have reported milk pH in clinical mastitis in does between 6.50 to 6.80 in their studies.

Table 3: Mean and standard errors of pH in various treatment groups at different intervals

Treatment	Interval (days)			Overall
	0	5	10	
Control	6.54^a±0.01	6.54^a±0.01	6.54^a±0.01	6.54^c±0.00
T1	7.56^a±0.04	6.99^b±0.06	6.84^b±0.11	7.13^a±0.08
T2	7.41^a±0.02	6.92^b±0.03	6.67^c±0.11	7.00^{ab}±0.08
T3	7.40^a±0.12	6.91^b±0.03	6.64^c±0.06	6.98^{ab}±0.09
T4	7.57^a±0.02	6.98^b±0.06	6.78^b±0.11	7.11^a±0.07
T5	7.22^a±0.04	6.69^b±0.05	6.56^b±0.04	6.82^b±0.07

Note: Means with same superscript within a row for different days do not differ significantly from each other (p≤0.01). Likewise means of groups within the overall column with same superscript do not differ from each other (p≤0.01).

Schalm *et al.,* (1971) [20], mentioned the explanation of elevated pH due to increased permeability of glands to blood components due to increased movements of bicarbonate ions into the milk.

Lactose

On the analysis of milk samples maximum increase in overall lactose % (4.36±0.06) was obtained in T_5 group followed by T_3 (4.24±0.08), T_2 (4.23±0.06), T_4 (4.11±0.06) and T_1 (4.09±0.08) groups Table 4.

Table 4: Mean and standard errors of Lactose (%) in various treatment groups at different intervals

Treatment	Interval (days)			Overall
	0	5	10	
Control	4.65^a±0.01	4.65^a±0.01	4.65^a±0.01	4.65^a±0.00
T1	3.80^b±0.12	4.18^{ab}±0.13	4.29^a±0.13	4.09^c±0.08
T2	3.99^b±0.08	4.30^a±0.09	4.40^a±0.11	4.23^{bc}±0.06
T3	3.90^b±0.11	4.35^a±0.10	4.47^a±0.12	4.24^{bc}±0.08
T4	3.88^b±0.05	4.19^a±0.11	4.26^a±0.11	4.11^c±0.06
T5	4.10^b±0.08	4.43^a±0.06	4.56^a±0.07	4.36^b±0.06

Note: Means with same superscript within a row for different days do not differ significantly from each other (p≤0.01). Likewise means of groups within the overall column with same superscript do not differ from each other (p≤0.01).

Lactose content was more indicative of SCC than fat, protein, SNF and total solids although lactose content decreased with higher SCC. The different level of high SCC representing severity of infection in goats did not affect the other milk components. The stage of lactation affects the composition and lactose content. In early lactation there is high production of milk (Shangguan 2010) [7]. Similar observations have been reported by Arora (2013) [21] as 4.45 % lactose content as normal. The decreased lactose content recorded in mastitic milk under the study is well explained by decreased lactate production by udder tissues. So, the alkalinity of mastitic milk gets reduced keeping the slight acidity of milk, as a result of acidic group of casein, citrate, phospohrous and dissolved CO_2 in milk (Kitchen *et al.,*1970) [13] and (Schalm *et al.,* 1971) [20]. There was a significant difference in fat and lactose percentage in milk of goat with SCM as compared to milk of uninfected animal, while the percentage of lactose in milk of uninfected is also compared to the milk of infected does. Lactose is synthesized in the gland cells of the udder from glucose and galactose during infection reduce secretary action of mammary cells due to destruction of epithelial cells by the

leukocytes. These changes were linked with many factor such as breed, feed, environmental condition and age (Hassan, 2013) [22].

Fat, Protein and SNF

The results found for Fat, Protein and SNF are presented in Table 5, 6 & 7. Under the study maximum increase in fat % (4.23±0.06) was obtained in T_5 group followed by T_3 (4.10±0.06), T_2 (4.08±0.05), T_4 (4.00±0.06) and T_1 (3.98±0.05) groups along with maximum increase in protein % (3.41±0.01) was obtained in T_5 group followed by T_3 (3.31±0.03), T_2 (3.29±0.03), T_4 (3.31±0.03) and T_1 (3.20±0.01) groups and also maximum increase in SNF % (11.25±0.03) was obtained in T_5 group followed by T_3, T_2, T_4 and T_1 groups followed by T_3 (11.15±0.03), T_2 (11.12±0.02), T_4(11.01±0.03) and T_1(10.98±0.04) groups.

Table 5: Mean and standard errors of Fat (%) in various treatment groups at different intervals

Treatment	Interval (days)			Overall
	0	5	10	
Control	4.52^{a}±0.02	4.52^{a}±0.02	4.52^{a}±0.02	4.52^{a}±0.00
T1	3.76^{b}±0.05	4.00^{a}±0.06	4.18^{a}±0.09	3.98^{c}±0.05
T2	3.88^{b}±0.06	4.11^{ab}±0.06	4.25^{a}±0.09	4.08^{bc}±0.05
T3	3.82^{b}±0.06	4.18^{a}±0.09	4.30^{a}±0.10	4.10^{bc}±0.06
T4	3.71^{b}±0.05	4.08^{a}±0.03	4.21^{a}±0.07	4.00^{c}±0.06
T5	3.96^{b}±0.05	4.32^{a}±0.09	4.42^{a}±0.09	4.23^{b}±0.06

Note: Means with same superscript within a row for different days do not differ significantly from each other (p≤0.01). Likewise means of groups within the overall column with same superscript do not differ from each other (p≤0.01).

These values of fat % were nearer to the values obtained by Hassan (2013) [22] and Getaneh *et al.*, (2016) [23] as 4.20 and 4.8 respectively. Whereas, the observations of Arora (2013) [21] were differed from these results as 3.80. Significant difference was lower in fat % of milk from infected animal with SCM had very high increase in the activity of enzyme called lipase, that cause milk fat breakdown and release free fatty acids that produce off flavors in milk and cause great loss to dairy industry. In goats the data indicated a decrease in total protein from uninfected to infected animals (Hassan, 2013) [22]. Milk protein% is increased as lactation progressed a significant low fat content of milk during mid lactation then that from early or late lactation with altered level of SCC and lactose percentage was observed by Shangguan (2010) [7].

Table 6: Mean and standard errors of Protein (%) in various treatment groups at different intervals

Treatment	Interval (days)			Overall
	0	5	10	
Control	3.52^{a}±0.01	3.52^{a}±0.01	3.52^{a}±0.01	3.52^{a}±0.00
T1	3.13^{c}±0.01	3.20^{b}±0.01	3.26^{a}±0.01	3.20^{d}±0.01
T2	3.17^{b}±0.02	3.32^{a}±0.04	3.38^{a}±0.05	3.29^{c}±0.03
T3	3.19^{b}±0.02	3.33^{a}±0.04	3.41^{a}±0.05	3.31^{c}±0.03
T4	3.14^{c}±0.01	3.20^{b}±0.01	3.26^{a}±0.01	3.20^{d}±0.01
T5	3.32^{c}±0.01	3.43^{b}±0.01	3.49^{a}±0.02	3.41^{b}±0.01

Note: Means with same superscript within a row for different days do not differ significantly from each other (p≤0.01). Likewise means of groups within the overall column with same superscript do not differ from each other (p≤0.01).

The results also showed with significant higher values in SNF % there was increase in protein percent obtained in uninfected milk. The study indicated decrease in SNF in infected ewe's milk depend on the destruction that occur by invasion of pathogens to the mammary tissues cause decrease in synthetic activity of Mammary gland (Hassan, 2013) [22]. The nutritional component of goat milk include higher chloride content and fat %. However, they are affected by age, breed, stage of lactation does of animal processing and preparation of produced milk and managmental facilities. Hence, the goat milk with its unique compostion could be a valuable alternate. The milk composition can be affected by a wide array of factors viz: breed, age, stage of lactation and diet of animal Marchel *et al.*, (2011) [24].

Table 7: Mean and standard errors of SNF (%) in various treatment groups at different intervals

Treatment	Interval (days)			Overall
	0	5	10	
Control	11.41^{a}±0.09	11.41^{a}±0.09	11.41^{a}±0.09	11.41^{a}±0.05
T1	10.71^{b}±0.02	11.09^{a}±0.03	11.14^{a}±0.02	10.98^{de}±0.04
T2	11.01^{b}±0.04	11.16^{a}±0.03	11.19^{a}±0.03	11.12^{cd}±0.02
T3	10.97^{b}±0.04	11.21^{a}±0.03	11.28^{a}±0.03	11.15^{bc}±0.03
T4	10.82^{b}±0.03	11.10^{a}±0.03	11.13^{a}±0.02	11.01^{de}±0.03
T5	11.07^{c}±0.02	11.31^{b}±0.01	11.39^{a}±0.02	11.25^{b}±0.03

Note: Means with same superscript within a row for different days do not differ significantly from each other (p≤0.01). Likewise means of groups within the overall column with same superscript do not differ from each other (p≤0.01).

In view of the parameters studied it is concluded that the increased milk parameters i.e. lactose, fat protein and SNF % are directly correlated with each other. Simultaneously, there is reduction of inflammation and impact of treatment have support them for their enhanced values at post treatment.

Efficacy of Treatment

The efficacy of therapeutic agents in clinical mastitis are presented in Table 8.

Group T_1

The treatment followed in this group comprised of Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD for 5 days. The post treatment curative effect obtained on animals basis was 50%.

Group T_2

The animals belonging to this group were treated with Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + Teat Dip Aloe Vera for 5 days as per the schedule. A total of 6 animals were treated for 5 days. The post treatment curative rate was found to be 66.66% effective.

Group T_3

In this group animals were treated with Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + HFT Dip 20% for 5 days The post treatment curative rate was found to be 66.66%.

Group T_4

The animal in this group were treated with Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + Teat Dip 0.4%chlorhexidine gluconate for 5 days combination was found to be 50% effective.

Group T_5

The treatment followed in this group comprised of Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + Teat Dip Aloe Vera + HFT Dip for 5 days the curative effect obtained was maximum in this group i.e. 83.33%.

Table 8: Efficacy of therapeutic agents in clinical mastitis

Groups	Name of therapy	Animals treated	Animals cured	Animals cure percent
T_1	Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD for 5 days.	6	3	50
T_2	Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + Teat Dip Aloe Vera for 5 days.	6	4	66.66
T_3	Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + HFT Dip 20% for 5 days.	6	4	66.66
T_4	Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + Teat Dip 0.4%chlorhexidine gluconate for 5 days.	6	3	50
T_5	Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + Teat Dip Aloe Vera + HFT Dip	6	5	83.33

Conclusion

Significant decrease in pH and Somatic Cell Count, with maximum increase in lactose %, fat %, protein %, SNF % and maximum cure rate was noticed in T5 (Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + Teat Dip Aloe Vera + HFT Dip) group. Hence, in view of the findings of present investigation, T5 (Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + Teat Dip Aloe Vera + HFT Dip) group proved the efficacy and superiority of the teat dip used in this group, over all the other teat dips used in T1 (Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD for 5 days.), T2 (Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + Teat Dip Aloe Vera for 5 days), T3 (Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + HFT Dip 20% for 5 days) and T4 (Inj-Enrofloxacin @ 5mg/kg b.wt. I/M BD + Teat Dip 0.4%chlorhexidine gluconate for 5 days) groups.

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