

Original Research Article

Evaluation of Therapeutic Response of Different Antibiotics in Subclinical Staphylococcal Mastitis in Goats

Abstract

Subclinical mastitis (SCM) is characterized by inflammation of the udder without visible signs of inflammation in the animal, udder and milk and hence could be diagnosed by laboratory examination only. Effective treatment of SCM in goats is crucial for maintaining optimal milk production. The present work was conducted in the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur (M.P.), India. A total of 236 lactating goats irrespective of age, breed, parity and stage of lactation belonging to both organized and unorganized sectors in and around Jabalpur were screened for a period of six months. On the basis of modified California mastitis test score, out of 236 lactating goats, 83 goats were found positive for subclinical mastitis with overall occurrence as 35.17% (83/236). A total of 48 milk samples positive for modified California mastitis test were further processed for bacterial isolation and identification. 34 (70.83%) milk samples were found positive for only *Staphylococcus* spp. while 12 (25%) were harbouring mixed infection (*Staphylococcus* spp. and Gram-positive bacilli). For therapeutic study, 18 goats, positive for subclinical Staphylococcal mastitis were divided into three groups i.e. G₁, G₂ and G₃ comprised of six goats in each group. Moreover, six apparently healthy goats were taken as healthy control group (G₄). Goats of group G₁ were treated with Inj. Enrofloxacin LA @ 7.5 mg/kg b. wt. I/M single dose. G₂ were treated with Inj. Ceftizoxime @ 10 mg/kg b. wt. I/V single dose and G₃ were treated with Inj. Oxytetracycline LA @ 20 mg/kg b. wt. I/M single dose. The response of therapeutic study was evaluated on the basis of somatic cell count and modified California mastitis test score. The most effective response for subclinical Staphylococcal mastitis in goats was elicited by G₂ treated with Ceftizoxime followed by G₁ treated with Enrofloxacin LA and the least in G₃ treated with Oxytetracycline LA.

Keywords: Subclinical mastitis, MCMT, SCC, Goats, *Staphylococcus* spp.

1. INTRODUCTION

Mastitis is the inflammation of the mammary gland parenchyma, marked by alterations in both the physical and chemical composition of the milk, usually involving the presence of bacteria and accompanied by pathological changes in the glandular tissues [1]. Subclinical mastitis is characterized by the absence of visible clinical symptoms, with the only indicators being an elevated somatic cell count, the presence of pathogenic organisms in the milk and an inflammatory response detectable only through screening or laboratory tests. It is a major infectious disease in small ruminants and poses a continuous risk of infection to the entire herd. With increasing demands for higher milk production and stricter standards for milk quality in dairy goat herds, it is crucial to prevent or identify udder infections at an early stage, not only for the benefit of the farmer but also to ensure consumer safety [2].

In dairy goats affected by subclinical mastitis (SCM), coagulase-negative *Staphylococcus* (CNS) species constitute between 44.7% and 95.9% of the isolated pathogens from milk samples. In contrast, *Staphylococcus aureus*, which is typically regarded as more pathogenic, accounts for 4.1% to 18.0% of the SCM cases in goats [3].

The treatment of subclinical mastitis (SCM) is crucial for maintaining milk production and various trials have been conducted using antibacterial drugs to address mastitis in goats. Cephalosporin antibiotics are favored due to their low toxicity and broad-spectrum antibacterial activity against bacteria (both gram-positive and gram-negative). Third-generation cephalosporins such as ceftizoxime, long-acting fluoroquinolones like marbofloxacin and tetracyclines are recommended for treating SCM in goats [4]. Additionally, certain treatments including a combination of vitamin E and selenium, organic selenium and enrofloxacin, have proven to be effective and beneficial for managing SCM in goats [5].

Additionally, one of the most significant challenges in treating and controlling subclinical mastitis is the rise of drug resistance, which is often caused by the indiscriminate use of antibacterial agents. This study addresses the therapeutic management of subclinical Staphylococcal mastitis in goats in and around the Jabalpur region of Madhya Pradesh.

2. MATERIALS AND METHODS

2.1. Location and place of work

The proposed study was carried out for six months at Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University (N.D.V.S.U), Jabalpur, Madhya Pradesh (M.P.). Located at 23.17° latitude and 79.57° East longitude, Jabalpur is 410.87 mean sea level in southern part of the second agro-climatic zone, which includes Satpura Plateau and Kaymore Hills. A tropical climate prevails here with an average annual rainfall of 1241 millimeters.

2.2 Screening

For this study, a total of 236 female goats were screened. Occurrence study was conducted in lactating goats of organized and unorganized sectors. Goats were screened on the basis of history, presence of clinical symptoms and MCMT. Complete history of the case including age, breed, parity, stage of lactation etc. was recorded.

2.3 Modified California Mastitis Test (MCMT)

An equal amount of milk (5 ml) and MCMT reagent (5 ml) was added to a plastic paddle and gently swirled in a horizontal motion with minimal agitation. The score was recorded after 10 seconds, while continuing to rotate the paddle. The reaction was graded by intensity of gel formation and colour change as follows [6]: (Table 01 and Figure 01).

Table 01: MCMT grading of milk

MCMT grade	Description
Negative	No change
Trace	Slime formation which disappeared with continuous movement of paddle
1+	Distinct slime but no gel formation
2+	Viscous with gel formation which adherent to the margin of the cup
3+	The gel formation with convex projection, the gel did not dislodge after swirling movement of the paddle

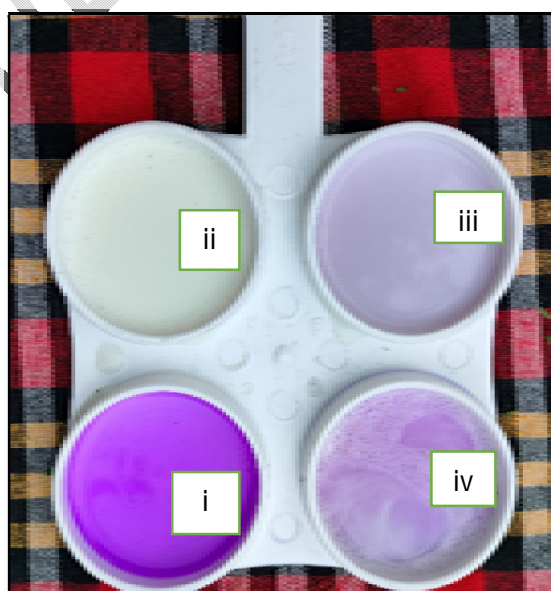


Figure01: Performing MCMT test

(i) Only reagent (ii) Only milk (iii) Negative sample (iv) Positive sample

2.4 Sample collection

For collection of milk, udder of each goat was thoroughly washed with potassium permanganate (0.01%) and wiped with clean cloth. About 10 ml of mid-stream milk was collected aseptically from each half of udder in sterilized vials and was transported (in ice) for further examination.

2.5 Processing of sample

2.5.1 Somatic Cell Count (SCC)

The somatic cell count was performed in milk samples on day '0' (pre-treatment) and on day '7' (post-treatment) in all the goats of different treatment groups. The SCC was performed with the help of somatic cell counter cum lactoscan (combo).

Procedure

1. Milk sample was homogenized on vortex mixture for one minute.
2. 20µl homogenized sample was taken with the help of micropipette in eppendorf tube and again homogenized for half a minute
3. Then 0.8µl milk sample was taken from the Eppendorf tube and was loaded on to the cartridge.
4. Excess of milk was removed by cotton
5. Cartridge was then inserted into the cartridge slot of lactoscan machine and press to continue button
6. Result was displayed on the screen

2.5.2 Isolation and identification of *Staphylococcus* spp.

Pre enrichment using Muller Hinton broth with 6.5 per cent sodium chloride

The culture media preparation and pre-enrichment of *Staphylococcus* spp. was done as per the procedure described by Markey *et al.* [7]. For pre-enrichment of *Staphylococcus* spp. milk samples were inoculated on Muller Hinton broth with 6.5 per cent sodium chloride and incubated at 37°C for 24 hours.

Inoculation of enriched sample on Mannitol Salt Agar

Pre enriched samples were cultured bacteriologically to isolate the *Staphylococcus* spp. on mannitol salt agar. These isolates were incubated aerobically at 37°C for 24 hours. The yellow or pink colony on mannitol salt agar was indicate the presence of *Staphylococcus* spp. [7].

Gram's Staining

Clean slide was prepared then a loopful of water was placed in the center of the slide. With the inoculating needle, aseptically very small amount of culture was picked up and mixed into the drop of water and was spread to 1/2-inch area. Slide was air dried and passed through a Bunsen burner flame three times to heat-fixed and to kill the bacteria. The heat fixed smear was placed on a staining receptacle and slide was covered with crystal violet solution for one minute and then washed off briefly with tap water and drained. The smear was then treated with few drops of Gram's Iodine and allowed to act for a minute. The slide was again washed in water and then decolorized in absolute ethyl alcohol. After the smear is decolorized, it was washed in water without any delay. The smear was finally treated with few drops of counter stain safranin and washed off with tap water. Excess water was removed using a blotting paper, dried in air and examined microscopically under the oil immersion lens. Gram positive cocci (violet color) arranged in grape-like clusters were seen [7].

2.6 Therapeutic regimen

A total of 18 goats, positive for subclinical Staphylococcal mastitis was divided into three groups G₁, G₂ and G₃ comprised of six goats in each group. Moreover, six apparently healthy goats, were taken as healthy control group (G₄). The following therapeutic regimen was followed (Table 02).

Table 02: Therapeutic regimen of subclinical mastitis in goats

Groups	No.of goats	Drug dosage
G ₁	06	Inj- Enrofloxacin LA @ 7.5 mg/kg b. wt. I/M single dose
G ₂	06	Inj- Ceftizoxime @ 10 mg/kg b. wt. I/V single dose
G ₃	06	Inj-Oxytetracycline LA @ 20 mg/kg b.wt. I/M single dose
G ₄	06	Healthy control

2.7 Statistical analysis

The alterations in different treatment groups at different intervals were analyzed using one-way ANOVA in R software using dplyr package and means were compared using post Hoc - Duncan's multiple range tests as per the standard procedure [8].

3. RESULTS AND DISCUSSION

3.1 Somatic cell count

Somatic cell count was recorded on day 0 (pre-treatment) and day 7 (post treatment) in all the 18 female lactating goats under therapeutic and compared with the healthy control goats.

The findings revealed that mean SCC on day 0 before treatment in goats of groups G₁, G₂ and G₃ were $14.63 \pm 0.80 \times 10^5$ cells/ml, $15.34 \pm 0.63 \times 10^5$ cells/ml and $14.10 \pm 0.89 \times 10^5$ cells/ml, respectively. After treatment the mean SCC on day 7 in goats of groups G₁, G₂ and G₃ were $11.57 \pm 1.52 \times 10^5$ cells/ml, $10.02 \pm 2.63 \times 10^5$ cells/ml and $12.42 \pm 1.72 \times 10^5$ cells/ml, respectively.

Significantly higher SCC was observed in goats having SCM on day 0 pre-treatment as compared to healthy control group. A significant reduction in the SCC of goats was observed maximum in group G₂ and G₁ on day 7 of post treatment. The details are summarized in table 03 and figure 02.

Table 03: Somatic cell count (Mean±SE) in goats in different treatment groups at different intervals

Groups (n=6)	SCC (10 ⁵ cells/ml)	
	Day '0'	Day '7'
G ₁	$14.63^{aA} \pm 0.80$	$11.57^{abB} \pm 1.52$
G ₂	$15.34^{aA} \pm 0.63$	$10.02^{abB} \pm 2.63$
G ₃	$14.10^{aA} \pm 0.89$	$12.42^{aA} \pm 1.72$
G ₄	$04.35^b \pm 0.16$	$05.28^b \pm 0.15$

Mean values with different superscripts between group (lowercase) and between days (uppercase) differ significantly (p<0.05)

The mean values of SCC recorded in Group I, Group II and Group III showed a significant difference (P≤0.05) when compared to apparently healthy goats. Similar findings were reported by Robertson and Muller [9], Min *et al.* [10], Koop *et al.* [11] and Persoon and Olofsson [12], who observed an increase in SCC in the milk of goats affected by SCM.

These changes indicate a higher SCC in the milk of goats with subclinical mastitis. The most significant subclinical abnormality observed is the elevated SCC, which is higher in goats than in cows

due to apocrine secretion [9]. Somatic cells primarily consist of milk-secreting epithelial cells shed from the mammary gland lining, along with white blood cells that enter the gland in response to injury or infection [13]. Given the direct correlation between inflammatory cells and intramammary infection, SCC is the most commonly used indicator for monitoring udder health [14]. Typically, somatic cells in milk comprise about 75% leukocytes and 25% epithelial cells. An elevated leukocyte count is a response to bacterial infection, tissue injury, stress and an increase in SCC indicates a decline in the quality of raw milk [13].

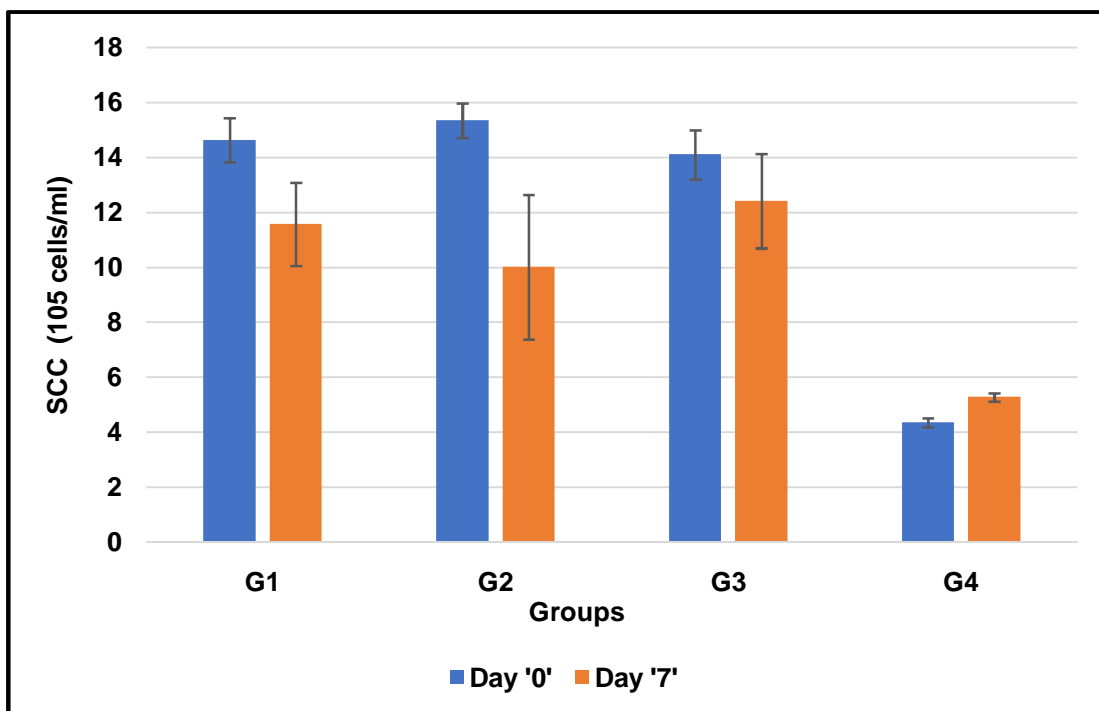


Figure 02: Mean somatic cell count in goats in different treatment groups at different intervals

3.2 Bacterial isolation and identification of *Staphylococcus* spp.

A total of 48 milk samples were found positive on MCMT in female lactating goats. Milk samples were collected for isolation and identification of bacteria on primary and selective media, respectively. 34 (70.83%) milk samples were found positive for only *Staphylococcus* spp. while 25 per cent were harbouring mixed infection (*Staphylococcus* spp. and Gram-positive bacilli). Based on the distinctive colour changes on mannitol salt agar (yellow or pink colour colony) and colony morphology (Gram positive cocci arranged in grape-like clusters). The details are summarized in table 04, figure 03 and 04.

Table 04: Isolation and identification of *Staphylococcus* spp. in milk samples found positive for SCM

Total number of milk sample examined positive for SCM	Growth on Muller Hinton broth + 6.5% NaCl	Growth on Mannitol salt agar	Gram's Staining	Mixed infection (<i>Staphylococcus</i> spp.+ gram positive Bacilli)	Only <i>Staphylococcus</i> spp.
48	48	46	46	12 (25%)	34 (70.83%)

These findings are closely similar with Najeeb *et al.* [15] and Omar and Mat-Kamir [16] who reported that *Staphylococcus* spp. isolated from SCM milk samples were 61.64 per cent and 73.2 per cent, respectively.

Staphylococcus bacteria are commonly found inside the teat canal, on udder skin and within mammary glands and are often transmitted through improper and unhygienic milking practices [17]. This can be due to increased risk of infection over time and the prolonged duration of infections, particularly in herds lacking a mastitis control program [18].

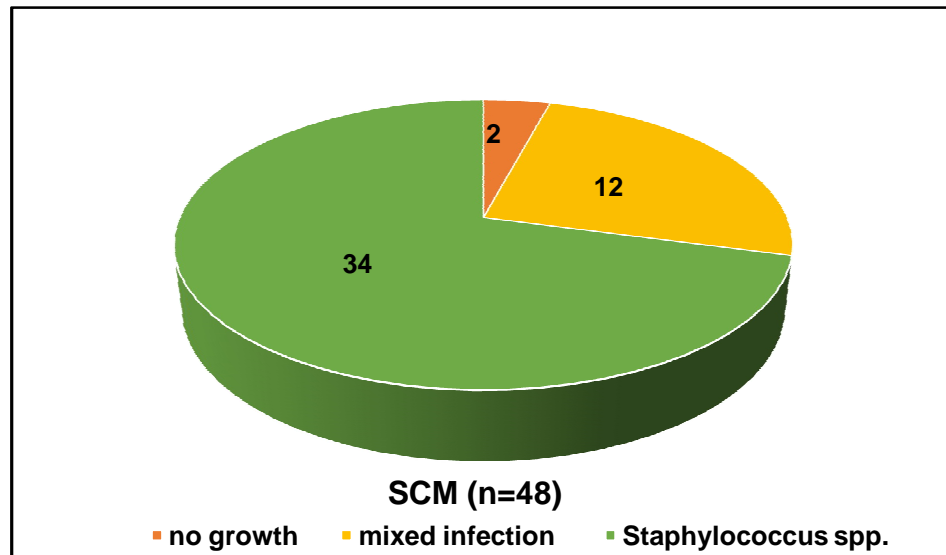


Figure 03: Distribution of bacterial growth on Mannitol Salt agar

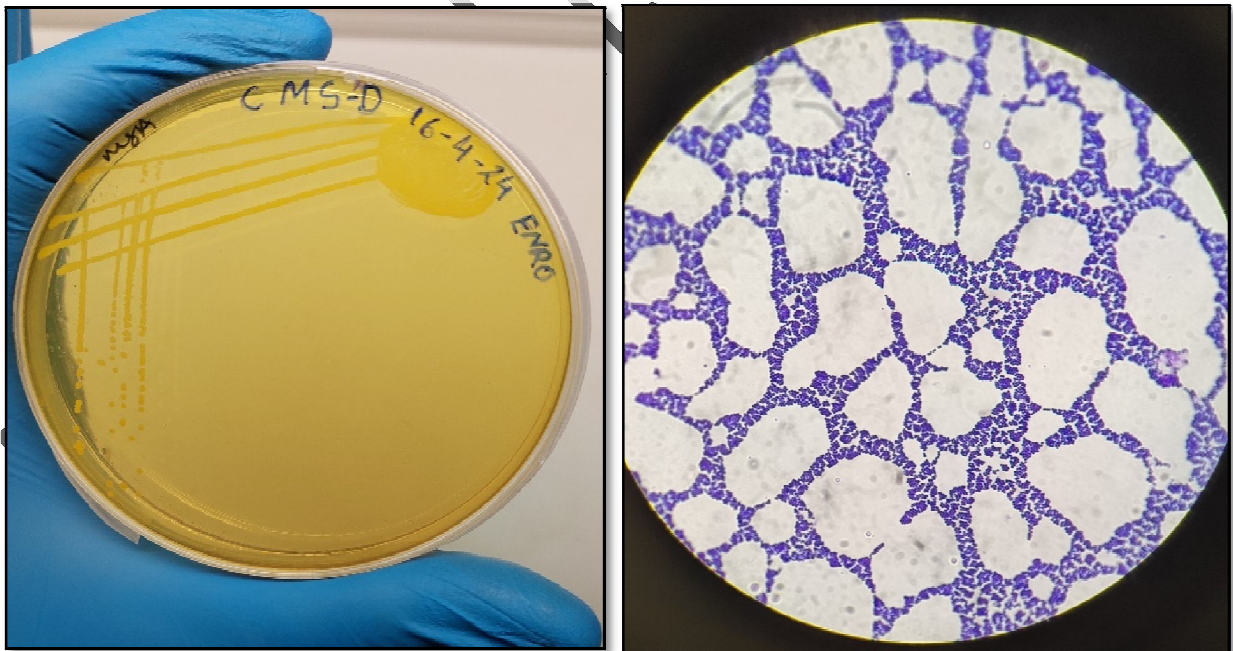


Figure 04:

(a) Yellow colored colonies of *Staphylococcus* on Mannitol salt agar

(b) Smear from pure colony showing Gram positive cocci in branches (100x)

3.3 Therapeutic response study

For the response of therapy in different treatment groups was evaluated on the basis of somatic cell count and modified California mastitis test. However, for the therapeutic study goats with sub clinical mastitis in early lactation stage was targeted in all the treatment groups. The details are summarized in table 05.

Table 05: Evaluation of therapeutic response in different treatment groups in subclinical Staphylococcal mastitis in goats

Groups	No. of goats	SCC (10^5 cells/ml)		MCMT grade		Ranking
		Day '0'	Day '7'	Day '0'	Day '7'	
G₁ (Enro. LA)		Day '0'	Day '7'	Day '0'	Day '7'	II
	1	15.60	07.40	3	1	
	2	16.00	15.90	3	3	
	3	12.20	11.98	2	2	
	4	11.99	06.99	2	1	
	5	15.80	12.20	3	2	
	6	16.20	15.00	3	3	
Mean±SE		14.63 ^{aa} ±0.80	11.57 ^{abb} ±1.52			
G₂ (Ceftizoxime)		Day '0'	Day '7'	Day '0'	Day '7'	I
	1	12.20	04.11	2	Negative	
	2	16.11	03.99	3	Negative	
	3	15.90	04.30	3	Negative	
	4	15.77	15.71	3	3	
	5	16.10	16.13	3	3	
	6	15.98	15.89	3	3	
Mean±SE		15.34 ^{aa} ±0.63	10.02 ^{abb} ±2.63			
G₃ (OTC LA)		Day '0'	Day '7'	Day '0'	Day '7'	III
	1	11.99	12.00	2	2	
	2	16.14	15.88	3	3	
	3	16.20	16.00	3	3	
	4	15.98	15.92	3	3	
	5	12.11	07.49	2	1	
	6	12.22	07.22	2	1	
Mean±SE		14.10 ^{aa} ±0.89	12.42 ^{aa} ±1.72			

Group G₁ (Enrofloxacin LA)

Out of 6 goats in two goats SCC significantly reduced i.e., from 15.6×10^5 to 07.4×10^5 and from 11.99×10^5 to 6.99×10^5 cells/ml. The similar goats showed MCMT score from +3 to +1 and +2 to +1 on day 7 (post treatment). However, in remaining 4 goats no significant difference was observed in terms of SCC and MCMT, on day 7 (post treatment).

The antibiotic used in the therapy of group G₁ suffered with subclinical Staphylococcal mastitis treated with Enrofloxacin LA @ 7.5 mg/kg b.wt. intramuscular single dose was also effective to treat SCM during lactation period, which agrees with the observation made by Owens *et al.* [19] and Halmandgeet *al.* [5]. This suggests that enrofloxacin may be an effective antibacterial agent for treating SCM in goats. Marin *et al.* [20] reported that enrofloxacin exhibited high in vitro sensitivity against isolates of caprine mastitis, further supporting its potential as a treatment option for SCM in goats. The mechanism of action of fluoroquinolones involves inhibiting DNA gyrase, which in turn inhibits bacterial cell division. In both beta-lactams and fluoroquinolones, the inhibition of bacterial replication is crucial to the development of persistence [21].

Group G₂ (Ceftizoxime)

5 out of 6 goats exhibited SCC of approximately 16×10^5 cells/ml in the milk samples, while the remaining goat had an SCC of approximately 12×10^5 cells/ml. Additionally, 5 of the 6 goats demonstrated a +3 grade on the MCMT, whereas single goat showed a +2 MCMT grade on day 0 (pre-treatment). However, 3 out of 6 goats exhibited SCC of approximately 16×10^5 cells/ml, while the other 3

goats had an SCC of approximately 4×10^5 cells/ml. Furthermore, 3 goats continued to show a +3 grade, while the remaining 3 goats tested negative for the MCMT on day 7 (post-treatment).

The antibiotic used in the therapy of group G₂ suffered with subclinical Staphylococcal mastitis treated with Ceftizoxime @10 mg /kg b.wt. intravenous single dose, potentially effective to get better recovery. The therapeutic effectiveness of ceftizoxime against the Staphylococcal mastitis is also noticed by Buragohain *et al.* [22]. In mastitis, the prolonged persistence of ceftizoxime in milk has been corroborated by the findings of Sar *et al.* [23], Karmakar *et al.* [4] and Kumar *et al.* [24]. Ceftizoxime is an amphoteric drug that exhibits increased persistence due to the shift towards alkaline pH in mammary secretions during mastitis, which promotes ion trapping. The concentration and pharmacokinetic parameters of ceftizoxime in milk suggest that it has a favorable antibacterial effect in cases of acute staphylococcal mastitis. Consequently, a single intravenous dose of ceftizoxime might be an effective treatment for the *S. aureus*. causing acute mastitis.

Group G₃ (Oxytetracycline LA)

3 out of 6 goats exhibited SCC of approximately 16×10^5 cells/ml in the milk samples, while the remaining 3 goats had an SCC of approximately 12×10^5 cells/ml. Additionally, 3 of the 6 goats demonstrated a +3 grade, whereas remaining 3 goats showed a +2 grade on the MCMT, on day 0 (pre-treatment). However, 3 out of 6 goats continued to exhibit SCC of approximately 16×10^5 cells/ml, Of the remaining goats, 2 had an SCC of approximately 7.5×10^5 cells/ml and one had SCC of approximately 12×10^5 cells/ml. Furthermore, 3 goats showed a +3 grade, while 2 goats showed +1 grade and a remaining goat represented +2 grade on the MCMT on day 7 (post-treatment).

The antibiotic used to treat the group G₃ with Oxytetracycline LA @ 20 mg /kg b.wt intramuscular single dose was not well efficient to treat the subclinical Staphylococcal mastitis. This finding was closely related with the observation of Kirkan *et al.*[25].This issue may stem from the misuse of antibiotics, as frequent use of the same antibiotics can lead to antibiotic resistance. Additionally, the bacteria may resist treatment because they can exist intracellularly, in L-forms, or within micro abscesses in the udder [26]. Viridis *et al.* [3] also reported that *Staphylococcus aureus* shows high resistance to doxycycline (28.0%), oxytetracycline (16.0%) and ampicillin (12.0%).

4. CONCLUSION

In the present study therapeutic response was evaluated on the basis of reduction of SCC and MCMT score, the most effective response for subclinical Staphylococcal mastitis in goats was elicited by G₂ treated with Ceftizoxime followed by G₁ treated with Enrofloxacin LA and the least in G₃ treated with Oxytetracycline LA. During the entire study period the overall production level of goat surged, reflecting healthier animal and enhanced milk quality. Moreover, the incidence of newborn kid mortality witnessed a significant decline, underscoring the profound impact of effective subclinical mastitis management on overall health and productivity. Hence, preventing or detecting udder infections at an early stage is essential, not only to safeguard the farmer but also to ensure consumer safety. Furthermore, a significant challenge in the treatment and control of subclinical mastitis is the rising issue of drug resistance, which has emerged due to the indiscriminate use of antibacterial agents.

FUTURE SCOPE

Thus, the current research offers pertinent data on the therapeutic management of subclinical Staphylococcal mastitis in goats in and around Jabalpur (M.P.). Detection of other *Staphylococcus* spp. responsible for subclinical mastitis in goats may be studied and alternative therapeutic strategies should be required to treat the cases of subclinical mastitis in terms to minimize drug residues in milk. Control programs to improve the udder health of goats are needed and focus should be on educating farmers on which breed should be selected for better udder and teat traits.

REFERENCES

1. Danmallam, F. A. and Pimenov, N. V. (2017). Species composition of microflora isolated from the mammary gland of a healthy and sick mastitis goat. *Vet. Zootekhniya*, **4**(2017): 6-12.
2. Bhanot, V., Mehla, O.P. and Kumar, R. (2017). Prevalence of mastitis in goats. *Journal of Krishi Vigyan*, **6**(1): 232-233

3. Virdis, S., Scarano, C., Cossu, F., Spanu, V., Spanu, C. and De Santis, E.P.L. (2010). Antibiotic resistance in *Staphylococcus aureus* and coagulase negative staphylococci isolated from goats with subclinical mastitis. *Veterinary Medicine International*, **2010**(1): 517060.
4. Karmakar, U., Datta, B.K., Patra, P.H., Prashant, M., Suman, S., Sar, T.K. and Mandal, T.K. (2011). Disposition kinetics of ceftizoxime in healthy and mastitic goats after intravenous administration. *Pharmacology Online*, **3**(1): 935-946.
5. Halmandge, S.C., Upendra, H.A., Kasaralikal, V.R. and Ramesh, P.T. (2021). Comparative therapeutic efficacy of subclinical mastitis in goats. *Indian Journal of Veterinary Medicine*, **41**(2): 34-37.
6. Radostits, O.M., Gay, C.C., Hinchcliff, K.M. and Constable, P.D. (2010). Diseases of mammary glands veterinary medicine: A text book of the diseases of cattle, sheep, goat, pig and horses, 10th Edn., Saunders Elsevier, London, pp 673-762.
7. Markey, B., Leonard, F., Archambault, M., Cullinane, A. and Maguire, D. (2012). *Clinical Veterinary Microbiology*, 2nd Edn., Elsevier Publishing Co., London, pp 118-344.
8. R Core Team (2013) R: A language and environment for statistical computing. r foundation for statistical computing, Vienna. <http://www.R-project.org/>
9. Robertson, N.H. and Muller, C.J.C. (2005). Somatic cell count in goat's milk as an indication of mastitis. *Animal Science*, **6**(1): 1-6.
10. Min, B.R., Tomita, G. and Hart, S.P. (2007). Effect of subclinical intramammary infection on somatic cell counts and chemical composition of goat's milk. *Journal of Dairy Research*, **74**(2): 204-210.
11. Koop, G., van Werven, T., Toft, N. and Nielen, M. (2011). Estimating test characteristics of somatic cell count to detect *Staphylococcus aureus* infected dairy goats using latent class analysis. *Journal of Dairy Science*, **94**(6): 2902-2911.
12. Persson, Y. and Olofsson, I. (2011). Direct and indirect measurement of somatic cell count as indicator of intramammary infection in dairy goats. *Acta Veterinaria Scandinavica*, **53**(15): 1-5.
13. Sharma, N., Singh, N. K. and Bhadwal, M.S. (2011). Relationship of somatic cell count and mastitis: an overview. *Asian-Australasian Journal of Animal Sciences*, **24**(3): 429-438.
14. Kaskous, S., Farschtschi, S. and Pfaffl, M.W. (2022). Physiological aspects of milk somatic cell count in small ruminants- a review. *Dairy*, **4**(1): 26-42.
15. Najeeb, M.F., Anjum, A.A., Ahmad, M.U.D., Khan, H.M., Ali, M.A. and Sattar, M.M.K. (2013). Bacterial etiology of subclinical mastitis in dairy goats and multiple drug resistance of the isolates. *The Journal of Animal and Plant Sciences*, **23**(6): 1541-1544.
16. Omar, S. and Mat-Kamir, N.F. (2018). Isolation and identification of common bacteria causing subclinical mastitis in dairy goats. *International Food Research Journal*, **25**(4): 1668-1674.
17. Manser, P.A. (1986). Prevalence, causes and laboratory diagnosis of subclinical mastitis in the goat. *The Veterinary Record*, **118**(20): 552-554.
18. 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. *Veterinary World*, **5**(2): 103-109.
19. 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. *Journal of Dairy Science*, **80**(2): 313-317.
20. 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. *Journal of Dairy Science*, **93**(11): 5243-5245.
21. Brauner, A., Fridman, O., Gefen, O. and Balaban, N.Q. (2016). Distinguishing between resistance, tolerance and persistence to antibiotic treatment. *Nature Reviews Microbiology*, **14**(5): 320-330.

22. Buragohain, R., Sar, T.K., Samanta, I., Biswas, U. and Mandal, T.K. (2019). Disposition of ceftizoxime in Staphylococcal mastitis in Indian crossbred cows. *The Veterinary Journal*, **245**(2018): 12-14.
23. Sar, T.K., Mandal, T.K., Samanta, I., Rahaman, A. and Chakraborty, A.K. (2010). Determination of ceftriaxone in plasma and ceftizoxime in milk of mastitic cows following single dose intravenous administration. *Indian Journal of Animal Sciences*, **80**(12): 1182.
24. Kumar, R., Sar, T.K., Patra, P.H., Samanta, I., Mishra, A. and Mandal, T.K. (2016). Disposition kinetics of ceftizoxime in acute mastitis in Murrah buffaloes. *Indian Journal of Animal Sciences*, **86**(2): 140-142.
25. Kirkan, S., Erbas, G., Savasan, S., Ucan, N., Gurpinar, S. and Ozavci, V. (2014). Detection of antimicrobial resistance in *Staphylococcus aureus* isolated from Saanen goats with subclinical mastitis. *Animal Health Production and Hygiene*, **3**(1): 284-287.
26. Sears, P.M., Fettinger, M. and Marsh-Salin, J. (1987). Isolation of L-form variants after antibiotic treatment in *Staphylococcus aureus* bovine mastitis. *Journal of the American Veterinary Medical Association*, **191**(6): 681-684.

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