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Therapeutic management of clinical mastitis in buffaloes

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Abstract
In total, 48 buffaloes were used in this investigation, all of whom had clinical mastitis as determined by the obvious abnormalities in milk discharges. The udder was cleaned, dried with a fresh towel, and the teats were sprayed with 70% ethanol before being examined. After that, the first few milk strips were discarded, and sterile vials containing 50 ml of milk samples from each quadrant were collected and transported immediately to the laboratory. Bacteriological examination and PCR confirms *S. aureus* (19) was discovered to be the main bacterium present in milk samples, followed by *E. coli* (13), *Streptococcus* spp., (09) and *Pseudomonas aeruginosa* (7). These buffaloes which were positive for clinical mastitis were considered for therapeutic trials and selected buffaloes were randomly allocated into six groups. Group I treated with enrofloxacin 5mg/kg for 5 days, group II with cetfizoxime 10mg/kg once in 3 days, group III with marbofloxacin 8mg/kg once in 3 days and groups IV, V and VI receives same antibiotic with Vit E and selenium respectively and all the groups receives same supportive therapy. antibiotics such as marbofloxacin and Ceftizixime along with antioxidants *i.e.* Vit E and Se showed early improvement by clinical, haematobiochemical and in oxidative enzyme status compared to antibiotics alone.

Keywords: Mastitis, therapeutic, enrofloxacin, cetfizoxime, marbofloxacin

Introduction
Mastitis is an udder parenchyma inflammation that causes physical and chemical alterations to the milk as well as pathological abnormalities in the mammary gland. The tremendous growth of dairy industry is crippled by bovine mastitis, the most frequent and costly status as it affects the dairy herd worldwide (Halasa *et al.*, 2007) [8]. The dairy industry and animal husbandry are both seriously threatened by the disease. Mastitis continue to pose a problem to academics despite decades of investigation. Given that it is a multifaceted disease with three primary components—host, environment, and pathogen and a complex interplay between them, little is known about it (Brand *et al.*, 1996) [3].
Bovine mastitis is broadly classified as two types, *viz.*, clinical, where changes of udder and in the milk is visible and subclinical, wherein the appearance of udder as well as milk is normal. Therefore detection of clinical cases of mastitis (CM) is easy than subclinical form (SCM). *Escherichia coli*, *S. dysgalactiae* and *S. uberis* are examples of environmental pathogens, while *Staphylococcus aureus* and *S. agalactiae* are examples of contagious infections that cause bovine mastitis. Controlling SCM is the best indirect strategy to lower clinical instances because subclinical cases may initially lead to clinical cases (Harmon, 1994) [10].
The present study was designed with a focus on effective therapeutic regimes for the clinical mastitis in buffaloes.

Materials and Methods
The current research involved dairy buffaloes that were raised by regional farmers in the villages and dairy farms of the Bidar district.

Milk Samples
A total 48 buffaloes with clinical mastitis based on the visible abnormalities of milk secretions were used in this study. The udder were examined washed, dried with a clean towel and the teats were sprayed with 70% ethanol. The udder was cleaned, dried with a fresh towel, and the teats were sprayed with 70% ethanol before being examined. The first few milk strips were then thrown away, and 50 ml of milk samples from each quarter were collected in sterile vials,

maintained at 4 °C and then transported immediately to the laboratory. Milk samples were centrifuged at 3000 rpm for 20 minutes after being incubated aerobically at 37 °C for 24 hours for the bacterial examination. A loopful of the sediment was streaked across the top of mannitol salt agar, blood agar and MacConkey agar plates after the supernatant fluid was discarded. The plates were checked for bacterial growth after 24-48 hours of incubation at 37 °C. The isolated colonies were identified as Finegold and Baron (1986) [7].

Fig 1: Milk samples of clinical mastitis show gross changes

Those buffaloes which were positive for clinical mastitis were considered for therapeutic trials and selected buffaloes were randomly allocated into six groups.

Table 1: Treatment regimen for clinical mastitis affected buffaloes

Group N=08	Therapeutic regimen
Group I	Inj Enrofloxacin @ 5 mg/kg body weight intramuscularly s.i.d. for 5 days.
Group II	Inj Ceftizoxime @10 mg/kg body weight intravenously single shot
Group III	Inj Morbofloxacin @ 8 mg/kg B.W. intramuscularly single shot
Group IV	Inj Enrofloxacin @ 5mg/kg body weight intramuscularly s.i.d. for 5 days with Vitamin E and Selenium (Eselium) 10ml intramuscular three doses on alternate days.
Group V	Inj Ceftizoxime @10 mg/kg body weight intravenously single shot with injection of Vitamin E and Selenium (Eselium) 10ml intramuscular three doses on alternate days.
Group VI	Inj Morbofloxacin @ 8 mg/kg B.W. intramuscularly single shot with injection of Vitamin E and Selenium (Eselium) 10ml intramuscular three doses on alternate days.

All the animals of different groups received supportive therapy as:
Inj Flumixin 1.1 mg/kg body weight Intramuscular for 5 days.
Inj antihistamine 0.5 mg/kg body weight Intramuscular for 5 days.
The samples were collected on 0^{th}, 5^{th} and 7^{th} day after treatment in all the groups and therapeutic efficacy of six treatments were assessed.
The Recovery were assessed based on absence of physical change in milk along with clinical changes in the udder, haematological and biochemical assessment.

Therapeutic efficacy
Therapeutic efficacy was determined by identifying recovery among the affected animals in each group in the form of clinical improvement (clinical recovery) and normalization of haemato-biochemical parameters (haemato-biochemical recovery) by using following formula; and interpreted the results as percent recovery.

$$\% \text{ recovery} = \frac{\text{No. of diseased animals - No of animals showing recovery}}{\text{No. of diseased animals}} \times 100$$

Statistical Analysis
All the data was analysis using chi square test, one way ANOVA, Spearman correlation and column analysis by using GraphPad Prism version 5.

Results and Discussion
Out of total 48 CM samples of mastitis affected buffaloes screened for detection of four bacterial species by PCR, the *S. aureus* (19) was found to be the main bacterium present in milk samples, followed by *E. coli* (13), *Streptococcus* spp., (09) and *Pseudomonas aeruginosa* (7). These findings were agreed with reports of Ali *et al*. (2015) [1]; Ali *et al*. (2021) [2]. The capacity of this bacterium to grow within micro-abscesses in the udder and to exist intracellularly may be factors in its high prevalence and resistance to antibiotic therapy (MacDonald, 1997) [16].

Table 2: Therapeutic efficacy of different groups at 5th and 7th day of treatment

Groups	Group I		Group II		Group III		Group IV		Group V		Group VI	
Period Parameter	Day 5	Day 7	Day 5	Day 7	Day 5	Day 7	Day 5	Day 7	Day 5	Day 7	Day 5	Day 7
Clinical Recovery (n=8)	4	6	5	6	5	6	6	7	7	7	8	8
% Efficacy	50.0	75.0	62.5	75.0	62.5	75.0	75.0	87.5	87.5	87.5	100	100
Haemato-biochemistry (n=8)	3	4	4	5	5	6	6	7	7	8	7	8
% efficacy	37.5	50.0	50.0	62.5	62.5	75.0	75	87.5	87.5	100	87.5	100
Overall % efficacy	43.75	62.5	56.25	68.75	62.5	75.00	75.00	87.5	87.5	93.75	93.75	100

Enrofloxacin

Enrofloxacin has demonstrated great bio-availability and excellent tissue penetration in goats affected by mastitis (Elsheikh *et al.,* 2002) [5], and fluoroquinolones distribute well into an inflamed mammary gland (Mavrogianni *et al.,* 2011) [17]. By taking cited literature in consideration, enrofloxacin was used as therapeutic regimen in present investigation. However, overall efficacy on 5th and 7th day after treatment was 43.75 percent and 62.50 percent respectively. In contrast, the reports of Suresh *et al.,* (2010) [25], Ramesh *et al.,* (2015) [23], Polveiro *et al.,* (2020) [21] and Yadav *et al.,* (2022) [26] observed higher efficacy varying from 91.60 percent to 100 percent. This difference could be attributed to the fact that frequent use of same antibiotic in a geographic area can result in development of antimicrobial resistance (AMR).

In the present work, the overall efficacy of enrofloxacin was improves to 75.0 percent and 87.50 percent on day '5' and day '7' after treatment when coupled with Vit E and Selenium (Group IV). Which corroborates the earlier findings of Sangary *et al.,* (2011) [6]; Latif *et al.,* (2014) [15]; Persson *et al.,* (2015) [19]; Ramesh *et al.,* (2015) [23] and Yadav *et al.,* (2022) [26].

Ceftizoxime

Ceftizoxime is newly introduced third generation cephalosporin with wide range of anti-microbial spectrum, hence was included in the therapeutic regimen of current research trial.

Overall clinical and haemato-biochemical recovery on 5th day post treatment was 56.25 percent and improves to 68.75 percent on day '7' post treatment. Bio-availability of Ceftizoxime in milk for a prolonged period has underlined its use in mastitis (Sar *et al.,* 2010, Kumar *et al.,* 2016 and Buragohain *et al.,* 2019) [24, 14, 4]. Use of Vit E and Selenium along with Ceftizoxime (Group V) further improved the efficacy to 87.50 percent and 93.75 percent on day '5' and day '7' after treatment. The present findings were in accordance with the findings of Karmakar *et al.,* (2011) [11]; Khangal *et al.,* (2015) [12] revealed better recovery rate.

Marbofloxacin

Since the introduction of marbofloxacin for Veterinary use, it is being first line of treatment of acute clinical mastitis (Pillet *et al.,* 2013, and Kromker and Leimbach, 2017). In present investigation the overall efficacy of marbofloxacin was recorded as 62.5 percent and 75.0 percent on day '5' and day '7' after treatment which is substantiated by earlier records of Patil *et al.,* (2021) and Rahimiyan *et al.,* (2021).

It was further improved to 93.75 percent and 100 percent on day '5' and day '7' when it was coupled with Vit E and Selenium (Group VI). Similar reports were also made by Halmandge *et al.,* (2021) and Patil *et al.,* (2021).

Conclusion

To conclude, This indicate that antibiotics such as marbofloxacin and Ceftizixime along with antioxidants *i.e.* Vit E and Se showed early improvement by clinical, haematobiochemical and in oxidative enzyme status compared to antibiotics alone such as Enrofloxacin and Ceftizoxime.

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