

## **Therapeutic studies on subclinical mastitis associated with subclinical hypocalcaemia and hypoglycemia in crossbred cattle**

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### **ABSTRACT**

*In the present research recorded the incidence of subclinical mastitis in 1599 quarter milk samples from 417 apparently healthy lactating cows by californian mastitis test and electrical conductivity test. Based on in vitro drug sensitivity of mastitis milk antibiotics were selected and treated with Enrofloxacin, Chloramphenicol, Amoxicillin + Sulbactam, Ceftriaxone + tazobactam, Ceftriaxone + Sulbactam and Cefprozime into the affected cows. For conducting therapeutic trials cows were grouped into different groups. Cows treated with combination of antibiotic along with calcium supplement (Calup gel) had improvement in calcium levels compared to antibiotic treated group only. In another group of animals glucose supplement (ketonil gel) was done along with routine antibiotic therapy. In this group also ketone bodies levels were reduced than compare with group treated with antibiotic only.*

**Key words:** Mastitis, Hypocalcaemia, Hypoglycemia, Cattle

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### **INTRODUCTION**

Mastitis in both clinical and subclinical forms is a frustrating, costly and extremely complex disease that results in a marked reduction in the quality and quantity of milk [1]. Subclinical mastitis is a major problem affecting dairy animals all over the world. It causes enormous losses for breeders and consequently influences the national income of the country [2]. In spite of a distinct variation between the etiology of metabolic disorders and an infectious disease like mastitis, several epidemiological studies demonstrated that there is a correlation between their occurrences. Evidence suggests that metabolic disease affects host defense, and therefore, impacts the common infectious diseases of dairy cows [3]. Literatures available on this works were very little in Andhra Pradesh. In this study was planned to record the relation between the sub clinical mastitis and common metabolic diseases like hypocalcaemia and hypoglycemia in cross bred cows.

### **MATERIALS AND METHODS**

Crossbred milch cows belonging to different dairy farms located in and around Tirupati, and lactating animals from individual holdings brought to Teaching veterinary clinical complex, Tirupati, Andhra Pradesh, were selected for the present study as early, mid and late lactation. Quarter milk samples from the above seven different organizations were subjected to CMT, EC, SCC and cultural test in order to detect subclinical mastitis. From all the SCM samples milk was collected in aseptic manner and isolated the causative microorganisms and ABST was done with antibiotic discs.

Among all the cattle 28 crossbred cows which were affected with sub clinical mastitis were randomly divided into 4 groups containing seven cows for treatment purpose. In Group-A cows suffering from SCM + culture positive and having hypocalcaemia (low serum calcium values) were treated with callup gel one tube a week (calcium containing gel) 3 weeks and appropriate antibiotic for 5 days based on ABST results, then at weekly intervals record the CMT

cultural test results and serum calcium values. In this group-B cows suffering from SCM+ culture positive and having Hypoglycemia were treated with ketonil gel (gel containing glucose precursors like propylene glycol and minerals from Neospark Company). One tube a week for 3 weeks and treated with suitable antibiotic for 5 days based on ABST results. Then record the observations at weekly intervals for 3 weeks. In this group-C cows suffering from SCM (positive for CMT) and having hypocalcemia (low serum calcium values) were treated with only antibiotic for 5 days and recorded the results every week for 3 weeks. In this group-D cows suffering from SCM (positive for CMT) and having hypoglycemia (higher BHBA values) were treated with suitable antibiotic and recorded the results.

#### Estimation of $\beta$ -hydroxybutyrate (d-3-hydroxybutyrate):

$\beta$ -Hydroxybutyrate (D-3-hydroxybutyrate) was estimated as per Mc Murray *et al.*, [4]. Principle involved in this method is on the oxidation of D-3-hydroxy butyrate to acetoacetate by the enzyme 3-hydroxy butyrate dehydrogenase. Concomitant with this oxidation the cofactor NAD<sup>+</sup> is reduced to NADH and the associated change of absorbance can be directly correlated with the D-3-hydroxy butyrate concentration. Sample cuvette contained 25  $\mu$ l of serum (or) plasma sample and 1000  $\mu$ l reagent R1. Mix sample and R1, incubate for 60 seconds at 37°C and then take first reading. Read again after 1 and 2 minutes. The standard was also treated in the same way and read at 340 nm against reagent blank. The values were expressed in mmol/L.

$$\text{D-3-hydroxy butyrate concentration (mmol. /L)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times \text{Standard Conc.}$$

#### Estimation of serum Calcium:

Calcium was estimated as per Moorhead *et al.* [5]. Principle involved in this method is OCPC reacts with calcium co-cresolphthalein complexone in alkaline solution to form a purple colored complex. The intensity of the purple colour formed is proportional to the calcium concentration and is measured photometrically between 540 nm and 600 nm with maximum absorbance at 575 nm. Sample cuvette contained 10 $\mu$ l of serum sample and 1000  $\mu$ l of working reagent. The contents were mixed well and read at 578 nm against reagent blank. The values were expressed in mg/dl.

$$\text{Calcium (mg/dl)} = \frac{\text{Abs. of test}}{\text{Abs. of standard}} \times \text{Concentration of standard (mg/dl)}$$

## RESULTS AND DISCUSSION

Out of 14 sub clinically infected quarters from 7 cows subjected to enrofloxacin treatment, milk from 12 quarters from 6 cows were test negative on both CMT and cultural examination on 8<sup>th</sup> day post treatment, milk from the remaining 2 teats was test negative (CMT, cultural) on 15<sup>th</sup> day post treatment. In group-2 Out of 14 sub clinically infected quarters from 7 cows subjected to chloramphenicol treatment, 11 quarters from 6 cows gave negative reaction on both CMT and cultural examination on 8<sup>th</sup> day post treatment. Milk from 3 quarters from one cow was test negative on 15<sup>th</sup> day post treatment. In group-3 Out of 14 sub clinically infected quarters from 8 cows subjected to ceftizoxime treatment, all the 14 quarters milk samples were test negative on both CMT and cultural examination on 8<sup>th</sup> day post treatment. In group-4 Fourteen sub clinically infected teats from 7 cows were treated with ceftrioxone + Tazobactam, and all the 14 milk samples were test negative with both CMT and cultural examination on 8<sup>th</sup> day post treatment. Whereas the milk from cows in Group-5 was test positive on 7<sup>th</sup>, 15<sup>th</sup> day post treatment, with CMT and cultural examination.

#### Subclinical mastitis in association with Hypocalcemia

In the present study seven cows with SCM + hypocalcemia were treated (Group –A). Of them, 6,7 and 7 cows were culturally negative on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> week post treatment respectively. The mean serum calcium levels before treatment was 7.16. The mean serum calcium levels during 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> week post treatment were 7.89, 8.32 and 8.68 mg/dl. In Group-C all the 7 cows were culture positive and had low serum calcium levels treated with only antibiotic and we can observed culture negative and had low serum calcium levels throughout the study period.

A total of 39 sub clinically infected cows were tested for hypocalcaemia of which 25 animals had low serum calcium levels. Batavani *et al.* and Zakil *et al.* reported that calcium levels were decreased in the serum of SCM affected quarters [6, 7]. In the present study seven cows with SCM +hypocalcemia were treated (Group –A). Of them. 6,7 and 7 cows were culturally negative on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> week post treatment respectively. While 3, 5 and 7 cows had normal calcium levels (9-11 mg./dl.) during 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> weeks post treatment. In Group-C all the 7 cows were culture negative and had low serum calcium levels when treated with only antibiotic throughout the study period.

Table 1: Therapeutic trials in Subclinical mastitis + Hypocalcemic cows treated with Calup gel + Antibiotic (GROUP – A)

Animal Number	Pre treatment	Post treatment					
		First Week		Second Week		Third Week	
		CMT/CULTURE	CALCIUM	CMT/CULTURE	CALCIUM	CMT/CULTURE	CALCIUM
F6	+	-	7.28	-	7.15	-	7.21
I4	+	-	8.12	-	8.76	-	8.83
C6	+	+	8.40	-	8.31	-	8.53
PF-4	+	-	7.13	-	8.28	-	8.73
JS-5	+	-	8.12	-	8.34	-	9.21
1677	+	-	7.90	-	8.81	-	8.96
62236	+	-	8.31	-	8.56	-	9.28
		Sum	55.26	Sum	58.21	Sum	60.75
		Average	7.89	Average	8.32	Average	8.68
		Standard deviation	0.50	Standard deviation	0.56	Standard deviation	0.70
		Standard error	0.19	Standard error	0.21	Standard error	0.26

Table 2: Therapeutic trials in Subclinical mastitis + Hypoglycemic cows Treated with ketonil gel + Antibiotic (GROUP – B)

Animal Number	Pre treatment	POST TREATMENT					
		First Week		Second Week		Third Week	
		CMT/CULTURE	BHBA	CMT/CULTURE	BHBA	CMT/CULTURE	BHBA
A14	+	-	1.481	-	1.326	-	1.018
18900	+	-	1.346	-	1.348	-	1.218
C18	+	-	1.517	-	1.289	-	1.402
D6	+	-	1.981	-	1.646	-	1.783
14078	+	+	1.589	-	1.348	-	1.108
C15	+	-	1.345	-	1.286	-	1.248
C14	+	+	1.732	+	1.482	+	1.209
		Sum	10.99	Sum	9.73	Sum	8.99
		Average	1.57	Average	1.39	Average	1.28
		Standard deviation	0.23	Standard deviation	0.13	Standard deviation	0.25
		Standard error	0.09	Standard error	0.05	Standard error	0.10

Table 3: Therapeutic trials in Subclinical mastitis + Hypocalcemic cows Treated with only Antibiotic.(GROUP – C)

Animal Number	Pre treatment	POST TREATMENT					
		First Week		Second Week		Third Week	
		CMT/CULTURE	CALCIUM	CMT/CULTURE	CALCIUM	CMT/CULTURE	CALCIUM
A7	+	-	8.42	-	8.18	-	8.20
B4	+	-	8.57	-	8.43	-	8.97
SVV-10	+	-	8.24	-	8.40	-	8.63
C17	+	-	8.82	-	8.25	-	8.53
B1	+	-	8.45	-	8.10	-	8.96
A6	+	-	8.26	-	8.82	-	8.24
B6	+	-	8.89	-	8.26	-	8.43
		Sum	59.65	Sum	58.44	Sum	59.96
		Average	8.52	Average	8.35	Average	8.57
		Standard deviation	0.26	Standard deviation	0.24	Standard deviation	0.31
		Standard error	0.10	Standard error	0.09	Standard error	0.12

Table 4: Therapeutic trials in Subclinical mastitis + Hypoglycemic cows Treated with only Antibiotic.(GROUP – D)

Animal Number	Pre treatment	POST TREATMENT					
		First Week		Second Week		Third Week	
		CMT/CULTURE	BHBA	CMT/CULTURE	BHBA	CMT/CULTURE	BHBA
C10	+	-	1.581	-	1.430	-	1.480
C5	+	-	1.496	-	1.842	-	1.248
F3	+	-	1.326	-	1.420	-	1.438
H-67	+	+	1.492	-	1.219	-	1.340
I4	+	-	1.523	-	1.327	-	1.481
C17	+	-	2.194	-	1.623	-	1.902
C7	+	+	1.680	-	1.567	-	1.660
		Sum	11.29	Sum	10.43	Sum	10.55
		Average	1.61	Average	1.49	Average	1.51
		Standard deviation	0.28	Standard deviation	0.21	Standard deviation	0.22
		Standard error	0.11	Standard error	0.08	Standard error	0.08

### Subclinical mastitis in association with hypoglycemia

In Group-B a total of 7 cows which SCM and hypoglycemia were treated with ketonil gel and antibiotics of them 5 cows were test negative cultural examination. The mean BHBA values before treatment were 2.00. The mean

BHBA values during 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> week was 1.57, 1.39 and 1.28 m.mols/lit. Seven animals in Group – D with SCM and high BHBA values were treated only with antibiotic. All the 7 cows were culturally negative and had higher BHBA during the study period (1 to 3 weeks).

A total of 39 sub clinically infected cows were tested for hypoglycemias of them 23 cows were found to have higher BHBA values. Leslie *et al*, reported that elevated somatic cell counts were higher in the subclinical ketotic cows compared to non-ketotic animals and there is mounting evidence from observational studies and clinical trials that increased rates of mastitis being associated with subclinical ketosis, further stated that hyperketonemia is hypothesized as one of the most important factors leading to reduced udder defenses, and is highly associated with several periparturient diseases, including subclinical and clinical mastitis [8]. Pritchard, investigated the relationship between elevated blood ketone levels and clinical mastitis and reported that a negative energy balance in dairy cows during the first two or more weeks postpartum can lead to ketosis and an increased incidence of mastitis [9].

Perusal of tables indicated that in Group-B a total of 7 cows which SCM and hypoglycemia were treated for hypoglycemia. of them, 5 cows were test negative on cultural examination and 3 cows had normal BHBA values below (1.4 m.mol./lt.) during the 1<sup>st</sup> week post treatment. All the seven cows were test negative for SCM on cultural examination and had normal BHBA on 2<sup>nd</sup> 3<sup>rd</sup> week post treatment. Seven animals in Group – D with SCM and high BHBA values week kept as untreated controls. All the 7 cows were culturally negative and had higher BHBA values (Mean) during the study period (1 to 3 weeks). Details therapeutic response was mentioned in the table 1 to 4.

#### Summary

Therapeutic trails indicative of treatment of sub clinical mastitis along with ketonil gel and calupgel will avoid the occurrence of milk fever and ketosis.

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