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Research Article

SUBCLINICAL MASTITIS INCATTLE AT AIZAWL, MIZORAM: PREVALENCE, ANTIBIOGRAM AND THERAPEUTICS

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ABSTRACT

The study was to find out the subclinical mastitis (SCM) cases in cattle of Aizawl district of Mizoram. Milk samples were screened and subclinical cases were found with Modified California mastitis test (MCMT). Overall, animal-wise prevalence of SCM was found to be 71%. Milk samples were subjected to culture and isolation of the etiological agent, followed by confirmation using biochemical tests. *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli* were isolated. Antimicrobial sensitivity testing of bacterial isolates was carried out and found that Cefoperazone and sulbactum combination were effective against *Staphylococcus, Streptococcus* and *Escherichia coli*. The animals were grouped into four groups of treatment as per the sensitivity results. Cefoperazone and sulbactum combination were effective against coagulase negative *Staphylococcus*. Animals treated with intramammary infusion along with parentral therapy of antibiotics showed more cure rate than either of them used alone. The results were recorded and statistical analysis has been done.

Key words: Antibiotic, Antimicrobial, Cattle, Milk, Sample, Subclinical mastitis, Treatment

Mastitis is the inflammation of parenchyma of mammary gland characterized by physio-chemical changes in milk and pathological changes in glandular tissues. It is a highly economically important disease of dairy animals. Sub clinical mastitis is 15-40 times more prevalent than clinical mastitis and it continues to be the most frequent diseases of milking cows (Abebe *et al.*, 2016). In India, the annual economic losses on account of udder infection were estimated to be Rs. 6053.21 crores and out of which loss of Rs. 4365.32 crore has been attributed to sub-clinical mastitis (Devakumar and Chhonkar, 2013). Mastitis may occur either in clinical or subclinical form. Subclinical mastitis (SCM) is the presence of infection without local inflammation resulting in an absence of visual signs and involves transient cases of inflammation and abnormal milk. The subclinical form is detectable mainly by tests applied to the milk for the demonstration of products of inflammation and by the changes in chemical compositions (Thompson-Crispi *et al.*, 2014). There are no systematic studies to understand the prevalence of SCM in cattle of Mizoram. The present study was undertaken with the objective to investigate the prevalence of SCM in cattle of Aizawl district of Mizoram state and to study the antibiogarm of bacterial isolates and to evaluate the efficacy of rational therapeutics.

MATERIALS AND METHODS

Study area and sampling: The study was conducted in 13 different livestock farms (intensive system of management) in Aizawl district of Mizoram during a period of nine months from August, 2019 onwards. Each selected unit had 5 to 15 milch cows and 100 cows were there from all units. The cows were either Jersey or Holstein-Friesian crossbreeds. Milk samples (5 ml each) were collected from all quarters from each cow in a sterile vial. The cows were screened for SCM using Modified California mastitis test (MCMT) as per the standard method (Reddy *et al.*, 2014). The cows with clinical mastitis were excluded from this study. The composite milk samples from the selected animals with SCM were collected in sterile vials. Inoculation and isolation of bacteria were done on nutrient agar (NA) plates (containing 10% sheep blood) as per the method given by Ferreira *et al.* (2018). The well-isolated and representative colonies showing different colony morphology were picked up and the smears were made on micro slides. Slides were stained with Gram's stain (Shah *et al.*, 2017). The gram-negative rods suspected for Enterobacteria were streaked onto MacConkey's Lactose agar plates and incubated at 37° C for 24 hours to differentiate lactose fermenters from that of non-lactose fermenters. The gram-positive cocci occurring in groups were streaked onto the plates of Mannitol Salt agar (for staphylococcal species). Gram positive cocci occurring in short to long chains were inoculated onto Edward's medium (for Streptococcal species). The well separated representative colonies from the NA plates were picked up and streaked onto NA slants, incubated aerobically at 37 °C for 24 hours and preserved in a refrigerator at 4 °C as

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stock cultures for further study.

Identification of organisms: The organisms isolated from the milk samples were identified to the genus and, species level on the basis of morphological, cultural and biochemical characteristics as per the method of Sastry and Bhat (2019). The biochemical tests performed for identification of the organisms were Catalase, Methyl red (MR), Indole, Voges Proskauer (VP), Nitrate reduction, Citrate utilisation, Motility, Glucuronidase, ONPG, Lysine utilization, Lactose, Glucose, Sucrose and Sorbitol fermentation tests (Cheesbrough, 2006). Tube coagulase test was performed and any evidence of clotting after 24 hours of incubation at 35° C was regarded as positive for coagulase production (Chamberlain, 2009). Christie Atkins and Munch-Peterson test (CAMP) for differentiation of streptococci was done and appearance of a half-moon zone or satellite effect of haemolysin in the haemolytic zone of staphylococci was indicative of a positive CAMP reaction (Guo *et al.*, 2019).

Antimicrobial sensitivity testing: Agar disc diffusion test were performed using eight antimicrobial sensitivity discs viz. Chloramphenicol (30 mcg), Ampicillin/sulbactum (10/10 mcg), Amoxycillin/clavulanic acid (20/10 mcg), Piperacillin/ Tazobactum (100/10 mcg), Cefoperazone/ sulbactum (75/10 mcg), Cefuroxime (30 mcg), Ticarcillin/clavulanic acid (75/10 mcg) and Cephotaxime (30 mcg) and the zone of inhibition was measured in millimeters (Saidi *et al.*, 2019).

Therapeutic protocol: The animals were grouped into 4 groups depending on the availability of animals in the same farm or locality and treatment given as per the following details. Four groups of cows with subclinical mastitis were made: Group I – 16, Group II – 15, Group III – 22 and Group IV – 18.

Group I (n=16): Cefoperazone and Sulbactum (2:1) combination at the rate of 10 mg/kg body weight by intramuscular route for 5 consecutive days.

Group II (n=15): Cefoperazone intra-mammary infusions at the dose rate of 250 mg per dose as a single dose.

Group III (n=22): Cefoperazone and Sulbactum (2:1) combination, parenterally for 5 consecutive days along with single dose of 250 mg Cefoperazone intra-mammary infusions.

Group IV (n=18): Untreated control group.

After completion of the treatment, milk samples were collected and MCMT was performed to ascertain whether the animal is free of mastitis or not. Statistical analysis (χ^2 test), was done as per method of Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Overall animal-wise prevalence of SCM on the basis of MCMT was 71%. A total 395 quarter samples were examined and 171 quarters (43.32%) were found positive for SCM. Devi and Dutta (2018) reported 66.67% of SCM in Assam. Highest prevalence was found in animals in their third lactation (25.35%), followed by animals in second lactation (18%) ($P \leq 0.05$) (Table 1). Kurjogi and Kaliwal (2014) reported high prevalence of mastitis in cows in their fifth lactation. Higher prevalence in third lactation may be related to peak milk production. There was a significant difference ($P \leq 0.01$) in the prevalence rate with stage of lactation in the present findings. The prevalence of SCM was significantly higher ($P \leq 0.01$) in the late stage (50.70%) (Table 2). Age group of 6 to 8 years shows more SCM cases with a significant difference. Similar findings were also recorded by Srinivasan *et al.* (2013). The susceptibility of the cows to SCM increases along with the advancement of age, and more prone to infections, since the defensive mechanism of the body is lowered which favors bacterial invasion and multiplication.

There was significant difference ($p \leq 0.05$) on the bacteriological status of SCM positive milk samples in the present finding (Table 4). Mastitis pathogens like coagulase negative *Staphylococcus* was found in 63 samples and coagulase positive *Staphylococcus aureus* was found in 37 samples (Table 3). Pumipuntu *et al.* (2019) reported 32 coagulase-positive and 197 coagulase-negative staphylococcal isolates in their work on SCM. The percentage of coagulase-negative staphylococci (38.18%) in this study was similar to the findings by other workers (Wang *et al.*, 2018). Prevalence of *Streptococcus agalactiae* (10.30%) in this study was similar to the findings of Mpatswenumugabo *et al.* (2017). *Escherichia*

Table 1

Prevalence of SCM on the basis of number of lactations

Lactation No.	No. of Animals affected	Percentage (%)	χ^2
1st	10[b]	14.08	68.62*
2nd	13[b]	18.00	
3rd	18[a]	25.35	
4th	8[b]	11.26	
5th	7[b]	9.85	
6th	3[c]	4.22	
7th	1[c]	1.40	
8th	4[c]	5.63	
9th	3[c]	4.22	
10th	4[c]	5.63	
Total	71	100	

Table 2

Prevalence of SCM on the basis of stage of lactations

Stage of Location	No. of Animals affected	Percentage (%)	χ^2
Early (1st to 3rd Month)	20^b	28.16	
Mid (4th to 6th Month)	15^b	21.12	
Late (above 6 months)	36^a	50.70	
Total	71	100	

Table 3

Different types of organisms isolated from Bovine Sub-Clinical Mastitis

No.	Type of Organisms isolated	No.	Percentage	χ^2
1	Coagulase negative *Staphylococcus*	63	38.18a	
2	*Staphylococcus aureus*	37	22.42b	
3	*Streptococcus agalactiae*	17	10.30c	
4	*Streptococcus* other than *S. agalactiae*	33	20.00b	
5	*Escherichia coli*	15	9.09c	
	Total	165	100	
	No. of samples examined	171		
	No. of samples culturally positive	158	92.39	
	No. of mixed infection	32	20.25	

coli were isolated from bovine udder but were less frequent cause of mastitis (Radostits *et al.*, 2007). These organisms were opportunistic pathogens to udder.

Sixteen quarters revealed mixed (double) infection out of which ten quarters had mixed infections with staphylococci and streptococci and the other six quarters had mixed infection with *E. coli*, staphylococci and streptococci. The identified species were *Streptococcus agalactiae* (17), streptococci other than *S. agalactiae* (33). All the *S. agalactiae* were CAMP positive and streptococci other than *S. agalactiae* were CAMP negative. Amer *et al.* (2018) reported different etiology for SCM like *streptococci, staphylococci* and coliforms. Coagulase negative *Staphylococcus* (90.47%) and *Staphylococcus aureus* (91.89%) were showing more sensitivity to Piperacillin/Tazobactum (Table 4). *Streptococcus agalactiae*, streptoccoccus other than *S. agalactiae* and *E. coli* were showing more sensitivity to Cefoperazone/ sulbactum (100%). The occurrence of antibiotic resistance was compared between mastitis pathogens (*Staphylococcus aureus*, non aureus staphylococci) from farms with organic and conventional dairy production. The resistance might be due to continuous use in veterinary practice and organism get resistance against chemotherapeutic agents. The antibiotic is penicillinase-labile penicillin and prevalence of such higher number of ampicillin resistant strains indicated that the organisms might have acquired

Table 4

Anti-Biogram of bacteria isolated from Bovine SCM milk sample

Species	No. of Isolates Tested	No. of Isolates sensitive to								Significance
		C	Cfs	Ac	Pt	As	Cu	Tc	Ce	
Coagulase Negative *Staphylococcus*	63	54^b (85.71)	63^a (100)	23^c (45.59)	57^b (90.47)	34^c (53.96)	55^b (87.30)	33^c (52.38)	56^b (88.88)	*
Staphylococcus aureus	37	29^b (78.37)	37^a (100)	12^c (32.43)	34^{ab} (91.89)	20^c (54.05)	32^b (86.48)	20^c (54.05)	32^b (86.48)	*
Total	100	83 (83.00)	100 (100)	35 (35.00)	91 (91.00)	54 (54.00)	87 (87.00)	53 (53.00)	88 (88.00)	
χ^2		0.98^{NS}	0.00**	0.17^{NS}	0.06^{NS}	0.01^{NS}	0.14^{NS}	0.03^{NS}	0.13^{NS}	
Streptococcus agalactiae	17	15^a (88.23)	17^a (100)	5^b (29.41)	16^a (94.11)	8^b (47.05)	13^a (76.47)	5^b (29.41)	15^a (88.23)	*
Streptoccoccus other than Strep agalactiae	33	28^b (84.84)	33^a (100)	11^c (33.33)	30^{ab} (90.90)	26^b (78.78)	30^{ab} (90.90)	12^c (36.36)	29^b (87.87)	*
Total	50	43 (86.00)	50 (100)	16 (32.00)	46 (92.00)	34 (68.00)	43 (86.00)	17 (34.00)	44 (88.00)	*
Escherichia coli	15	10^b (66.66)	15^a (100)	6^c (40)	13^a (86.66)	9^{bc} (60.00)	12^{ab} (80.00)	9^{bc} (60.00)	13^{ab} (86.66)	*
χ^2		0.11^{NS}	0.00^{NS}	0.08^{NS}	5.19*	5.19*	1.94^{NS}	0.24^{NS}	0.01^{NS}	

Table 5

Percentage of reduction of subclinical mastitis quarter-wise after treatment

S. No.	Name	No. of cows	Total No. of quarters examines	No. of infected quarters	Total no. of quarters			Cure rate %
					Cured as per MCMT	Not cured	χ^2	
1	Group I	16	64	41	36	5	94.84**	87.80
2	Group II	15	60	43	28	15		65.11
3	Group II	22	85	51	51	-		100
4	Group IV	18	70	36	6	30		16.66

penicillinase, an enzyme which is very often acquired by the penicillin-resistant staphylococci. The present findings were in agreement with the findings of Elmonir *et al.* (2019). None of the strains was resistant to amoxicillin-clavulanic acid (Bolte *et al.*, 2020). Cephotaxime in the present study shows sensitivity to *Staphylococcus* (88%). Dubal *et al.* (2010) reported 91.11% sensitivity with cephotaxime against the pathogens associated with bovine mastitis. Table 5 shows pre and post therapy status of SCM in cows.

In group I, after therapy the milk from each quarter were checked with MCMT and found that all the affected quarters of cows did not respond to the treatment. Hossain *et al.* (2017) observed that success rate of parentral therapy in mastitis cases is influenced by rate of dispense of drugs from blood to reach into foci of infection. Another reason can be the difference in efficacy of Cefoperazone and Sulbactum against different etiological agent. In group II, the percentage of reduction of SCM was 51.32%. This finding was in conformity of Scaccabarozzi *et al.* (2015) who performed intra-mammary treatment with Cefoperazone which was effective in goats. This may be due to rapid release base of antibiotics which make quick dispersal throughout the udder and then absorbed into the bloodstream. The cure rate in group III was 100% in quarter-wise and 100% in animal-wise in this group. The combined effect of intramuscular and intra-mammary route is considered to have synergism. Statistically, there was a significant difference ($p \leq 0.01$). In group IV, 5 of the 18 cows could become free of infection naturally. The self cure might be due to the immunological changes in the mammary glands (Table 5). The overall percentage of reduction of SCM among the treated groups was 77.43%.

The cases of SCM were studied and etiologies were found. Antimicrobial sensitivity tests were performed. The treatments were given as per the anti microbial sensitivity tests. Cefoperazone and Sulbactum when given as parentrally and intramammary infusion showed higher efficacy and cured 100% SCM cases.

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