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Mastitis: Havoc to dairy cattle in Mizoram

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Abstract
Mastitis is the widely prevalent malady of dairy cattle. The present study aimed at finding the prevalence of bovine mastitis in Mizoram and to explore the treatment options with different antibiotics after determining the etiology. Hundred animals from 18 different farms were screened using modified California Mastitis test and recorded as positive and negative based on appearance of precipitation. An overall incidence rate of mastitis was observed as 72%. The milk samples were collected from positive animals, and microbial etiology were determined using appropriate laboratory techniques. The different organisms isolated were *Staphylococci*, *Streptococci* and *E. coli*. Animals were grouped into three groups and treated with Ceftizoxime, Cefoperazone sodium, Cefquinome sulphate and Cefquinome sulphate. It is observed that the third group where, Cefquinome sulphate and Cefquinome sulphate used showed 100% cure. These observations necessitate the requirement of regular screening of cattle and application of preventive and control measures for mastitis.

Keywords: Mastitis, Mizoram, cattle, diagnosis, treatment, milk

Introduction
Mastitis is the inflammation of mammary gland and is one of the most prevalent diseases of dairy cattle characterized by pathological changes in glandular tissues of udder and physical, chemical, and microbiological changes in milk. It is characterized by heat, redness, swelling, hardness, and pain with abnormalities in milk like increased somatic cells, especially leukocytes, in the milk and by pathological changes in the mammary tissue [24]. Bovine mastitis is categorized as one of the most widely spread and costly diseases affecting the dairy industry throughout the world [1]. India stands third after European Union and United States in milk production with 91.3 million metric tons production in 2019 [28]. The total cattle population of India is 192.5 million [17]. First available literature on mastitis from Mizoram is in 2003 [16].

There are 137 species and subspecies of potential pathogens associated with infection of the mammary gland [10]. Most of the mastitis is caused by *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Streptococcus bovis* and *Klebsiella pneumonia* [30]. However, the most recognized pathogen in the majority of mastitis cases in most countries is *Staphylococcus aureus* [9]. In India, *Staphylococci* have been reported as the chief etiological agent in both clinical and subclinical bovine mastitis [23]. *Escherichia coli* have been reported as the predominant coliform organisms encountered in bovine mastitis [7].

Economic loss due to mastitis is a sum of production loss, treatment loss and loss of animal value [13]. The overall morbidity rates in cattle due to mastitis were 15.5% and losses due to mastitis per lactation in nondescript cow and crossbred cow were ₹ 868.34 and ₹ 1, 314.10 respectively [5]. The present study was to elucidate the prevalence of bovine mastitis in dairy cows in rural, peri-urban and suburban regions of Aizawl district of state of Mizoram, to identify the causative bacteria from the affected quarter and also to evaluate the efficacy of different antibiotics for clinical cure.

Materials and Methods
Study area
The milk samples were collected from cows from different farms with or without any clinical signs of mastitis in and around Aizawl district of Mizoram. The laboratory works were done in Department of Veterinary Medicine as well as in Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Selesih, Aizawl.

Collection of milk samples for bacteriological examination

A total of 400 samples from each quarter of 100 animals were screened using Modified California Mastitis Test (MCMT) [18] from 18 different farms in and around Aizawl. The result was interpreted as:

Negative (-): No evidence of gel formation or precipitation

Positive (+): Trace of precipitation to distinct gel formation

After identification of animals with mastitis, all the clinical parameters and detailed history of animals were recorded and collected the milk samples for microbiological examination. Milk samples were collected aseptically [29] from the affected quarter of those cows which shows trace (T) or positive (+) reactions in the MCMT. About 4 mL of milk was collected separately from the teat of the affected quarters in sterile screw-capped plastic vials. Plastic vials were marked LF (Left fore), LH (left hind), RF (right fore) and RH (right hind).

Isolation and identification of bacteria

Collected samples were cultured on media such as Nutrient agar (NA), Eosin Methylene Blue agar (EMBA), Slantz and Barley agar and Baird Parkar agar (BPA). For isolation of the bacteria, NA was used as primary and MacConkey Agar (MCA) as differential media. The milk samples were mixed thoroughly and a loopful of milk sample from each quarter was streaked on the NA and MCA. The inoculated plates were incubated aerobically at 37 °C for 24-48 hours. After incubation, the plates were examined for presence of bacterial growth.

The well isolated and representative colonies showing golden yellow growth were picked up and then re-streaked on BPA for purification and selection of *Staphylococci* and incubated at 37 °C for 24 to 48 hours. After recording the colony morphology, the colonies were picked up and smears made on microslides were stained with Gram's stain for further identification procedures.

The lactose fermenting colonies suspected for *E. coli* in MacConkey agar were further re-streaked into EMBA and incubated at 37 °C for 24 h. After recording the colony morphology, the colonies were picked up and smears made on microslides were stained with Gram's stain for further identification procedures.

The Hotis test positive samples were inoculated into the Slantz and Barley Agar which allowed only S*treptococcal* species to grow. For further confirmation, Gram staining was done. The purified cultures were preserved in NA slants as stock cultures. The well separated representative colonies from the NA plates were picked up and streaked onto NA slants, incubated aerobically at 37 °C for 24 h and preserved in a refrigerator at 4 °C as stock cultures for further study.

Identification of organisms

The bacterial isolate was identified to the genus level with standard procedures applicable to the genera concern [8]. The biochemical tests performed for identification of the micro organisms were Catalase Test, Methyl Red (MR) Test, Voges Proskauer (VP) Test, Indole Test, Citrate Utilisation Test and Triple Sugar Iron (TSI) Test.

Treatment

Thirty cows with mastitis were divided randomly into three groups (10 cows each) I, II and III which shows trace (T) or positive (+) reactions of one or more quarters in the MCMT.

Group I: Ceftizoxime 1500mg IV repeated after 3 days @5mg/kg body weight

Group II: Cefoperazone sodium 250mg 10mL syringe by intramammary infusion (IMI) at 12 hrs interval for 5 consecutive days

Group III: Cefquinome sulphate 25mg IM @ 1mg/kg body weight or 2ml/50kg body weight and Cefquinome sulphate 75mg IMI for 5 days

After the completion of the treatment, MCMT of the milk samples was performed again after 24 to 48hours of the last treatment by collecting milk samples from all the treated animals to ascertain whether the animals were free of mastitis or not. The data after collection and arranging were subjected to statistical analysis [27].

Results

Prevalence of Mastitis

A total number of 100 cows were screened using MCMT and 72 animals were found positive for mastitis, out of which, 84.72% was subclinical mastitis (61/72) and 15.28% were clinical mastitis (11/72). Overall incidence of mastitis was 72% (72/100). The incidence of bovine mastitis in relation to number of lactations is shown in table 1. Highest incidence was found in cows in their 3rd lactation (25%) and lowest incidence was seen in 7th lactation (1.39%).

Table 1: Prevalence of mastitis in relation to lactation number

Lactation no.	No. of animals affected	Percentage (%)
1st	10	13.89
2nd	8	11.11
3rd	18	25.00
4th	11	15.28
5th	9	12.50
6th	5	6.94
7th	1	1.39
8th	3	4.17
9th	4	5.55
10th	3	4.17
Total	72	100

The influence of stage of lactation on Bovine mastitis is presented in table 2. It was observed that the incidence was higher in cows in late stage of lactation (51.39%) and lower in mid lactation (19.44%).

Table 2: Prevalence of bovine mastitis in relation to the stage of lactation

Stage of lactation	No. of affected animals	Percentage (%)
Early (1st to 3rd Month)	21	29.17^{b}
Mid (4th to 6th Month)	14	19.44^{b}
Late (above 6 Month)	37	51.39^{a}
Total	72	100

The percentages bearing different superscripts differ significantly ($P \leq 0.05$).

The incidence of bovine mastitis in relation to the age of the cow was recorded in table 3 and low in 2 to 4 years of age group (13.89%).

Table 3: Age-wise prevalence of bovine mastitis

Age group (years)	No. of affected animals	Percentage (%)
2-4	10	13.89[b]
4-6	15	20.83[ab]
6-8	19	26.39[a]
8-10	15	20.83[ab]
10-12	13	18.06[ab]
Total	72	100

The percentages bearing different superscripts differ significantly ($P \leq 0.05$).

The quarter wise incidence of bovine mastitis showed highest in Right hind quarters (RH) (29.63%) and lowest in Right fore quarters (RF) (21.60%)

Isolation of bacteria

Out of 400 milk samples, 162 milk samples which show positive (+) or trace positive (+) for bovine mastitis by MCMT were collected and cultured for identification and isolation of organisms. Out of 162 milk samples, 152 were culturally positive (93.83%). A total of 152 different strains belonging to three different type of species were isolated which is given in table 4. The different organisms isolated were *Staphylococci*, *Streptococci* and *E. coli*. The most commonly isolated organisms were *Staphylococci* (86.18%) followed by *E. coli* (9.21%) and *Streptococci* (4.61%). Mixed infection was found to be 10.52% in the collected samples.

Table 4: Different types of organisms isolated from samples of bovine mastitis

No	Types of organisms isolated	No.	Percentage
1.	*Staphylococci spp.*	131	86.18[a]
2.	*E. coli*	14	9.21[b]
3.	*Streptococci spp.*	7	4.61[c]
	Total	152	100
	No. of samples examined	162	
	No. of samples culturally positive	152	93.83
	No. of mixed infection	16	10.52

The percentage the bearing different superscripts differ significantly ($P \leq 0.05$).

Treatment

Group I: Among 10 cows with mastitis in this group, 8 (80%) cows were cured.

Group II: Among 10 cows with mastitis in this group, 5 (50%) cows were cured

Group III: Among 10 cows with mastitis in this group, 10 (100%) cows were cured (Table 5)

Table 5: Percentage of cure rate and reduction of mastitis after therapy

Group	Route	No. of cows	Quarters examined	Infect ed quarters	Total no. of quarters		Cure Rate %	Percentage of prevalence		% of reduction of mastitis
					Cure d	Not cured		At the beginning (A)	At the end (B)	
I	Intravenous	10	40	26	21	5	80.76	65.00	19.23	70.41[a]
II	Intramammary	10	40	26	16	10	61.54	65.00	38.46	40.83[b]
III	Intramuscul ar and intra- mammary	10	40	27	27	-	100	67.50	-	100[c]

The percentage bearing different superscript differ significantly ($P \leq 0.01$).
Percentage of reduction = (A-B) x100/A Where, A= % of prevalence at the beginning
B= % of prevalence at the end

Discussion

Prevalence of mastitis

The overall incidence rate of mastitis was found to be 72% by MCMT which was comparable with the findings in a study at Bangladesh [14]. On the contrary, the overall incidence of mastitis was 38% as reported by earlier workers [26]. The variation in the prevalence of mastitis between this study and the others might be due to differences in environmental and management practices or difference in mastitis control programmes.

An incidence of SCM of 84.72% was found. The lower rate of incidence of SCM was reported by many workers; 31.25% [3] 50.4% [21] and 32.92% [19]. An incidence of CM of 15.28% was found in the present study which was similar to earlier findings [2] where they reported the incidence as 10%.The incidence of SCM was more than CM which could be attributed to the fact that little attention was given to SCM as the infected animal shows no obvious symptoms and secretes apparently normal milk and farmers, especially small holders, are not well informed about invisible loss from SCM.

Quarter-wise prevalence of mastitis 40.50% was found in the present study which was comparable with the previous findings [26]. The variability in the prevalence of bovine mastitis among the findings could be attributed to difference in management of the farms and breeds considered. The variation in the incidences of mastitis might be due to the fact that infectious agent might not have entered in all the quarters at the same time or due to the injury, defective sphincters, absence of udder washing, milking of cows by common milkers which have cuts and chaps on their hands and using of common udder clothes.

The highest incidence was in 3rd lactation and 4th lactation which agreed with earlier observations [14, 15] where reported the highest incidences in 3rd and 4th lactation. The mastitis prevalence was higher during the late stage of lactation in present study ($P \leq 0.05$). This agreed with the previous reports [1]. Highest incidences in late lactation may be due to the fact that after several lactations the skin of the udder may become stretched and is less tightly applied to the underlying tissues thus it allows becoming loose and more susceptible to injury and causes mastitis. It was observed that none of the farmers practice teat dipping as a preventive measure.

Maximum numbers of affected cows (26.39%) were in the age group of 6-8 yrs. The percentage bearing different superscript differ significantly ($P \leq 0.05$) and prevalence of mastitis increases with advancement of age which agreed with the prior observations [4] where they reported risk of mastitis increases with age of cows. The higher prevalence of mastitis with advancement of age may be due to increased potency of teats and increased degree and frequency of previous exposure in multiparous old cows and also may be due to that older cows have largest teats and more relaxed sphincter muscles, which increase the accessibility of infectious agent in the cows' udder.

The quarter-wise incidence of bovine mastitis was higher in Right hind quarter (RH) (29.63%) and Right forequarter (RF) (21.60%) was found to be the least affected. There was no significant difference in the infection rate of the quarters. In the present study the overall incidence of mastitis was higher in the hind compared to the fore quarters in the cows which agreed with the earlier observations [4]. Highest incidences of hind quarters might be due to the high production capacity of the hind quarters and the high chance of getting fecal and urine contamination.

Etiology of Bovine mastitis

Out of 162 milk samples from mastitic quarters which shows positive to MCMT, 152 milk samples were found culturally positive (93.83%) and from these samples 152 different strains belonging to three different type of species were isolated. No bacteria could be isolated from 10 mastitic quarters which may be due to that the animals were pre-medicated or these 10 quarter milk samples were sterile and were considered as infection with mycotic or mycoplasma infections and the utilized specific media could not detect it. Similar type of nonspecific mastitis was reported before also [11].

Mixed infection with two strains in the four quarters had made a total of 152 strains from 152 bacteriologically positive quarters (Table 4). One strain of bacteria could be isolated from each of the remaining bacteriologically positive quarters. The most commonly isolated organisms were *Staphylococci* (86.18%) followed by *E. coli* (9.21%) and *Streptococci* spp. (4.61%) ($P \leq 0.05$). Predominance of *Staphylococci* in milk of cows with mastitis is reported by workers [6]. The high prevalence of *Staphylococci* may be partly explained by the presence of these agents on the skin and mucus membranes of various parts of the animal body or their contagious nature or may be attributed to the fact that the principal reservoirs of *Staphylococci* are the skin of the infected gland.

In the present study, incidence of *E. coli* mastitis was quite high. *E. coli* is one of the important environmental pathogens causing mastitis and it arises due to poor disposal of litter, poor hygiene, no practice of teat dipping and misuse of antibiotics [22].

Mixed (double) infection was found in eight of the quarters. Out of which, five of the quarters had mixed infections with *Staphylococci* and *E. coli* and the other three of the quarters had mixed infection with *Staphylococci* and *Streptococci.* Earlier also the mixed growth or infection in bovine mastitis was reported [12].

Treatment

In the group I, the cure rate was 80.76% quarter-wise and 80.00% animal-wise. The percentage of reduction of mastitis was 70.41%. Post-treatment examinations of milk samples showed that all the affected quarters did not cure or respond to the treatment. Ceftizoxime is believed to have high affinity to bind and accumulate in the adipose tissue of mammary gland [25].

Group II showed 50% cure rate. The cure rate of mastitis quarter-wise and animal-wise was 61.54% and 50%, respectively. The percentage of reduction of mastitis was 40.83%. The lesser efficacy of Cefoperazone sodium was observed. The drug is unable to penetrate deep into mammary tissue and encapsulation of microbes causing mastitis [20].

Group III showed 100% cure. The drug was used less frequently so no or low change of developing resistance by the bacteria. Moreover, the uses of combinations of intramuscular and intramammary antibiotic which have synergistic effect [10].

Conclusion

This study envisages the need for rampant awareness programmes and regular screening in herd for timely treatment and preventive control measures. The necessity of cow side mastitis detection facilities is need of the hour. Earlier the detection less is the loss in terms of treatment and production loss. Judicious use of antibiotics is another necessity that can save the time and loss incurred in connection with occurrence of mastitis. Even though the cattle number in Mizoram is less, a healthy herd keeps the requirement of the people in a satisfactory level.

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