RESEARCH ARTICLE

Prevalence of Subclinical Mastitis, Associated Risk Factors and Pathogens in Dairy Cattle of West Coastal India

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Abstract

Mastitis is one of the most expensive diseases of dairy animals and subclinical mastitis occupies the major proportion of mastitis. Prevalence of mastitis is highly dependent upon type of breed, climatic and housing conditions, management practices, stage of lactation, etc. and hence a study was conducted to screen the prevalence of subclinical mastitis (SCM), associated risk factors, and prevalent pathogens in small backyard and large organized dairy units in four districts in the west coast of India. A total of 240 cattle were screened for subclinical mastitis and milk samples from SCM cases were screened for the presence of major mastitis-associated pathogens. Information on managemental practices was collected and risk factors were analyzed statistically. The prevalence of clinical and subclinical mastitis was 9.93% (27/272) and 30.83% (74/240), respectively. *Staphylococcus aureus*, coagulase-negative *Staphylococci*, *E coli,* and *Bacillus* spp. were the major pathogens isolated from SCM cases. The study revealed that SCM is highly prevalent in dairy farms of coastal districts and higher in crossbred than the indigenous cattle. The absence of regular shed cleaning and the use of milking machines were significantly associated with higher SCM prevalence. Post-milking iodine teat dip was carried out by 27.5% and 88.89% of the small and large farmers and none of the farmers had adopted dry cow therapy. Around 10.0% of small farmers and 77.78% of the large farmers used milking machines. The high prevalence of SCM shows the need for active surveillance programs and the application of preventive measures in the area.

Key words: Cattle, Mastitis pathogens, Risk factors, Subclinical mastitis, West coastal India.

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Introduction

Mastitis, the inflammation of udder tissue is one of the most expensive diseases of dairy animals (Bardhan, 2013). Subclinical mastitis (SCM) characterized by a low level of persistent inflammation and reduced milk yield, forms the major proportion of mastitis and contributes to the major share of economic loss (Birhanu *et al.,* 2017). As the SCM is not easily detected, affected animals remain untreated for prolonged periods, leading to a persistent loss in milk production and affecting fertility and hence causing economic losses in dairy farming (Fernandes *et al.,* 2021). Although, incidences of SCM are reported from dairy herds in different parts of the country little information is available on its prevalence in dairy herds in the west coastal region of India. The occurrence of mastitis highly depends upon climatic and housing conditions, management practices, type of breed, stage of lactation, and awareness of dairy farmers about the disease. The west coast of India has a warm humid climate and a heavy rainfall pattern with a long rainy season from June to November. The animal housing conditions and dairy breeds maintained vary among different regions of the country. Though a few studies have reported the prevalence of SCM in different regions of India, there are no in-depth studies on the prevalence and associated risk factors of SCM in the coastal region of India. Identification of predisposing factors and bringing managemental interventions will help to reduce the economic loss to farmers and help to reduce antibiotic use in dairy farming. Hence the present research was carried out to identify the prevalence of SCM in small backyard and large dairy farms in coastal districts of Goa,

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Kerala, and Maharashtra, and the associated risk factors and major SCM-associated bacterial pathogens.

Materials and Methods

Study Area and Sampling

Randomly 10 numbers each of small backyard dairy farmers (maintaining a dairy unit of 3 to 10 cattle located adjacent to residential houses), the members of Dairy Cooperative Societies of South Goa, North Goa, Kozhikode, and Ratnagiri districts, and 9 large dairy farmers (having more than 25 cows and sheltered in organized commercial farms and Goshalas) in the North Goa district were selected for milk sampling and survey. The information on various parameters about animal, herd, and management practices was collected using a semi-structured questionnaire from the owner or farm attendant. A total of 272 lactating cows including 176 from 40 small backyard dairy farms and 96 from 9 large organized farms from 4 districts (North Goa, South Goa, Ratnagiri, and Kozhikode) were screened for mastitis. The breeds maintained by the farmers were grouped into two types, the crossbreds of Jersey and HF, and the indigenous Gir, Sahiwal, Deoni, and local non-descript cows.

Screening for Mastitis and Sample Collection

Overall, 272 animals were examined, 27 animals were found suffering from clinical mastitis (CM) and 240 healthy animals for which all information could be collected were further screened for SCM with the California Mastitis Test (CMT) kit (DeLaval, USA) as per the manufacturer's instruction. A quarter was considered as CMT positive if it had a score of ≥2+ and a cow was defined as CMT-positive when it had at least 1 quarter with a CMT-positive score of ≥2+. From the CMT-positive animals, 5 mL of milk was collected aseptically from each quarter and was pooled and transported in ice to the laboratory for microbial isolation.

Milk Culturing

About one mL of composite milk sample collected from SCM-positive cows was added into 9 mL of BHI broth and incubated at 37°C for 24 h. Cultures showing turbidity were inoculated in selective media for Coliforms (MacConkey agar), *Bacillus* spp. (Bacillus cereus agar), and *Staphylococcus* spp. (Mannitol salt agar) at 37°C for 24 h. Pure colonies were inoculated in BHI broth before proceeding for further tests. Cultures were subjected to biochemical tests and were confirmed by genus-specific PCR targeting *gap* gene for Staphylococcus (Yugueros *et al.,* 2000) and *E coli* was confirmed by PCR targeting *E coli Alanine Racemase* (*ecalr*) gene (Yokoigawa *et al.,* 1999). The *Bacillus* genus was identified by colony characteristics, Gram staining, and biochemical tests.

Statistical Analysis

The data was statistically analyzed by using PROC MEANS and PROC FREQ of SAS (2012) version 9.2. The possible association between the prevalence of SCM and risk factors was determined by a Chi-square test. Values were considered significant at $p<0.05$.

Results and Discussion

The overall prevalence of clinical mastitis was 9.93% (27 /272), The CMT screening test of the remaining animals showed that 30.83% (74/240) cows and 21.88% (210/946) quarters examined were positive for subclinical mastitis, whereas Bangar *et al.* (2015) and Krishnamoorthy *et al.* (2021) reported higher prevalence of 46.35% and 45.00%, respectively. A higher prevalence of SCM ranging from 43.33 to 67.27% and 30.73% to 39.55% on animal and quarter basis, respectively, has been reported from different regions of India (Sudhan *et al.,* 2005; Hegde *et al.,* 2013; Mir *et al.,* 2014; Jena *et al.,* 2015). Similar to the present study, SCM prevalence of 31.4% and 34.2% was reported in the cattle population of coastal regions in Malaysia and Bangladesh, respectively (Saeed *et al.,* 2022; Islam *et al.,* 2019). The highest proportion of the SCM-affected cattle (40.54%, 30/74) had all 4 quarters affected followed by 27.03% (20/74) having 2 quarters affected, 17.57% (13/74) having 3 quarters affected and only 14.86% (11/74) had one quarter affected with SCM.

The managemental practices followed in two different types of farms, *viz.,* small backyard and large farms were found to be highly different. In small farms, animals were taken care of by family members, whereas activities in large farms were attended by hired labourers. Maintenance of shed hygiene by proper cleaning and washing was done in 90.0% and 88.89% of the small and large farms, respectively (Table 1). The study revealed that 88.89% of the large farmers followed practices like pre-milking udder wash, use of post-milking teat dip, and proper shed cleaning and washing, whereas among small farmers, 90.0% were following udder

Table 1: Management practices followed on the small and large dairy farms

Management practices	Small farms (40)		Large farms (9)	
	Number	Percent	Number	Percent
Maintenance of shed hygiene	36	90.00	8	88.89
Pre-milking udder wash	36	90.00	8	88.89
Post-milking teat dip	11	27.50	8	88.89
Use of machine milking	4	10.00	7	77.78

washing and proper shed cleaning, and only 27.5% used post-milking teat dip. None of the farmers used dry cow therapy to prevent mastitis. Around 10.0% of small farmers and 77.78% of the large farmers used milking machines.

The association between the presence or absence of various risk factors and the prevalence of SCM among the animals studied is presented in Table 2. A significantly ($p<0.025$) higher (34.62%) prevalence of SCM was observed in crossbred cows as compared to indigenous cows (18.96%). Similar to the present finding higher prevalence among crossbred cows than that of indigenous cows was reported in earlier studies (Sharma *et al.,* 2018, 2023). A significantly ($p<0.017$) lower (29.44%) prevalence of SCM was observed in farms with regular washing and cleaning sheds as compared to farms without this practice (66.67%), and also with the practice of hand milking than machine milking (22.97% Vs. 43.47%; $p<0.001$). However, no statistically significant ($p>0.05$) difference was found in the prevalence of SCM among two farm types, *viz.* small backyard (28.57%) and large farms (34.88%), and between two farming systems, *viz.* intensive (27.98%) and semi-grazing (37.50%). This shows that SCM is a matter of equal concern in both intensive and semi-grazing-type farming systems and small and large farms.

The prevalence of SCM was 33.06% and 28.45%, respectively, in cows with and without the use of post-milking iodine teat dips, which was also not statistically significant. Similar to the present results, association of teat dip with higher SCM prevalence was reported in some earlier studies (Khasanah *et al.,* 2021; Navaneethan *et al.,* 2023), while Busato *et al.* (2000) did not find significant association. Teat dipping was reported to be protective against SCM by Ramirez *et al.* (2014)), but was not equally efficient against all bacterial pathogens (Smith and Hogan, 1993; Osteras *et al.,* 2008).

Furthermore, no significant differences were found among groups with and without the use of floor rubber mats (35.87 vs. 27.70%), pre-milking udder wash (30.17 vs. 50.00%), and farms with or without proper cement flooring and drainage (29.95 vs. 33.96%). The west coastal region has a hot humid climate throughout the year with a high rainfall. In these weather conditions, lack of regular cleaning can easily lead to the build-up of bacterial population on the animal body and shed premises increasing the chance of getting mastitis. The results show that the incidence of SCM depends on a variety of factors, mainly maintaining udder health and hygiene in individual cows and farm practices. Poor sanitation and hygiene are the foremost causes of SCM in dairy animals (Sinha *et al.,* 2014).

The bacterial culture study showed that out of 74 cultured composite milk samples from SCM-affected cattle, 57 samples (77.03%) showed positive bacterial isolation. Bacterial colonies were identified by Gram staining, biochemical tests and confirmed by PCR. Multiple bacterial pathogens were isolated from 22 of 57 (38.60%) samples. The study could isolate 80 bacterial isolates. PCR amplification produced a 900 bp product of *gap* gene in case of *Staphylococcus* spp. and 360 bp product of *Ecalr* gene in case of *E coli* (Fig. 1, 2).

Table 2: Association between the risk factors and incidence of SCM (n=240)

Risk Factor	Levels	Number of cows	SCM positivity % (95% CI)	*p*-value
Breed type*	Crossbred	182	34.62	0.025
	Indigenous	58	18.96	
Farm type	Small/backyard	154	28.57	0.18
	Large	86	34.88	
Farming system	Intensive	168	27.98	0.14
	Semi grazing	72	37.50	
Milking type**	Hand milking	148	22.97	0.001
	Machine milking	92	43.47	
Washing the udder before milking	Yes	232	30.17	0.23
	No	8	50.00	
Use of post-milking iodine teat dip	Yes	124	33.06	0.44
	No	116	28.45	
Regular washing and cleaning shed*	Yes	231	29.44	0.017
	No	9	66.67	
Use of Rubber mat	Yes	92	35.87	0.18
	no	148	27.70	
Proper cement flooring & drainage	Yes	187	29.95	0.08
	no	53	33.96	

*$p<0.05$, **$p<0.01$ between levels

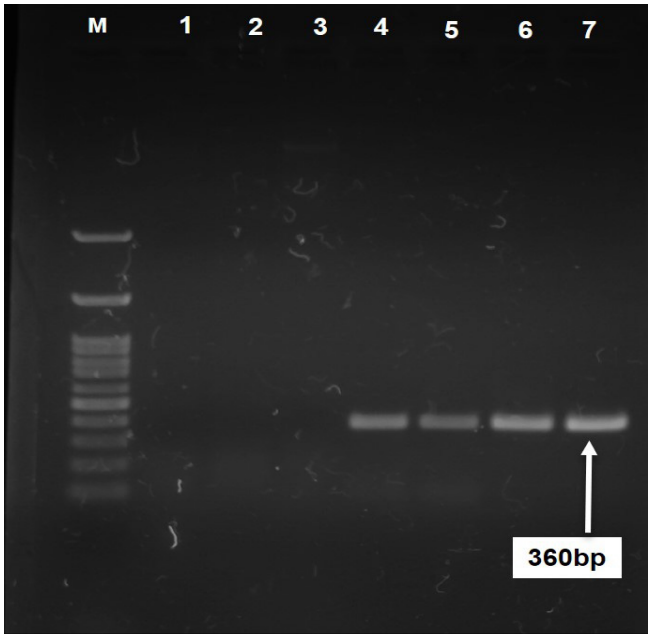

Fig. 1: PCR amplification of *ecalr* gene of *E coli* (360 bp), Lane M, 100 bp ladder, 1 to 3 negative, 4 to 7 *E coli* samples

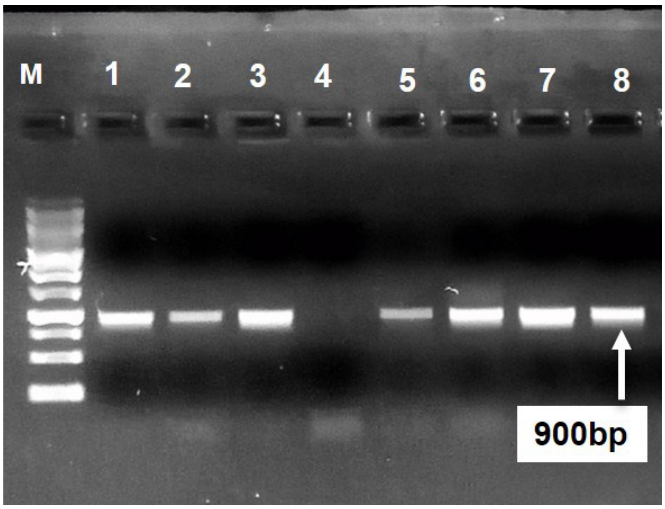

Fig. 2: PCR amplification of *gap* gene of *Staphylococcus* genus (900 bp), Lane M, 1 kb DNA Ladder, 1 to 8 samples, lane 4 is negative.

Among the bacterial pathogens targeted, the most isolated pathogen was *Staphylococcus* spp. (66.21%) followed by CNS (45.94 %), Enterobacteriaceae (24.32%), *S. aureus* (20.27%), *Bacillus* sp. (17.56%) and *E coli* (14.86%). Earlier studies also showed that *Staphylococcus* was the most prevalent bacterial pathogen isolated (Harini and Sumathi, 2011; Hegde *et al.,* 2013; Birhanu *et al.,* 2017), and a similar prevalence of *S. aureus* was reported by others (Chung *et al.,* 2021; Saeed *et al.,* 2022). Least prevalence of *E coli* was noticed identical to previous reports by various workers (Harini and Sumathi, 2011; Hegde *et al.,* 2013; Bhandari *et al.,* 2021). The present study showed a prevalence of *Bacillus* spp., which is less than that reported by Chung *et al.* (2021) and higher than that of Harini and Sumathi (2011).

Table 3: Percent positivity of samples for bacterial pathogen (n=74)

Pathogen isolated	No of isolates (%)
Staphylococcus sp.	49 (66.21)
S. aureus	15 (20.27)
CNS	34 (45.94)
Enterobacteriaceae	18 (24.32)
E coli	11 (14.86)
Bacillus sp.	13 (17.56)

Conclusion

In conclusion, the findings from the study revealed that subclinical mastitis is highly prevalent in dairy farms of coastal states and is associated with bacterial infection more frequently of *Staphylococcus* sp., and to a lesser extent of *E coli* and *Bacillus*, sp. A significant association was found between SCM prevalence and factors like breed, regular cleaning of the shed, and use of the milking machine. However, only 27.5% and 10% of small farmers practiced post-milking teat dips and used machine milking, while frequency of other practices were almost the same in both small and large farms.

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