

PREVALENCE OF SUBCLINICAL MASTITIS IN DAIRY COWS: AN EXPLORATORY STUDY IN THENI DISTRICT OF TAMIL NADU, INDIA

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ABSTRACT

*Mastitis is one of the major economic diseases in the dairy cattle, with subclinical mastitis become increasingly common, especially in crossbred cows. The current study was conducted to assess the prevalence of subclinical form of bovine mastitis, associated risk factors and dominant bacterial pathogens among cattle in south-west part of Tamil Nadu. Among the total of 104 animals milk samples screened for subclinical mastitis and it was found a prevalence of 57.69%. Out of 416 quarters screened, 106 quarters were found positive with overall prevalence of 25.48%. The prevalence was higher in hindquarters (26.41%) than the forequarters (23.58%). Upon bacteriological examination, affected milk samples were affected with *Staphylococcus* spp. (53.33%), *Streptococcus* spp. (13.33%) and *Escherichia coli* (3.33%). Combined infection of *Escherichia coli* & *Staphylococcus* spp. and *Staphylococcus* spp. & *Streptococcus* spp. were found to be 10.00 & 3.33%.*

Key words: *Subclinical mastitis, Dairy cow, Prevalence, Risk factors, Bacterial isolates*

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INTRODUCTION

Livestock is an integral source of income for landless and marginal farmers and plays an important role in Indian national economy. According to the 20th Livestock

Census, India has 302.79 million bovine population, which include 192.49 million cattle and stands as a largest dairy producer in the world with 221.06 million tones milk production during 2021-22 (Government of India, Department of Animal Husbandry and Dairying, Annual report 2021-22). Among the various factors causing huge loss to the dairy industry, mastitis occupies major cause of concern (Singh *et al.*, 2021). A multi etiological complex, causes inflammation

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of parenchyma of mammary glands result in mastitis, characterized by physical, chemical changes in milk and pathological changes in udder (Constable *et al.*, 2017). In contrast, Subclinical Mastitis (SCM) is presented with absence of physical changes both in udder and milk (Orlandini and Bijgaart, 2011). Although the milk appears normal, subclinical mastitis is responsible for greater loss to the dairy industry (Kader *et al.*, 2002) with reduced milk production in compromised quality (Salvador *et al.*, 2014) and found 15 to 40 times more prevalence than clinical mastitis (Seegers *et al.*, 2003). About 75-80 percent mastitis is subclinical, characterized by a significantly increased leukocyte count in milk (Bradley, 2002) and about 70-80% economic loss has been attributed due to subclinical mastitis alone in India (Dua, 2001).

Staphylococcus aureus, non-aureus *Staphylococci* and *Streptococcus spp.*, are the most prevalent cause of SCM. The improper management practices such as high stocking density, cracked floors, poor drainage systems, stall feeding, poor hygiene and sanitation and peri-parturient diseases have also been contributing to the prevalence of SCM (Bari *et al.*, 2022).

For detecting subclinical mastitis, there are several direct and indirect tests, which include the California Mastitis Test (CMT), the modified white side test (MWT), the bromothyl blue card test, determination of electrical conductivity, the chloride estimation test, the modified Aulendorfer mastitis probe test (MAMP), inline monitoring of SCC, infrared thermography

and cultural identification of causal agents (Langer *et al.*, 2014 and Kamphuis *et al.*, 2008).

California Mastitis Test is considered as one of the oldest, low-priced, easily-applicable cow-side test and widely adopted as a milk grading test in various countries to detect different stages of udder inflammation under field conditions. This test enables the subjective evaluation of the somatic cells present in a milk sample to assess the probability and severity of intramammary infection (IMI) (Aprajita *et al.*, 2023 and Dingwell *et al.*, 2002).

Identification of a bacterial pathogen in milk from a cow with mastitis is regarded as the definitive diagnosis of an Intramammary (IM) infection (Oliver *et al.*, 2004). Primary identification of bacteria was done based on cultural colony morphology, type of hemolysis and Gram's staining (Dasohari *et al.*, 2017).

There is always a need for regular surveillance and monitoring of subclinical mastitis time to time and the present study was designed to assess the prevalence of bovine sub-clinical mastitis with a cultural isolation of bacterial pathogen under field condition.

MATERIALS AND METHODS

A total of 104 animals from five taluks of Theni district, Tamil Nadu were screened for subclinical mastitis. In the present study (Table 1). Udder and teats were cleaned and each teat end is scrubbed

with cotton gauze moistened with 70% ethyl alcohol, then milk samples were collected from all four quarters after discarding first two streams of milk and screened for subclinical mastitis using California mastitis test (Make and model: Delaval).

The test was carried out with 3 ml of milk samples collected from each quarter into the respective 4 cups in CMT paddle. Then equal amount of Modified California Mastitis Test reagent was added to each cup of paddle. The content was mixed by gentle circular motion of paddle in the horizontal plane. Depending on the degree of precipitation and formation of gel, readings of positive test were categorized as negative, trace, weak positive (+), distinct positive (++) and strong positive (+++), respectively (Ruegg and Reinemann, 2002 and Kandeel *et al.*, 2018) (Table 2 and Fig 1).

Positive samples were collected using sterile vials and were transported to the laboratory for microbiological examinations. Milk samples from positive quarter was inoculated in Nutrient broth and incubated at 37°C for 24 hours. Further isolation was done by streaking in Mannitol salt agar, Edward's medium and EMB agar plates to identify 3 main genera of mastitis-causing pathogens *Staphylococcus* spp., *Streptococcus* spp., and *E. coli*. (Rana *et al.*, 2022).

From the bacterial growth, single colony was subjected to Gram's staining. Staining was performed as per standard procedures described by Hucker and Cohn (1923) and stained slide was examined

under microscope to identify them as Gram positive and negative. The size, shape and arrangement of bacteria were observed.

RESULT AND DISCUSSION

Among the total of 104 cows milk samples screened for subclinical mastitis, 60 were found positive for SCM by CMT with prevalence of 57.69%(Table 2). Prevalence of SCM in the present study was in agreement with the earlier study on the prevalence as 62.80%, 60.25% and 52.48%, respectively in West Bengal (Roy *et al.*, 1989), Rajasthan (Chahar *et al.*, 2005) and Madhya Pradesh (Ghose and Sharda, 2003).

Out of 416 quarters screened, 106 quarters were found positive with overall prevalence of 25.48 percent (Table 3), whereas Mir *et al.* (2014) and Savita (2016) reported quarter-wise prevalence as 31.02 per cent, 30.73 per cent, and 29.50 per cent, respectively.

The variation in the prevalence of SCM reported across different studies could be attributed to various risk factors such as herd size, milking method adopted, overall poor hygiene, variation in agro-climatic conditions of the region and owner's literacy levels (Ergun *et al.*, 2004 and Singh *et al.*, 2021).

Various risk factors could be attributed to the variation in the prevalence of SCM in different studiessuch as poor hygiene, herd size, method of milking, variations in agro-climatic conditions of the region andowner's literacy level.

Individual quarter wise prevalence of SCM

The quarter wise prevalence of SCM in cattle was illustrated (Table 4). Out of 106 positive quarters, the individual quarter wise prevalence was found to be highest in left hind quarter (34.61%) followed by left fore quarter (28.85%), right hind quarter (19.23%) and right fore quarter (19.23%) (Table 4). The prevalence was higher in hindquarters (26.92 per cent) than the forequarters (24.04 per cent).

Singh *et al.* (2021) and Pooja *et al.* (2022) reported the SCM quarter-wise prevalence in left hind quarter (15.70% & 25.51%), right hind quarter (5.37% & 40.62%), left fore quarter (5.78% & 23.23%) and right fore quarter (4.54 & 32.32%) in dairy cows respectively. Sudhan *et al.* (2005) and Sharma *et al.* (2012) also reported that hind quarters were affected more frequently than fore-quarters. Highest prevalence in the left hind quarter could be attributed to greater exposure to dung and urine contamination and relatively high production capacity of hind quarters (Singh *et al.*, 2021 and Radostits *et al.*, 2010)

Bacteriological examination of milk samples

Based on selective culture media and gram staining technique, affected animal milk samples were cultured for identifying bacterial species. Out of 60 samples studied, *Staphylococcus spp.*, *Streptococcus spp.* and

Escherichia coli were found to be 53.33%, 13.33% & 3.33% and combined infection of *Escherichia coli* & *Staphylococcus spp.* and *Staphylococcus spp.* & *Streptococcus spp.* were found to be 10.00 & 3.33% (Table 4) (Fig. 2a,2b,3a,3b,4a,4b). This finding is in agreement with Khasapane *et al.* (2023) who reported a high prevalence of *Staphylococcus spp.* (43.7%) followed by *Streptococcus spp.* (18.2%) and *Escherichia spp.* (9.5%). On the other hand, Harini and Sumathi (2011) also reported predominant bacterial isolate as *Staphylococcus aureus* (58%) and *Escherichia coli* (23.5%) followed by *Streptococcus spp.* (5.5%). *S. aureus* with high prevalence could be attributed due to common practice of hand milking and the lack of dry cow therapy. *S. aureus* and *Streptococcus agalactiae*, are commonly found on skin of mammary gland, in teat canals, in infected udders and usually acquired during milking (Schnitt and Tenhagen, 2020).

Coliforms especially *Escherichia coli* cause an environmental mastitis and associated with moisture, mud, faeces, and other organic matter primarily acquired by poor hygiene, husbandry, and milking technique (Jang *et al.*, 2017).

CONCLUSION

Prevalence study on subclinical mastitis among dairy cows of Theni district, Tamil Nadu proved the existence of subclinical mastitis under field condition with *Staphylococcus spp.* identified as

predominant etiological factor in this geographical region. Subclinical mastitis leads to substantial economic losses in the dairy sector due to reduced milk yield and quality. In the light of above, routine regular screening of dairy animals is essential for the early detection, precise timely intervention and effective management of subclinical mastitis.

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Table.1. Screening of subclinical mastitis in dairy cows of Theni district

Taluk	No. of dairy farmers	Total No. of animals	No. of animals screened	No. of affected animals	No. of affected quarters
Theni	9	57	24	12	15
Bodinayakkanur	6	91	30	12	17
Uthamapalayam	5	31	14	12	22
Periyakulam	3	29	15	12	32
Andipatti	5	51	21	12	20
Total	28	259	104	60	106

Table.2. California Mastitis Test Interpretation (Ruegg and Reinemann, 2002)

CMT Score	Visible Reaction	SCC Range (cells per mL)
Negative	No evidence of precipitate - Mixture remains liquid	0 – 200,000
Trace	Slight precipitate, best seen by tipping, disappears with continued movement	150,000 – 500,000
1+	Distinct precipitate but no tendency toward gel formation	400,000 – 1,500,000
2+	Mixture thickens immediately, moves toward center	800,000 – 5,000,000
3+	Gel forms and surface becomes convex	>5,000,000

Table.3. Prevalence of sub clinical mastitis in dairy cattle

Category	Total Number - Screened	Total Number - affected	Percentage of SCM
Animals	104	60	57.69%
Quarters	416	106	25.48%

Table.4. Individual quarter wise prevalence of SCM

Quarter's position	Position of teat	No. of quarter screened	No. of quarter positive	Prevalence (%)
Fore quarter	Right	104	20	19.23
	Left	104	30	28.85
Hind quarter	Right	104	20	19.23
		104	36	34.61

Table.5. Distribution of CMT score on prevalence of sub clinical mastitis

CMT Score	Number of Affected Quarters	Percentage of Affected Quarters
Trace	06	5%
1+	19	18%
2+	58	55%
3+	23	22%
Total Number of Affected Quarters	106	

Table.6. Bacteriological isolates of subclinical mastitis affected milk samples

S.No	Type of bacteria	Number of quarters		
		Tested	Positive	Percent (%)
1	<i>Staphylococcus spp.</i>	60	32	53.33
2	<i>Streptococcus spp.</i>	60	8	13.33
3	<i>Escherichia coli</i>	60	2	3.33
4	<i>Escherichia coli</i> + <i>Staphylococcus spp.</i>	60	6	10.00
5	<i>Staphylococcus spp.</i> + <i>Streptococcus spp.</i>	60	2	3.33
	Total	300	50	16.66

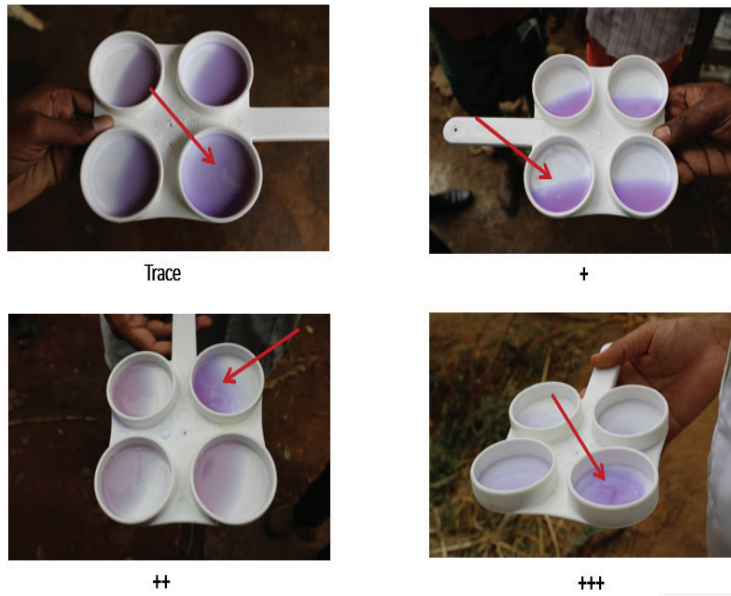


Fig.1. California Mastitis Test Interpretation



Fig.2a. *Staphylococcus* spp., isolates with golden yellow pigments on Mannitol salt agar

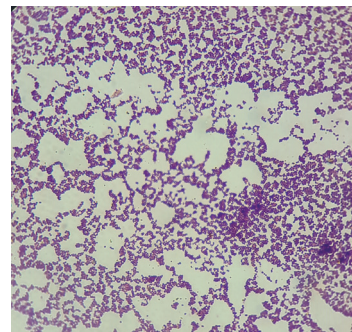


Fig. 2b. *Staphylococcus* spp., with characteristic bunches of grapes on Gram staining



Fig.3a. *Streptococcus* spp isolates with small pinpoint colonies on Edward's medium

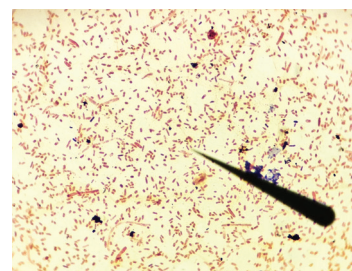


Fig.3b. *Streptococcus* spp with characteristic chain like cocci on Gram staining



Fig.4a. *Escherichia coli* isolates with metallic sheen on EMB agar

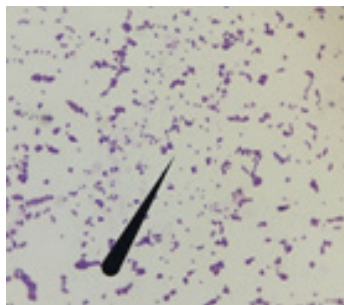


Fig.4b. *Escherichia coli* with characteristic rod shaped red colonies on Gram staining

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