

PREVALENCE OF SUBCLINICAL MASTITIS IN CATTLE ON THE BASIS OF DIFFERENT MASTITIS DIAGNOSTIC TESTS[#]

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ABSTRACT

The present study was undertaken to evaluate prevalence of subclinical mastitis on the basis of commonly used mastitis diagnostic tests. A total of 592 functional quarters of 150 apparently healthy lactating cows were screened by modified California mastitis test, somatic cell count, electrical conductivity and milk pH. The animal-wise highest prevalence of SCM was found in somatic cell count (47.33%) followed by electrical conductivity (46%), modified California mastitis test (44.66%) and milk pH (42.67%). The quarter wise highest prevalence of SCM was recorded in somatic cell count (23.98%) followed by electrical conductivity (23.47%), modified California mastitis test (22.80%) and milk pH (21.45%). Right side quarters were found more affected than the left side quarters. Hind quarters were more affected than the fore quarters. Parity wise highest prevalence of SCM in each mastitis detection tests was observed in Vth parity. Whereas, lactation stage wise highest prevalence of subclinical mastitis was found in early lactation stage followed by mid lactation and least in late lactation stage.

Key words: Subclinical mastitis, modified California mastitis test, SCC, EC, parity and lactation stage

Introduction

India has emerged as the largest producer of milk with 20.17 per cent share in total milk production in the world and also has the largest population of milch animals in the world (Basic Animal Husbandry Statistics, 2022). Worldwide, mastitis is associated with economic losses of \$35 billion every year. It adversely affects milk production whereby losses due to subclinical mastitis are more severe than those due to clinical cases (Muhammed *et al.*, 2011). Annual losses in dairy industry due to mastitis were approximately 2 billion dollars in USA and 526 million dollars in India, in which subclinical mastitis is responsible for approximately 70% of economic losses (Varshney and Naresh, 2004).

Subclinical mastitis is 3-40 times more common than clinical mastitis and causes the greatest overall losses in most dairy herds. Sub-clinical mastitis is usually unnoticed but it is highly prevalent than clinical mastitis and decreases the production and can easily advance into clinical mastitis under adverse conditions so identification and control of sub-clinical mastitis is very important from farmers' point of view (Islam *et al.*, 2012). It is a very difficult task for the farmers to detect sub-clinical mastitis, but its detection is crucial to save lots of troubles to farmers as well as animals (Kabir *et al.*, 2017).

Subclinical mastitis is difficult to be detected by visual inspection and palpation of the udder due to the absence of visible changes in the udder or milk, which makes SCM more challenging. California mastitis test (CMT) is a simple, inexpensive, rapid and highly sensitive test that accurately predicts the inflammatory cell counts in milk from individual quarters or pooled milk samples (Madut *et al.*, 2009). SCC is used as an indicator of milk quality (Hamann and Kromker, 1997). Electrical conductivity (EC) and pH of milk have been used as indicators of mastitis since last two decades (Hamann and Zeconi, 1998). The EC is determined by the concentration of anions and cations. If the cow suffers from mastitis, the

concentration of Na⁺ and Cl⁻ in the milk increases, which leads to increased EC of milk from the infected quarter (Kitchen, 1981). The diagnosis of mastitis according to the International Dairy Federation (IDF) recommendations is based on the somatic cell counts (SCC) and microbiological status of the quarter. The objectives of the present study were to find out prevalence of SCM on the basis of different diagnostic tests and occurrence of SCM in lactating cows.

Materials and Methods

Source of animals

The present study was carried out in apparently healthy cows of organized farm in and around Bikaner district of Rajasthan. A total of 592 quarters of 150 animals were screened by modified California mastitis test, Somatic cell count, Electrical conductivity and pH for subclinical mastitis.

Collection of milk sample

The individual quarter milk sample (about 60 ml) was collected in sterilized test tubes after cleaning the teat orifice with 70% ethyl alcohol. Milk samples were collected after discarding the first few streams of milk and immediately brought to laboratory and kept in refrigeration (4°C) until analysed.

Diagnostic procedures

A total of 592 quarters of 150 lactating animals were screened by modified California mastitis test (MCMT), Somatic cell count (SCC), Electrical conductivity (EC) and pH. The milk samples were subjected to MCMT as per the procedure given by Schalm and Noorlander (1957) using a modified CMT reagent. In the present study, Ezee was used in place of aryl sulphate as an anionic surface active agent. Cresol red was replaced by bromocresol purple (Chahar, 2001 and Savita, 2016)

The somatic cell count of milk samples was performed

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as described by Schalm *et al.* (1971). However, for staining of milk smear, Giemsa stain was used (Chahar, 2001; Bhat *et al.*, 2016).

Electrical conductivity of milk samples was measured by Pen type EC-035 (ATC) Conductivity meter of ERMA instruments. The pH of milk was determined immediately using single electrode Pen type digital pH meter [PH-035 (ATC) of ERMA Instruments.

Results and Discussion

In present study, prevalence of subclinical mastitis was estimated by various indirect mastitis detection tests *viz.* modified California mastitis test, SCC, EC and pH.

Animal wise prevalence of SCM on the basis of different diagnostic tests

The data of animal wise prevalence of subclinical mastitis on the basis of are shown in Table 1.

Table 1: Prevalence of Subclinical mastitis in cattle based on different diagnostic tests

	Diagnostic tests	Animal wise prevalence		Quarter wise prevalence	
		SCM affected animals	Percentage	SCM affected quarters	Percentage
1	MCMT	67	44.66	135	22.80
2	SCC	71	47.33	142	23.98
3	EC	69	46	139	23.47
4	PH	64	42.67	127	21.45

Out of 150 cows, 67, 71, 69 and 64 cows were found to be positive for subclinical mastitis by MCMT, SCC, EC and pH, respectively. The animal-wise per cent prevalence of subclinical mastitis on the basis of MCMT, SCC, EC and pH was recorded as 44.66 per cent, 47.33 per cent, 46 per cent and 42.67 per cent, respectively. The animal wise prevalence of subclinical mastitis by MCMT was in close approximation to the study of Ayano *et al.* (2013), Sekhar Reddy *et al.* (2014), Sulieman *et al.* (2018), Sharma *et al.* (2018), Marwaha (2018), Yadav (2018), Kachhawa (2019), Savita *et al.* (2020) and Gupta (2021) wherein they reported animal wise prevalence as 41.02 per cent, 48.14 per cent, 48.8 per cent, 41 per cent, 48 per cent, 38.33 per cent, 49.78 per cent, 38 per cent, and 43.07 per cent, respectively. Badiuzzaman *et al.* (2014) found animal-wise prevalence as 72.07 per cent, 66.67 per cent by MCMT, and SCC, respectively which was higher than the present study. Difference in prevalence of SCM might be due to climatic factors (humidity, temperature, and seasonal variations), geographical locations, difference of breed, management systems, the tests used for screening of the milk samples and other associated conditions.

Quarter wise prevalence of SCM by different diagnostic tests

The prevalence of subclinical mastitis on the basis of quarter wise is shown in Table 1. Out of 592 functional quarters of 150 cows, 135(22.80%), 142 (23.98%), 139 (23.47%) and 127 (21.45%) quarters were found to be positive for subclinical mastitis by MCMT, SCC, EC and pH, respectively. The quarter wise prevalence of subclinical mastitis by MCMT was similar to the study of Kushwaha (2016), Savita (2016), Sharma *et al.* (2018), Marwaha (2018), Savita *et al.* (2020) and Gupta *et al.* (2022) wherein, they reported quarter wise prevalence as 16.21 per cent, 29.50 per cent, 31.16 per cent, 19.50 per cent, 29.50 per cent, 22.45 per cent, respectively. Shelke *et al.* (2019)

recorded quarter wise prevalence of SCM as 22.50 per cent, 25.78 per cent, 17.18 per cent and 18.99 per cent by MCMT, SCC, EC and pH, respectively which was similar to present study. Savita (2018) reported quarter wise prevalence as 43.50 per cent, 38 per cent and 37.50 per cent by SCC, EC and culture examination which was higher than results of present investigation. Varatanovic *et al.*, (2015) concluded that MCMT represents important diagnostic method in detection of subclinical mastitis in cows and out of 4432 quarters, 2166 (48.87%) were found positive to MCMT which was higher than the result of present study.

Quarter type wise prevalence of SCM by different diagnostic tests

The quarter type-wise prevalence of subclinical mastitis in cows recorded with different diagnostic tests as MCMT, SCC, EC and pH was presented in Table 2.

Table 2: Overall quarter type wise prevalence of subclinical mastitis in cattle based on different diagnostic tests

S. No.	Diagnostic tests	Quarter wise prevalence												Total (592)
		RH(147)		LH(149)		Total hind quarters		RF(149)		LF(147)		Total Fore quarters		
		Positive	%	Positive	%	Positive	%	Positive	%	Positive	%	Positive	%	
1	MCMT	48	32.65	37	24.83	85	28.71	28	18.79	22	14.96	50	16.89	135
2	SCC	52	35.37	39	26.19	91	30.74	28	18.79	23	15.66	51	17.22	142
3	EC	50	34.01	39	26.19	89	30.06	28	18.79	22	14.96	50	16.89	139
4	PH	44	29.03	34	22.81	78	26.35	30	20.13	19	12.92	49	16.55	127

Quarter type wise prevalence of SCM was recorded in the RH quarter as 32.65 per cent, 35.37 per cent, 34.01 per cent and 29.03 per cent, in the LH quarter 24.83 per cent, 26.19 per cent, 26.19 per cent and 22.81 per cent, in the RF quarter 18.79 per cent, 18.79 per cent, 18.79 per cent and 20.13 per cent and in the LF quarter 14.96 per cent, 15.66 per cent, 14.96 per cent and 12.92 per cent by MCMT, SCC, EC and pH, respectively. The highest quarter type wise prevalence of SCM was recorded in hindquarters as 28.71 per cent, 30.74 per cent, 30.06 per cent and 26.35 per cent than fore quarters as 16.89 per cent, 17.22 per cent, 16.89 per cent and 16.55 per cent by MCMT, SCC, EC and pH, respectively. Lowest quarter wise prevalence of SCM was found in Left fore (LF) quarter by all the diagnostic tests that represented as 14.96 per cent, 15.66 per cent, 14.96 per cent and 12.92 per cent. Right side quarters were found more affected than the left side quarters. The results of present study are in agreement with Badiuzzaman *et al.* (2014), Bhat *et al.* (2016), Zeryehun and Abera (2017), Swami *et al.* (2017), Sharma *et al.* (2018) and Gupta (2021) wherein, they recorded highest prevalence in right hind quarter than other quarters. Shelke *et al.* (2019) and Tripura *et al.* (2014) recorded highest prevalence in left hind quarters than right hind quarters which may be due to increased exposure of both hind quarters to dung and urine of animals and could be attributed to the high production capacity (Radostits *et al.*, 2000). In the contrary, Srinivasan *et al.* (2013) recorded higher prevalence in fore quarters than hind quarters. The variations in the quarter side prevalence were attributed to the common practice of the milkmen based on the side of

approach at the time of milking and greater vulnerability to direct trauma and relatively more closeness to the floor as compared to fore quarters (Akhtar *et al.*, 2012).

Parity wise prevalence of SCM by different diagnostic tests

Data of parity wise prevalence of sub-clinical mastitis are presented in Table 3.

Parity wise prevalence of SCM was recorded as 23.07 per cent, 23.07 per cent, 21.42 per cent, and 23.07 per cent in Ist parity, 26.66 per cent, 40.0 per cent, 26.66 per cent and 26.66 per cent in IInd parity, 45.45 per cent, 45.45 per cent, 45.45 per cent and 40.90 per cent in IIIrd parity, 52.94 per cent, 55.88 per cent, 55.88 per cent and 47.05 per cent in IVth parity, 53.13 per cent, 56.25 per cent, 56.25 per cent and 53.13 per cent in Vth parity, 48 per cent, 48 per cent, 48 per cent and 48 per cent in VIth parity and 33.33 per cent 33.33 per cent 33.33 per cent and 33.33 per cent in VIIth parity by MCMT, SSC, EC and pH, respectively. The higher parity wise prevalence of SCM was recorded in Vth to VIth parity than decreased with subsequent parities and lower prevalence was recorded in Ist and IInd by all the diagnostic tests. The results of present study are in correlation with the findings of Siddiquee *et al.* (2013), Savita (2016), Kushwaha (2016), Badiuzzaman *et al.* (2014), Savita (2018), Kachhawa *et al.* (2019), Choudhary (2019) and Gupta (2021) who reported highest prevalence in IVth or Vth parity. Higher incidence of subclinical mastitis during late lactation might be due to lowered resistance of animals and weakening of teat sphincter.

Lactation stage wise prevalence of SCM by different diagnostic tests

Data of lactation stage wise prevalence of sub-clinical mastitis are presented in Table 4. The lactation stage wise prevalence of subclinical mastitis in early lactation was 55.38 per cent, 58.46 per cent, 56.92 per cent and 49.23 per cent by

MCMT, SCC, EC, and pH, respectively while, in mid lactation prevalence was 39.62 per cent, 41.51 per cent, 41.51 per cent and 37.73 per cent and in late lactation the prevalence of subclinical mastitis was 31.25 per cent, 34.37 per cent, 31.25 per cent and 31.25 per cent by MCMT, SCC, EC, and pH, respectively. The higher prevalence on the basis of lactation stage was reported in early lactation stage followed by mid lactation and late lactation by all the diagnostic tests.

The results of present study are in agreement with Choudhary (2019) and Mourya *et al.* (2020) and Gupta (2021) who reported highest prevalence in early lactation stage as 63.25 per cent and 56.25 per cent, 52.50 per cent, respectively. Kathiria *et al.* (2014) recorded the highest prevalence of subclinical mastitis during the early lactation in comparison to mid and late lactation stages. Negative energy balance and post partum rapid physiological change in the mammary tissue causes low / reduced udder resistance and higher prevalence of SCM. According to Sheikh *et al.* (2019) during early stage of lactation cow goes through many physiological changes in body leading to constant stress and decreased immune status.

Conclusions

The highest animal wise prevalence of SCM was found with somatic cell count (47.33%) followed by electrical conductivity (46%), modified California mastitis test (44.66%) and least in milk pH (42.67%). The quarter's wise prevalence of SCM in present investigation was 22.80%, 23.98%, 23.47% and 21.45% with modified California mastitis test, somatic cell count, electrical conductivity and, milk pH, respectively. The highest quarter type wise prevalence of SCM was recorded in hindquarters than fore quarters and right side hind quarters were more affected than the left side fore quarters in each mastitis detection tests. Parity wise highest prevalence of SCM in each mastitis detection tests was observed in Vth parity. Whereas, lactation stage wise highest prevalence of subclinical

Table 3: Parity wise prevalence of subclinical mastitis in cattle based on different diagnostic tests

S. No.	Diagnostic tests	Quarter wise prevalence												
		RH(147)		LH(149)		Total hind quarters		RF(149)		LF(147)		Total Fore quarters		Total (592)
		Positive	%	Positive	%	Positive	%	Positive	%	Positive	%	Positive	%	
1	MCMT	48	32.65	37	24.83	85	28.71	28	18.79	22	14.96	50	16.89	135
2	SCC	52	35.37	39	26.19	91	30.74	28	18.79	23	15.66	51	17.22	142
3	EC	50	34.01	39	26.19	89	30.06	28	18.79	22	14.96	50	16.89	139
4	PH	44	29.93	34	22.81	78	26.35	30	20.13	19	12.92	49	16.55	127

Table 4: Lactation stage wise prevalence of SCM in cattle based on different diagnostic tests

S. No.	Lactation stage (Month)	No of cow screened	Diagnostic tests							
			MCMT		SCC		EC (mS/cm)		pH	
			Cow positive	Prevalence (%)	Cow positive	Prevalence (%)	Cow positive	Prevalence (%)	Cow positive	Prevalence (%)
1	Early Lactation (1-3 Months)	65	36	55.38	38	58.46	37	56.92	34	49.23
2	Mid Lactation (3-6 Months)	53	21	39.62	22	41.51	22	41.51	20	37.73
3	Late Lactation (6-8 Months)	32	10	31.25	11	34.37	10	31.25	10	31.25
Total		150	67	-	71	-	69	-	64	-

mastitis was found in early lactation stage followed by mid lactation and least in late lactation stage.

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Conflict of interest

There is no conflict of interest as per my knowledge and belief since the manuscript is a part of original Ph.D research work

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