Journal of Animal Research: v.9 n.2, p. 303-309. April 2019

DOI: 10.30954/2277-940X.02.2019.14

Incidence and Factors Affecting Subclinical and Clinical Mastitis in Selected Organised Dairy Farms Located in Tamil Nadu

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Received: 13 Nov., 2018 **Revised:** 22 Feb., 2019 **Accepted:** 23 Feb., 2019

ABSTRACT

The study is undertaken in five organised dairy farms located in Chennai, Trichy and Karur districts of Tamil Nadu to assess the incidence of clinical and sub clinical mastitis and predisposing factors such as Herd, Breed, Parity, stage of lactation and the efficiency of two screening tests for the diagnosis of sub clinical mastitis (SCM) in the dairy farms. A total of 159 cows with 636 quarters samples screened for clinical mastitis signs and SCM by modified California mastitis test (mCMT) and somatic cell count (SCC).The results showed that 9.4 per cent of animals had clinical mastitis, 66.7 per cent had SCM by mCMT and 77.4 per cent by SCC. The quarter wise incidence of SCM was 47.6 per cent as screened by mCMT and 51.7 per cent by SCC. There are no significant differences observed in the incidence of clinical mastitis and SCM in farm and breed wise. Parity ($p<0.01$), Stage of lactation by SCC method ($p<0.05$), Quarter-wise ($p<0.05$) incidence have significant differences of occurrence. SCM as screened by mCMT and SCC were compared for agreement by Cohen's Kappa statistics and the per cent agreement for the two methods of screening SCM was 54.7 per cent with a P-value of 0.000. The study conclude that the higher SCM incidence in organised dairy farms as 66.7 per cent by mCMT and 77.4 per cent by SCC and necessitate the need of effective mastitis control program to be implemented at the farm level.

Keywords: Organised dairy farms, subclinical and clinical mastitis, factors

Mastitis is usually known to be an economically devastating disease results in huge losses to the dairy farmers and industry. Clinical mastitis is an individual problem and it is characterised by changes in udder and milk drawn from it whereas subclinical mastitis is herd problem because it constitutes a reservoir of infection which could be transmitted to other animals of herd. Average decrease in milk yield due to clinical and subclinical mastitis was estimated to be 50% and 17.5% respectively (Joshi and Gokhale, 2006).

The herd level prevalence of subclinical mastitis and causative factors influencing mastitis invariably differ from place to place, herd to herd and time to time. Studies on mastitis from various parts of the country reflect high incidence over the decades (Ali *et al*., 1989; Mir *et al.*, 2014; Sharma *et al*., 2018). Subclinical mastitis at farm level mostly goes unrecognised due to in-apparent signs of incidence. Hence the routine surveillance and constant monitoring of the animals are very much essential to control subclinical mastitis in dairy farms.

Predisposing factors for incidence of subclinical mastitis highly depend upon type of breed, parity, stage of lactation, management practices, and awareness of farmers. Due to widespread adoption of crossbreeding in Tamil Nadu

most of dairy animals are crossbred population of Holstein Friesian (HF) and Jersey, which are more prone to subclinical mastitis compared to the indigenous animals. The California mastitis test (CMT) has the potential to be rapid, accurate and economically feasible test for fresh cows (Dingwell *et al*., 2003). As a quantitative method, the measurement of somatic cells in milk samples is performed to assess subclinical mastitis (Harmon, 2001). Somatic cell count (SCC) was the most accurate test for the diagnosis of subclinical mastitis followed by the modified California mastitis test (mCMT) (Sharma *et al*., 2008; Sharma *et al*., 2011). This study was conducted to assess subclinical mastitis using mCMT and SCC as methods of screening the organised dairy farms.

MATERIALS AND METHODS

Location and animals

The study was conducted on one hundred and fifty nine cows maintained in five organised dairy farms located at Chennai, Trichy and Karur districts of Tamil Nadu. The study area broadly falls into two agro-climatic zones, North eastern-zone under hot and humid tropical climate (Chennai) and Cauvery delta zone with hot dry tropical climate (Trichy and Karur). Information on animals such as breed, age, parity, stage of lactation, type of housing and adoption level of hygienic milking practices was recorded during the period of March 2018 to June 2018.

Management

The study animals were managed under intensive farming system. The housing system was tie stall with head to head system. They were milked twice a day. Both machine and hand milking were practised. The animals were provided either with green grass CO-4 or crop residues such as paddy straw or sorghum straw according to availability. They were fed with rice bran, gram husk, wheat bran, groundnut oilcake along with commercial cattle feed. Feeding ration was almost similar in all the farms.

Screening for sub-clinical mastitis

As per method described by Rosenberger (1979), fresh samples of approximately 10 ml milk from each quarter of apparently healthy cows at afternoon milking of each dairy farm were collected aseptically in separate glass tubes and labelled quarter wise (LF- Left fore, LH- Left hind, RF- Right fore and RH- Right hind) with respective cow number. Immediately after collection, milk samples were subjected to physical examination with naked eye to detect any abnormalities in colour, odour, consistency and presence of clot, blood, flakes and other visible abnormalities. These aseptically collected milk samples were subjected to mCMT and SCC.

For mCMT, the reagent was prepared by adding 2.0 ml stock solution of 0.5% Bromocresol purple reagent to make a volume of 100 ml by adding the stock solution of 3% Sodium lauryl sulphate and pH was adjusted to 8.0 - 8.2 (Shukla, 1980). R*esults were scored as N (Negative), T (Traces) scores of 1+, 2+ and 3+. In this study, mCMT scores of 'N' and trace 'T' were taken as negative or normal whereas, mCMT scores of 1+, 2+ and 3+ were considered as indicators of subclinical mastitis* (Radostits *et al*., 2010).

For SCC, modified Newmann's stain (HiMedia) was used to stain the cells and the procedure described by Harmon (2001) was followed. The acceptability break point for SCC was taken as 5×10^5 cells /ml as per Hegdae e*t al*. (2013) to confirm the animal as subclinically mastitic. Milk samples showing gross changes in colour and consistency, and swelling of udder were considered as clinically affected cases. A subclinical mastitis case was defined as an animal with at least one of the quarters showing cut off scores as described above.

Statistical analysis

Prevalence of SCM and CM was calculated as the number of positive cases of subclinical/ clinical mastitis to number of animals tested multiplied by 100, and both animal and quarter-wise incidences were studied. The association of predisposing factors such as farm, breed, parity, stage of lactation, with the mCMT and SCC positivity were analysed by Chi–square test. The results obtained from these two tests were compared for agreement using Cohen's Kappa statistics. All the statistical analyses were done using SPSS statistics *ver*25 software.

RESULTS AND DISCUSSION

Incidence of SCM

The results of incidence of subclinical mastitis as screened by mCMT and SCC were compared for agreement and Cohen's Kappa statistics are presented in Table 1. The per cent agreement of the two methods of screening SCM was 54.7 per cent (moderate agreement as per Viera and Garrett, 2005) with a P-value of 0.000.

Results of animal wise incidence of SCM and CM are presented in Table 2. Overall animal wise incidence of SCM observed in this study was 66.7 per cent by mCMT and 77.4 per cent by SCC which were comparatively high. Hadrenberg, (2016) observed that the overall incidences as 35.4 per cent of SCM in Bihar and as low as 27.3 per cent, 35.8 per cent and 37.8 per cent during early, mid and late lactations respectively by using CMT method.

The quarter wise incidence of SCM was 47.6 per cent as screened by mCMT and 51.7 per cent as screened by SCC (Table 3). Hegdae e*t al*. (2013) reported that 45 per cent of milk samples were positive for SCM by adopting SCC method. Kayesh *et al*. (2014) recorded that quarters of 40.57 per cent were positive by CMT. Langer *et al*. (2014) reported the incidence of 45.2% on quarter level of SCM, by adopting SCC at 5,00,000 cut off value level which is found similar to this study. SCC has been considered as a better method in identifying SCM based on the observations. Sharma *et al*. (2018) recorded the incidence of 43.37 per cent quarters of SCM positivity by CMT, which is similar to the present study showing 47.6 per cent positivity. Quarter-wise incidence of clinical mastitis was 2.83 per cent.

The overall incidence of clinical mastitis was 9.4 per cent in the present study. Similar to this finding, 11.6 per cent prevalence of clinical mastitis on cow basis was reported

Table 1: Kappa analysis for agreement between mCMT and SCC

		Somatic cell count		Total	Kappa value
		Negative	Positive		
Modified California mastitis test	Negative	21	17	38	0.547**
	Positive	6	100	106	
Total		**27**	**117**	**144**	

Table 2: Overall animal wise incidence of subclinical and clinical mastitis

Number of cows	Subclinical mastitis (Per cent)		Clinical mastitis (Per cent)
	mCMT	SCC	
159	66.7	77.4	9.5

Table 3: Quarter-wise prevalence of clinical and sub clinical mastitis by mCMT and SCC in dairy cows of organised farms

Quarters	No. screened	Sub clinical mastitis (Per cent)				Clinical (Per cent)	
		mCMT for SCM Positive (Per cent)	Sig	SCC for SCM Positive (Per cent)	Sig		Sig
LF	159	53.46	*	50.9	*	1.89	*
LH	159	38.99		45.3		3.77	
RF	159	47.80		53. 5		0.63	
RH	159	50.31		57.2		5.03	
Overall	**636**	**47.64**		**51.7**		**2.83**	

* Significant at 5% level ($p<0.05$).

by Hadrenberg (2016). Sharma *et al*. (2018) reported a high incidence of 13.5 per cent in their study at Jammu region. However the subclinical mastitis identified by mCMT in their study was slightly lower with 59 per cent incidence. Badiuzzaman *et al*. (2015) reported 66.67 per cent cow wise prevalence of SCM by SCC. This study is in close agreement with Jena *et al*. (2015) who recorded the incidence of sub clinical mastitis as 67.27 per cent using CMT and 74.55 per cent using SCC with a total of 110 lactating cows of rural, peri-urban and suburban regions of Jaipur District in the state of Rajasthan and concluded that SCC showed the highest efficacy in diagnosis of SCM.

Predisposing factors of SCM

Factor wise incidence of SCM as detected by mCMT and SCC are presented in Table 4. There was no significant difference between incidences of SCM in various farms. This could be due to the uniformity in management practices.

Breed

The farms under this study mostly consist of crossbred of HF, Jersey and few native cattle breeds. The incidence of SCM was assessed breed wise by mCMT and SCC, and results are presented in Table 4. The incidence of SCM observed by mCMT for the breeds HF cross, Jersey cross and native cattle were 68 per cent, 66 per cent and 50 per cent respectively and those by SCC method were 80 per cent, 75.5 per cent and 50 per cent respectively. The per cent incidences of clinical mastitis in these breeds were 9 per cent, 9.49 per cent and 16 per cent respectively.

However, there was no significant difference between incidence of SCM among crossbreds of HF, Jersey and native cows by both the methods. The number of native cattle were too less and there was not much difference between Jersey and Friesian Crossbreds. Kurjogi and Kaliwal, (2014) detected the prevalence of SCM in HF, Jersey and native breeds as 54.7 per cent, 47.8 per cent and 40.8 per cent respectively. Sanotharan *et al*. (2016)

Table 4: Predisposing factors of incidence of subclinical and clinical mastitis in cows by mCMT and SCC

Factors	No. of cows screened	mCMT	Sig.	SCC	Sig.	Clinical mastitis	Sig.
		Positive for SCM (%)		Positive for SCM ≥ 5×10^5 (%)		Positive (%)	
Farm^{ns}							
Breed							
HF cross	100	68	NS	80	NS	9	NS
Jersey cross	53	66		75.5		9.4	
Native	6	50		50		16	
	159	66.7		77.4		9.4	
Parity							
1	39	53.8	**	59	**	12.8	**
2	27	70.4		74.1		0	
3	35	77.1		94.3		28	
4	25	44		64		20	
5	23	87		100		8.6	
6 and above	10	80		80		20	
	159	66.7		77.4		9.4	
Stage of lactation							
Early lactation (10 days-3 month)	49	63.3	NS	69.4	*	10.2	NS
Mid lactation (>3 months-6 months)	46	63		76.1		6.5	
Late lactation (>6 months-dry)	64	71.9		84.4		11	
Total	**159**	**66.7**		**77.4**		**9.4**	

NS- Non significant * Significant at 5% level ($p<0.05$) ** highly significant ($p<0.01$).

reported that 60.7 per cent European cross breeds were positive by CMT.

Parity/ Number of lactation

The observations of paritywise incidence of SCM and clinical mastitis assessed by mCMT and SCC method are presented in Table 4. Parity had a highly significant effect on incidence of mastitis. In general an increased prevalence of SCM with increased number of parity was observed in this study. The increasing trend of SCM incidence was observed from first parity to third parity and again from fifth parity and above. Similar observation by Sanotharan *et al*. (2016) reported the highest (75 per cent) prevalence in > 5 parity.

As far as clinical mastitis is concerned, the first and fourth parity have higher incidence when compared with other stages. Oliveira *et al*., 2015 reported clinical mastitis in both multiparous and primiparous cows and observed that cows with parity number 3 and above were more likely to have clinical mastitis. Breen *et al*. (2009) observed that a significantly increased risk of clinical mastitis with increasing parity number and decreasing month of lactation. The higher incidence of clinical mastitis in the first parity might have been due to sudden exposure of mastitis pathogens at the physiological risk period, stocking along with persistently infected subclinical mastitic cows, clinically affected cases and negligence of hygienic milking practices followed in the farms.

Stage of lactation

Influence of stage of lactation on the incidence of SCM is presented in the Table 4. The number of SCM positive cattle in three stages of lactation such as early, mid and late lactation by mCMT method was 63.3 per cent, 63.0 per cent and 71.9 per cent, respectively. There was no significant difference on the incidence of SCM between three stages of lactation diagnosed by mCMT method. However, the incidence of SCM as influenced by stage of lactation was significantly different at 5% level ($p<0.05$) using SCC. Again the SCC method was found to screen more cases of SCM as reported in earlier studies (Bodiuzzaman *et al*., 2015). A uniform trend of high incidence of SCM was observed in late lactation in this study. As seen in the present study, high mean SCC was noticed at third stage of milking in crossbred mastitis cows by Saravanan *et al*. (2015) in Bangalore and Ongole. Usually SCC increases with progressing lactation (late lactation) regardless of whether the cow is infected or not (Dohoo and Meek, 1982).

Contrary to our finding, the stage of lactation wise incidences reported by Badiuzzaman *et al*. (2015) were 78.43 per cent, 67.86 per cent and 65.63 per cent for early, mid and late lactations, respectively. The gradual increase of SCC in late lactation is accentuated if an infection is present. The high incidence of SCM in late lactation might be due to repeated exposure of udder to mastitis pathogens while lactation stages advance.

Sanotharan *et al*. (2016) reported that the highest prevalence of SCM by CMT positivity was recorded in late stage of lactation (71.1 per cent) and the lowest prevalence was found in early stage of lactation (27.7 per cent). This study also agrees with Sharma *et al*. (2018) as reported that late stage of lactation showed high prevalence rate of mastitis followed by early and mid-lactation. The high prevalence of SCM during late lactation might be due to the fact that this period is more vulnerable to infection.

CONCLUSION

Subclinical mastitis at farm level is a complex problem due to in-apparent nature, because of increased crossbred stock of our farms necessitates the better understanding of the factors responsible of the incidence of the problem at farm level. The present study concludes that there is no significant difference at 5% level ($p<0.05$) on the incidence of subclinical mastitis and clinical mastitis by farm, breed, by mCMT and SCC, and stage of lactation by mCMT. But significant differences have been observed at quarter level, parity and stage of lactation by mCMT and SCC methods. SCC method has given the better indicative of incidence of subclinical mastitis. Since Kappa statistics revealed moderate agreement for the two methods of screening SCM, mCMT can be utilised as a screening test for the incidence of subclinical mastitis at farm level. Further the study indicates the need for effective mastitis control program to be implemented at the farm level.

ACKNOWLEDGEMENTS

Authors are thankful for the owners of five study farms for their kind permission and co-operation during the

study period. Authors also acknowledge the Dean, Faculty of Basic sciences, Tamil Nadu Veterinary and Animal Sciences University, Chennai for the approval of this study as a part of Ph.D. research programme and providing necessary facilities to carry out research.

REFERENCES

Ali, S.L., Supekar, P.G. and Shukla, P.C. 1989. A study of the incidence of subclinical mastitis in cows in Mhow region. *Gujarat Vet.*, **16:** 16–28.

Badiuzzaman, M., Samad, M.A., Siddiki, S.H.M.F., Islam, M.T. and Saha. S. 2015. Subclinical mastitis in lactating cows: comparison of four screening tests and effect of animal factors on its occurrence. *Bangl. J. Vet. Med.*, **13(2)**: 41-50.

Breen, J.E., Green, M.J. and Bradely, A.J. 2009. Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom. *J. Dairy Sci*., **92**: 2551-2561.

Dingwell, R.T., Leslie, K.E., Schukken, Y.H., Sargeant, J.M. and Timms, L.L. 2003. Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. *Can. Vet. J*., **44**: 413–416.

Dohoo, I.R., Meek, A.H. and Martin, S.W. 1984. Somatic cell counts in bovine milk: relationships to production and clinical episodes of mastitis. *Can. J. Comp. Med.*, **48:** 130-135.

Hardenberg, F. 2016. Clinical and subclinical mastitis in dairy cattle and buffaloes in Bihar, India, Degree project in Veterinary Medicine submitted at Swedish University of Agricultural Sciences. Uppsala. *http://stud.epsilon.slu.se.*

Harmon, R.J. 1994. Physiology of mastitis and factors affecting somatic cell counts. *J. Dairy Sci*., **77:** 2103-2112.

Hegde, R., Isloor, S., Nithin Prabhu, K., Shome, B.R., Rathnamma, D., Suryanarayana, V.V.S., Yatiraj, S., Renuka Prasad, C., Krishnaveni, N., Sundareshan, S., Akhila, D.S., Gomes, A.R. and Hegde, N.R. 2013. Incidence of Subclinical Mastitis and Prevalence of Major Mastitis Pathogens in Organized Farms and Unorganized Sectors. *Indian J. Microbiol*., **53**: 315–320.

Jena, B., Pagrut, N.K., Sahoo, A. and Ahmed, A. 2015. Subclinical Bovine Mastitis in Rural, Peri-Urban and Suburban Regions of Jaipur District of Rajasthan. *Indian J. Anim. Res*., **5**: 175-182.

Joshi, S. and Gokhale, S. 2006. Status of Mastitis as an Emerging Disease in improved and Periurban Dairy Farms in India. *Ann. NY. Acad. Sci*., **1081**: 74–83.

Kurjogi, M.M. and Kaliwal, B.B. 2014. Epidemiology of Bovine Mastitis in Cows of Dharwad District. *Int. Sch. Res. Notices*, **2014**: 1-9.

Oliveira, C.S., Hogeveen, H., Botelho, A.M., Maia, P.V., Coelho, S.G. and Haddad, J.P. 2015. Cow-specific risk factors for clinical mastitis in Brazilian dairy cattle. *Prev. Vet. Med*., **121**: 297–305.

Olivera, J., Mir, A.Q., Bansal, B.K. and Gupta, D.K. (2014). Subclinical mastitis in machine milked dairy farms in Punjab: prevalence, distribution of bacteria and current antibiogram. *Vet. World*, **7**: 291-294.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D. 2010. Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats, 10th Edn., Elsevier Publishing Co., NY, pp. 686-687.

Rosenberger, G. 1979. Clinical examination of cattle. 1st Edn, Verlag, Paul Parey, Berlin, Germany, pp. 215-220.

Sanotharan, N., Pagthinatan, M. and Nafees, M.S.M. 2016. Prevalence of Bovine Subclinical Mastitis and its Association with Bacteria and Risk Factors in Milking Cows of Batticaloa District in Sri Lanka. *Int. Inno. Sci. Res*., **3**: 2313-3759.

Saravanan, R., Das, D.N., De, S. and Panneerselvam, S. 2015. Effect of season and parity on somatic cell count across zebu and crossbred cattle population. *Indian J. Anim. Res*., **49**: 383-387.

Sharma, N., Maiti, S.K. and Pandey, V. 2008. Sensitivity of indirect tests in the detection of subclinical mastitis in buffaloes. *Vet. Pract*., **9**: 29-31.

Sharma, N., Singh, N.K. and Bhadwal, M.S. 2011. Relationship of somatic cell count and mastitis: An overview. *Asian-Austral. J. Anim. Sci.*, **24**(3): 429-438.

Sharma, N., Singh, S.G., Huma, Z.I., Sharma, S., Misri, J., Gupta S.K. and Hussain, K. 2018. Mastitis occurrence pattern in dairy cows and importance of related risk factors in the occurrence of mastitis. *J. Anim. Res*., **8**: 315-326.

Shukla, P.C. 1980. A Study to evaluate the relative sensitivity of some of the indirect diagnostic tests available for detection of subclinical cases of mastitis. M.V.Sc. and A.H. thesis (Veterinary Medicine), Jawaharlal Nehru Krishi Vishvavidhyalaya, Jabalpur.

Viera, A.J. and Garrett, J.M. 2005. Understanding inter-observer agreement: The Kappa statistic. *Fam. Med*., **37(5)**: 360-363.