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Short Communication

Causal association of epidemiological factors with the incidence of bovine clinical and subclinical mastitis

R. Navaneethan[1], S. Saravanan[2*] and P. Suresh[3]

[1]Kurichi Veterinary Dispensary, Kumbakonam division, Tanjore district- 612 504, Tamil Nadu, India; [2]Department of Veterinary Public Health, Veterinary College and Research Institute, Tamil Nadu Veterinary Animal Sciences University (TANUVAS), Namakkal- 637 002, Tamil Nadu, India; [3]Department of Veterinary Microbiology, Veterinary College and Research Institute, Tamil Nadu Veterinary Animal Sciences University (TANUVAS), Then- 625 531, Tamil Nadu, India

Abstract

The prevalence of clinical and subclinical mastitis associated with risk factors and their extent of association was studied based on the positivity of 454 mastitis milk samples by polymerase chain reaction (PCR), collected from clinical and subclinical mastitis cows which were brought to the Veterinary College and Research Institute (VC&RI), Namakkal, Tamil Nadu. The prevalence of clinical mastitis was high in cows at 4th lactation and above (55.8%, OR-3.37), late lactation (65.3%, OR-5.55), of H-F crossbreed (39.0%, OR-1.81), with the previous incidence of mastitis (48.5%), under concrete bedding (30.4%, OR-0.99), without the practice of pre-teat dipping (41.3%, OR-28.0), with post-teat dipping (39.8%, OR-0.06), under hand milking (34.0%) and in the winter season (41.3%, OR-2.50). The prevalence of subclinical mastitis was high in cows at 4th lactation and above (44.1%, OR-2.99), late lactation (34.6%, OR-2.93), of other breeds (29.7%, OR-1.46), under soil bedding (24.9%, OR-1.60), without practice of pre-teat dipping (31.9%, OR-58.59), with post-teat dipping (28.5%, OR-0.24), under hand milking (23.0%) and in the winter season (34.4%, OR-2.9). The prevalence of clinical and subclinical mastitis was statistically significant in cows between ages of lactation, stages of lactation and seasons, whereas no significant difference could be observed between breeds of cows.

Keywords: Bovine mastitis, Clinical and subclinical, Causal association, Epidemiological factors, Odds ratio (OR)

Highlights

- Clinical and subclinical mastitis cases in bovines were identified.
- Various epidemiological risk factors like age, breed, season and stage of lactation were recorded.
- Odds ratio was calculated for each of the epidemiological factors.
- The extent of association of these risk factors with the incidence of mastitis was interpreted.

Mastitis is generally regarded as one of the costliest diseases in dairy herds (Oliveira *et al.*, 2015). The average loss due to mastitis per animal per month accounts for INR 3206.55, INR 2119.67 and INR 1708.89 in the optimistic scenario and INR 3549.59, INR 2448.03 and INR 1934.78 in the pessimistic scenario in the case of crossbred cows, indigenous cows and buffaloes, respectively (Bardhan, 2013). The loss contributed by sub-clinical mastitis is very hard to quantify, but experts agree that it accounts for more financial losses in the herd than do clinical cases (Cheng and Han, 2020).

Animal health and welfare, quality of milk, antibiotic usage and the image of the dairy sector are considered to be important issues in mastitis control (Jingar *et al.*, 2017). Enhanced milking hygiene, implementation of post-milking teat disinfection and maintenance of milking machines are some of the measures to prevent bovine mastitis. Several risk factors, which include pathogen, host and environmental factors, are known to have significant associations with the incidence of

*Corresponding Author, E-mail: sarvet.25@gmail.com

bovine mastitis and play an important role in mastitis control programs (Klaas and Zadoks, 2018).

Since effective mastitis control programs rely more on prevention rather than treatment (Cheng and Han, 2020), knowledge of various predisposing factors in the epidemiology of bovine mastitis is of paramount importance to formulate prevention strategies. Nevertheless, there is a paucity of information on the extent of the risk associated with various predisposing factors in the incidence of bovine mastitis. Hence, the study aims at reporting the various epidemiological risk factors associated with the occurrence of clinical and subclinical bovine mastitis and their causal association.

Milk samples (n=454) from clinical and subclinical mastitis cases during the period from October 2016 to March 2017 brought to the Veterinary College and Research Institute, Namakkal, which were positive for *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* by polymerase chain reaction (PCR) were taken for the study. The prevalence at the quarter level was recorded to be 29.95 and 23.12 per cent for clinical and subclinical mastitis, respectively. The degree of association between the epidemiological factors and the occurrence of clinical and subclinical mastitis in dairy cattle was assessed by calculating the odds ratio (OR) as described by Smith (1995) and interpreted. The prevalence of mastitis at the quarterly level associated with various epidemiological factors was determined, and the extent of risk involved was calculated by odds ratio (OR) as a measure of association (Table 1). The study did not involve any animal experiments and was conducted following ethical guidelines.

The prevalence of clinical and subclinical mastitis was found to be highest in cows at 4th lactation and above (55.8 and 44.1%, respectively), followed by other lactations. Statistically, a significant difference (P<0.05) could be observed in the prevalence of mastitis between the ages of lactations. A similar finding was recorded by Biffa *et al.* (2005) and Tufani *et al.* (2012), who reported the highest incidence of mastitis in cows of 10 years old (44.6%) and 4th parity (26.98%), respectively in crossbred Jersey cows in Srinagar, India. The high prevalence at increased age of lactation could be associated with the lengthening of the teat, the distance between the udder and floor, the pendulous udder and loss of sphincter potency (Sharma *et al.*, 2012).

Cows with clinical and subclinical mastitis were 3.4 and 3 times, respectively, more likely to be at 4th lactation and above than cows without clinical and subclinical mastitis, respectively.

The prevalence of clinical and subclinical mastitis was high in late lactation (65.3 and 34.6%, respectively), followed by other lactations. Statistically, a significant difference (P<0.05) could be observed in the prevalence of mastitis between the stages of lactation. In contrast, Biffa *et al.* (2005), Tufani *et al.* (2012) and Eirahman (2013) recorded a high prevalence of mastitis in early lactation (45.8, 52.38 and 38.1%, respectively), followed by mid and late lactations in crossbred Jersey cows in Ethiopia, India and Egypt, respectively.

The high prevalence reported by the previous authors could be attributed to high infection rate in the dry periods, periparturient immune suppression due to decreased neutrophils and lymphocytes, negative energy balance and oxidative stress (Sharma *et al.*, 2012). In contrast, in this study, the high prevalence at late lactation could presumably be associated with poor managemental practices. Cows with clinical and subclinical mastitis were 5.5 and 3 times, respectively more likely to be at late lactation than cows without clinical and subclinical mastitis, respectively.

The prevalence of clinical mastitis was found to be highest in cows of H-F cross (39.0%) followed by other breeds, whereas the prevalence of subclinical mastitis was highest in cows of other breeds (29.7%), followed by cross breeds. However, statistically, no significant difference (P>0.05) could be observed in the prevalence of mastitis between

Table 1. Epidemiological factors associated with mastitis in bovines brought for treatment

Sl. no	Epidemiological factors	Categories	Total no. of samples screened (n=454)	Samples from clinical mastitis (n= 136)	Odds ratio (OR)	Per cent positivity at quarter level (29.95)	Samples from subclinical mastitis (n= 105)	Odds ratio (OR)	Percent positivity atquarter level (23.12)	P value
1.	Age of lactation	1st lactation	59	29	3.37	49.1	13	2.99	22.0	0.01*
		2nd lactation	170	37		21.7	48		28.2	
		3rd lactation	182	46		25.2	25		13.7	
		4th lactation and above	43	24		55.8	19		44.1	
2.	Stage of lactation	Early lactation	167	45	5.55	26.9	24	2.93	14.3	0.02*
		Mid lactation	246	57		23.1	63		25.6	
		Late lactation	52	34		65.3	18		34.6	
3.	Breed	Jersey cross	284	75	1.81	26.4	57	1.46	20.0	0.55
		HF cross	133	52		39.0	37		27.8	
		Others	37	9		24.3	11		29.7	
4.	Teat	Obstruction/injury/other lesions	17	-		-	-		-	
5.	Previous illness/disorders	Previous incidence of mastitis	35	17	2.38	48.5	-		-	
		Ketosis/Indigestion	-	-		-	-		-	
		Recumbent cow	-	-		-	-		-	
		Milk fever	5	-		-	-		-	
	Housing	Soil bedding	349	104	0.99	29.7	87	1.60	24.9	
		Concrete bedding	105	32		30.4	18		17.1	
7.	Teat hygiene	No pre-teat dipping	329	136	28.00	41.3	105	58.59	31.9	
		Pre-teat dipping	125	-		-	-		-	
		No post-teat dipping	125	5	0.06	4.0	11	0.24	8.8	
		Post-teat dipping	329	131		39.8	94		28.5	
8.	Milking techniques	Hand milking	367	125	3.56	34.0	88	1.29	23.9	
		Machine milking	87	11		12.6	17		19.5	
9.	Season	Winter	186	77	2.50	41.3	64	2.90	34.4	0.16
		Summer	164	36		21.9	29		17.6	
		S-W monsoon	89	23		25.8	12		11.5	
		N-E monsoon	15	5		33.3	-		-	

* $P<0.05$

different breeds. This finding in clinical mastitis agrees with that of Sharma (2003), Biffa *et al.* (2005) and Abdel-Rady and Sayed (2009), who observed the Friesian breeds to be more susceptible to the mastitis (90.0, 56.5 and 20.43%, respectively) than native breeds in India and Ethiopia. In contrast, Tufani *et al.* (2012) reported the incidence to be the highest in crossbred Jersey (61.90%), followed by other breeds of cows in Srinagar in India.

The high prevalence of the disease in the H-F breeds of cattle could be associated with low innate udder immunity and poor immune response by cellular factors due to increased intramammary pressure (Tufani *et al.*, 2012). Whereas, the highest prevalence in breeds other than cross breeds of cows in subclinical mastitis could presumably be due to the poorer attention paid to them with husbandry, hygienic milking process and dietary management when compared to high-yielding cows. Cows with clinical and subclinical mastitis were 1.8 and 1.5 times, respectively more likely to be H-F breeds and other breeds, respectively than cows without clinical and subclinical mastitis, respectively. Whereas, Biffa *et al.* (2005) recorded an OR of 3.3 and 1.2 for prevalence in H-F and local breeds of cows, respectively.

A high prevalence of clinical mastitis (48.5%) was recorded in cows that had previous incidences of mastitis, whereas, no prevalence could be recorded for subclinical mastitis. This finding is in accordance with that of Rowlands *et al.* (1986) and Biffa *et al.* (2005), who also recorded a prevalence of clinical mastitis with 38.0 and 57.0 per cent, respectively in cows that previously experienced mastitis.

In this study, recurrent episodes of clinical mastitis might be due to the persistent intramammary colonization of pathogens associated with ineffective antibiotic therapy during previous occurrence or persistence of the pathogens in the mammary gland. Cows with clinical mastitis were 2.4 times more likely to be exposed to mastitis previously than cows without clinical mastitis.

The prevalence of clinical mastitis was higher in cows managed in a house under concrete bedding (30.4%) than in a house under soil bedding (29.7%), however with relatively a similar prevalence. Whereas, in subclinical mastitis, the prevalence was higher in cows managed in a house under soil bedding (24.9%) than in a house under concrete bedding (17.1%). These findings corroborate with Khanal and Pandit (2013), who also recorded a high prevalence of subclinical mastitis in dairy livestock associated with poor management and unhygienic shed. Bartlett *et al.* (1991) also observed the incidence of mastitis to be high on flooring with soil, followed by concrete and sand bedding.

The relatively high prevalence of clinical mastitis on the concrete floor could be associated with dirty bedding, inadequate removal of slurry and straw bedding, all of which are potential sources for environmental mastitis (Sharma *et al.*, 2012). Cows with subclinical mastitis were 1.6 times more likely to be exposed to soil bedding than cows without subclinical mastitis. Whereas, cows with clinical mastitis were found to have no risk under concrete bedding than cows without clinical mastitis.

The prevalence of clinical and subclinical mastitis was high in cows without the practice of pre-teat dipping (41.3 and 31.9%, respectively) with teat sanitizer, whereas no prevalence could be recorded in cows with pre-teat dipping. This finding agrees with that of Doherr *et al.* (2007) and Hogan and Smith (2003), who stated that germicidal sanitizers on teats immediately prior to milking could reduce the incidence of new coliform infections and could prevent mastitis due to prolonged intervals between milking associated with leaking of milk and chance for bacteria to colonize teat ends and teat canals.

In contrast, the prevalence of clinical and subclinical mastitis was high in cows with the practice of post-teat dipping (39.8 and 28.5%, respectively). Hogan and Smith (2003) also stated that teat antiseptic applied post-milking to control coliform mastitis was found to be unsuccessful. In contrast, Sudhan and Sharma (2010) stated that post-milking teat dipping could be effective in eliminating environmental organisms, *E. coli* and *Str. Uberis* on the teats after milking.

In this study, the high prevalence recorded in cows without the practice of pre-teat dipping could be associated with more chances for the spread of *Staphylococcus* spp. from the skin of teats into the teat canals. Whereas, the high prevalence recorded in cows with the practice of post-teat dipping could be associated with the poor efficiency of teat dipping to eliminate Staphylococci which already colonized the teat canals and also an inefficient concentration of sanitizer used by farmers. Cows with clinical and subclinical mastitis were 8.8 and 58.6 times, respectively more likely to be without the practice of pre-teat dipping than cows with the practice of pre-teat dipping. Cows with clinical and subclinical mastitis were found to have no risk when there was no practice of post-teat dipping than cows without clinical and subclinical mastitis, respectively.

The prevalence of clinical and subclinical mastitis was high in cows practiced with the technique of hand milking (34.0 and 23.0%, respectively), as most of the farmers in this study adopted knuckling method. This finding agrees with that of Kavitha *et al.* (2009), who recorded the incidence of clinical and subclinical mastitis to be high when knuckling was practiced as a method of milking rather than stripping, as these methods could induce damage to the teat tissue, increasing the risk for intramammary infections (Sudhan and Sharma, 2010). Cows with clinical and subclinical mastitis were 3.6 and 1.3 times, respectively, more likely to be with the practice of hand milking than cows without clinical and subclinical mastitis, respectively.

The prevalence of clinical mastitis was highest in winter (41.3%) and lowest in summer (21.9%). Whereas, the prevalence of subclinical mastitis was recorded as high in winter (34.4%) and low in S-W monsoon (11.5%) among the seasons winter, summer and S-W monsoon only. Statistically, there was a significant difference ($P<0.05$) in the prevalence between different seasons. This finding is in concurrence with that of Biffa *et al.* (2005), who reported that cows were at greater risk (OR, 2.6) of acquiring udder infection in the rainy season. However, Ranjan *et al.* (2011) reported a more or less equal incidence of bovine mastitis during winter (47.37%) and summer (42.26%) seasons and the least cases of bovine mastitis during the rainy season (7.37%) in Ranchi, India. In contrast, Palanivel *et al.* (2008) reported the highest incidence in summer (53.8%) followed by other seasons in Tamil Nadu.

The high prevalence in the winter season could be associated with increased humidity favouring high bacterial load in the cow's environment (Sharma *et al.*, 2012). Cows with clinical and subclinical mastitis were 2.5 and 2.9 times, respectively more likely to be infected during the winter season than cows without clinical and subclinical mastitis, respectively.

The risk factors like age of lactation, stage of lactation, previous illness, housing, teat hygiene, milking technique and season were found to have an influence on the occurrence of clinical or subclinical mastitis in bovines. Creation of awareness for farm owners about regular testing for subclinical mastitis, pre- and post-dipping, udder washing, and proper disinfection of bedding is of paramount importance to prevent mastitis. In addition, regular diagnosis and treatment of subclinical mastitis at an early stage could help in preventing the development of clinical mastitis in a herd.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: RN: Carried out the detection of mastitis cases and the risk factors. SS: Involved in the statistical analysis, interpretation of causal association and draft of the manuscript. PS: Involved in draft and revision of the manuscript.

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