The Pharma Innovation Journal 2023; 12(3): 4213-4216

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(3): 4213-4216

www.thepharmajournal.com
Received: 22-12-2022
Accepted: 26-01-2023

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Epidemiological and clinico-etiological studies in recurrent bovine mastitis

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Abstract
A total of 110 lactating dairy cows with a history of recurrent and / or unresponsive clinical mastitis were selected for the study. The information regarding age, breed, parity, stage of lactation of the animals and their clinical signs were recorded and analysed. The prevalence of mastitis was found to be highest in animals of 5 to 6 years age (77.27%), in fourth parity (42.73%) and in the first three months of lactation (45.45%). Majority of the animals showed changes in the milk only (50.90%) whereas changes in the milk and udder without systemic signs were observed less frequently (35.35%). Flakes in milk with discolouration was exhibited by 50 percent of the animals and there was moderate swelling in majority of the animals. Cultural isolation and biochemical characterisation of the bacterial isolates from the milk samples revealed the presence of *Staphylococcus* spp (74.54%), *E. coli* (7.27%), *Klebsiella* spp (6.36%), *Micrococcus* spp (1.81%). and *Streptococcus* spp (1.81%). Staphylococci were the predominant isolate in the cases of clinical mastitis. Hence moderate changes in the udder or no changes in the udder can be correlated with recurrent staphylococcal mastitis.

Keywords: Mastitis, epidemiological data, clinical signs, etiological agents

Introduction
Mastitis is economically the most significant disease of dairy cattle which leads to reduction in potential production, losses in quality and quantity of milk, losses due to discarded milk, premature culling, veterinary costs and labour costs (Hogeveen *et al*., 2011) [5]. Mastitis is the inflammation of the parenchyma of the mammary gland regardless of the cause and characterized by a range of physical and chemical changes in the milk and pathologic changes in the glandular tissue (Radostits *et al.*, 2010) [12]. Among the bacterial etiological agents identified, staphylococci and streptococci were the most common Gram- positive bacteria and *E coli* and *Klebsiella pneumoniae* were the most common Gram- negative bacteria (Contreras and Rodriguez., 2011) [3]. The various pathological changes identified in mastitis occur as a result of the host interactions with the invading pathogens. Virulence factors of *Staphylococcus aureus* like structural components (collagen, fibrinogen, elastin binding proteins, capsules, slimes), various enzymes and toxins (Abril *et al*., 2020) [1] contribute to the clinical signs. *Staphylococcus aureus* was the most common cause of both subclinical mastitis and chronic mastitis. Many cases were characterized by induration, presence of clots in milk and slow progression of the disease over a period of months. The clinical signs in coliform mastitis are caused due to the release of lipopolysaccharides and various inflammatory mediators. Coliform mastitis rarely results in chronic infections (Suojala *et al*., 2013) [18]. The present study was carried out to assess the various epidemiological factors associated with occurrence of recurrent and or unresponsive clinical mastitis and to the study the clinical signs and changes in milk in relation to the etiological agents.

Materials and Methods
The present study was carried out in the Department of Veterinary Epidemiology and Preventive Medicine during the period from January 2021 to July 2022. A total of 110 dairy cows affected with a history of recurrent clinical mastitis were selected for the study. The information regarding age, breed, parity and stage of lactation of the clinically affected animals were recorded and analysed. The clinical signs, appearance of udder and milk were scored (Pyorala *et al*., 1994; Radostits *et al*., 2010) [10, 12]. Details regarding the consistency of udder, color and consistency of milk were recorded.

Isolation and identification of the bacterial agents

Milk samples collected asceptically from bovine mastitis cases were streaked on to Brain heart infusion agar and incubated at 37 °C for 24 hours for primary isolation. Among the 110 samples, 101 samples showed bacterial growth and nine samples did not produce colonies. Out of the 101 culture positive samples Gram -positive and Gram- negative isolates were recovered from 86 and 15 samples respectively. The staphylococci isolates were identified based on colony morphology, Gram-staining and biochemical tests as per Quinn *et al.* (2013) [11].

Fifteen Gram- negative rods isolated from clinical mastitis cases were then subjected to biochemical tests for identification. The ability to ferment lactose was detected by their colony characteristics on Mac Conkey agar and all the fifteen isolates were lactose fermenters and yielded pink colonies. The lactose fermenting colonies were further subcultured in to EMB agar and the eight isolates which showed metallic sheen were identified as *E coli*. The lactose fermenting, mucoid colonies were presumptively identified as *Klebsiella* spp and were further confirmed by indole test, methyl red test (MR test), Voges Proskauer test (VP test), citrate utilization test {IMViC (++- -), urease test, nitrate reduction test and triple sugar Iron tests.

Results and Discussion

The present study revealed that occurrence of mastitis was highest in the animals of 5 to 6 years of age (77.27%) followed by the age group of 3 to 4 years (12.73%) and the lowest was observed in the age group of 7 years and above (10%). This is in accordance with Mourya *et al*. (2020) [9] who recorded a similar finding. The possible reasons for this increased occurrence of mastitis may be the weakened immune response as age advances (Fig.1)

Among the animals showing mastitis, majority of animals were in the fourth parity (42.73%), followed by 33.64 percent animals in the third parity and 13.63 percent were in the second parity. Jingar *et al*. (2014)[7] also reported higher incidence of mastitis in animals of higher parity in a study conducted at National Dairy Research Institute, Karnal. Sinha *et al*. (2021) [17] also observed maximum incidence of mastitis in fourth parity animals. Higher incidence of mastitis in multiparous animals may be age induced, or due to decreased efficacy of the teat as a barrier to the infectious agents or high milk yield in the animals (Fig.2).

The affected animals were crossbred which included four crossbred Jersey and rest were crossbred HF. Similar findings were earlier reported in different studies by Kurjogi and Kaliwal (2014) [8] where highest incidence of mastitis was recorded in Holstein Friesian and Jersey crossbreds. The reason may be the comparatively lower disease resistance of the cross bred animals compared to the indigenous breeds.

Occurrence of clinical mastitis was highest (45.45%) in the animals in the first three months of lactation followed by four to six months of lactation (32.72%) and the occurrence was lowest in more than seven months of lactation (21.81%). This is in accordance with Islam *et al*. (2010) [6] and Shaik *et al*, (2019) [15] who reported a higher prevalence of mastitis in the early lactation. The milk production is comparatively higher during the first three months of lactation period and this might have contributed to the increased prevalence of mastitis. In contrast to the present findings. Sharma *et al*. (2018) [16] reported a higher prevalence of mastitis in animals in mid to late lactation (Fig.3).

The clinical signs shown by the animals were classified based on their severity such as systemic signs like fever and reduced feed intake, changes in the udder and changes in the milk. Most of the animals (50.90%) showed changes in the milk without changes in the udder whereas changes in the udder and milk without and with systemic signs were observed in 35.45 percent and 13.64 percent animals respectively. Clinical manifestation of the mastitis mainly depends on the degree of reaction of the udder tissue towards the invading pathogens. Similar findings were reported by Zigo *et al*. (2021) [19]. Clinical mastitisis characterised by abnormalities in milk secretion, abnormalities of the mammary gland and systemic signs. Samples collected in the present study were from recurrent, chronic and subacute infections where majority of the animals didn't show any changes in the udder. Rainard *et al.* (2018) [13] reported the persistence of infection during the lactation period and possibly in the subsequent lactations with more or less intense clinical flare ups in chronic staphylococcal mastitis. The present study recorded systemic signs in Gram-negative bacterial infections (Table.1).

The animals with mastitis in this study were classified and scored on the basis of the appearance of the udder. The animals affected with clinical mastitis showed moderate swelling in 39.09 percent of the cases, severe swelling and fibrosis in 09.09 percent of the cases and gangrenous udder in 0.90 percent of the cases while and 50.90 percent of the cases showed no changes in the udder. In staphylococcal mastitis, udder changes vary according to the severity of the infection with severe changes in per acute and gangrenous mastitis. Staphylococcal mastitis resulted in permanent parenchymal damage with fibrosis and micro abscess formation (George *et al.*, 2008) [4]. (Table.2)

Most of the milk samples were collected from animals suffering from recurrent/subacute/chronic mastitis which might be the reason for absence of severe changes in the udder which occurs in per acute or acute mastitis.

The development of induration of the udder is slow in chronic mastitis which might also be the reason for the absence of udder changes in the majority of the cases. In advanced chronic form, the inflammation is restricted to the epithelium of the ducts which is replaced by connective tissue proliferations, blockage and atrophy of the glands. (Radostits *et al.* (2010) [12]. Salih and Ahmed (2015) [14] reported one percent gangrenous mastitis due to *Klebsiella* spp in Khartum, Sudan which is in agreement with our finding.

The animals affected with mastitis were scored with values one to four. based on the changes in the colour and consistency of milk. Ten percent of the animals showed discolouration of the milk only and flakes in milk with discolouration was seen in 50 percent of the cases, serous or clotty milk in 32.73 percent cases and Haemagalactia was observed in 6.36 percent of the cases whereas 10 percent of the samples were normal. The changes in milk occurred due to the inflammatory response against the invading pathogens. Radostits *et al*. (2010) [12] described various changes in milk like clots, flakes and purulent material in clinical mastitis. Haemagalectia was noted in majority of the Gram -negative infections. The character of the secretion was no way pathognomonic for any specific intramammary organisms (Radostits *et al.* (2010) [12] (Table.3)

Relationship between clinical signs and etiological agents

Cultural isolation and biochemical characterisation of the bacterial isolates from the milk samples revealed the presence of Staphylococcus spp (74.54%), *E. coli* (7.27%), *Klebsiella* spp (6.36%), *Micrococcus* spp and *Streptococcus* spp (1.81%).

Changes in the milk with and without changes in the udder was observed in 85.14 percent of the samples which was caused by Staphylococcus/*Streptococcus* spp. Systemic signs with changes in the udder and milk was caused by *E. coli* and *K. pneumoniae* (14.85%).

Relationship of clinical signs in mastitis with etiological agents has been well documented. The occurrence of clinical signs could be related to the bacterial load and there is a strong relationship between the etiological agents and clinical signs (Radostits *et al*., 2010; Sujoula *et al*., 2013) [12, 18]. The clinical manifestations of the mastitis depend on the interplay between the immune status of the cow and the virulence of the etiological agent.

When cows are affected with a large number of virulent pathogens, clinical mastitis follows, whereas lower number of less virulent pathogens result in subclinical mastitis (Zigo *et al*., 2021) [19]. As the majority of the etiological agents isolated in clinical mastitis cases were staphylococci, the finding that moderate changes in the udder or no changes in the udder can be correlated which has been reported in recurrent staphylococcal mastitis (Rainard *et al.* 2018) [13].

E coli infections caused severe irreversible tissue damage resulting in fibrosis and gangrene of the udder (Cheng and Han., 2020) [2]. Abscess formation and gangrene were observed in an animal affected with *K. pneumoniae*. Severe swelling and fibrosis were mostly associated with E. *coli* or *K. pneumoniae* mastitis.

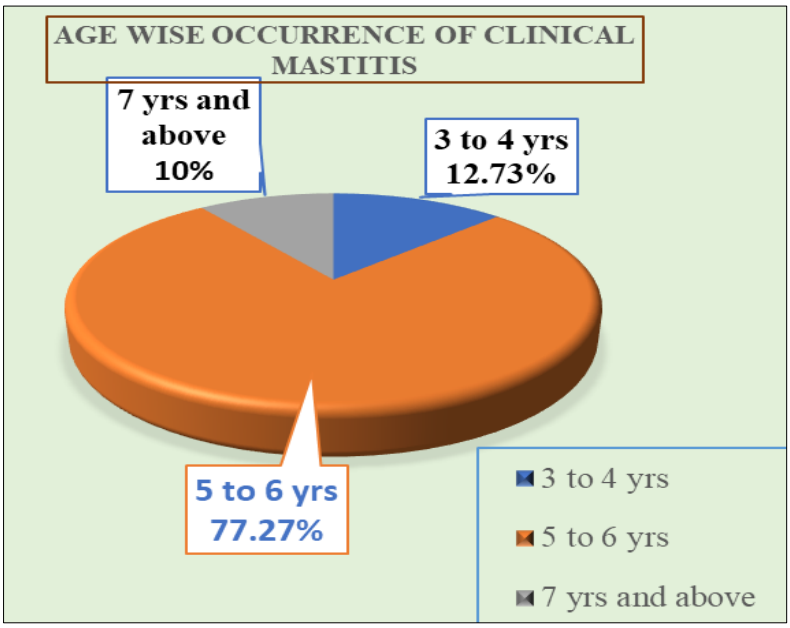

Fig 1: Age wise occurrence of clinical mastitis

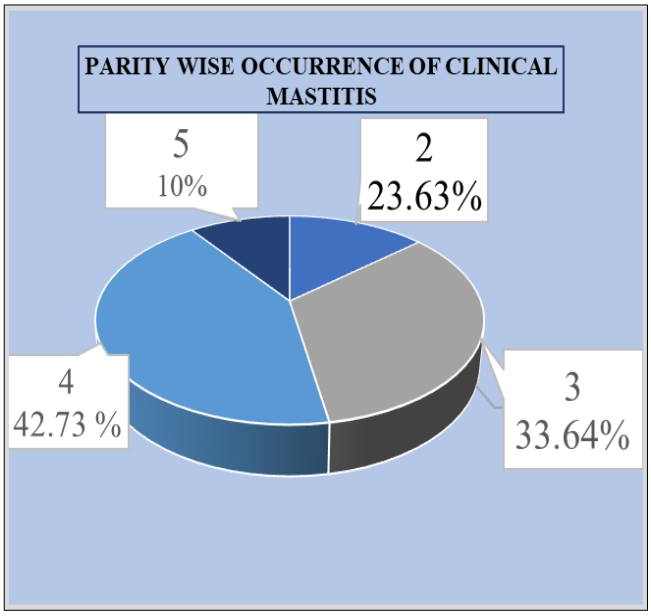

Fig 2: Parity wise occurrence of clinical mastitis

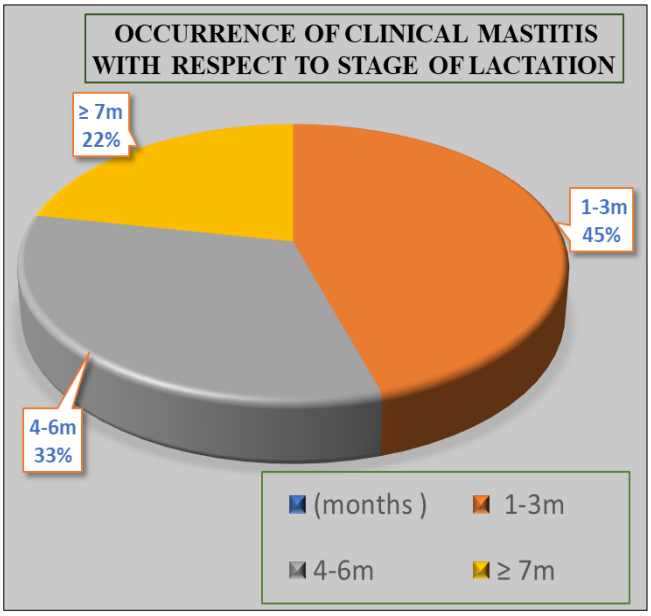

Fig 3: Occurrence of clinical mastitis based on stage of lactation

Table 1: Clinical signs exhibited by animals in clinical mastitis

SI. N	Clinical signs	No. of animals affected	Percent (%)
1	Changes in milk without changes in udder	56	50.90
2	Changes in the udder and milk without systemic signs	39	35.45
3	Systemic signs with changes in the udder and milk	15	13.64
	Total	110	100

Table 2: Abnormalities in udder in the animals with clinical mastitis

SI. No	Abnormalities of udder	No. of animals affected	Percent (%)
1	No changes in the udder	56	50.90
2	Moderate swelling	43	39.09
3	Severe swelling and fibrosis	10	09.09
4	Abscess formation, gangrene and atrophy	1	00.90
	Total	110	100

Table 3: Abnormalities in milk of the animals with clinical mastitis

SI. No	Abnormalities of milk	No. of animals affected	Percent (%)
1	Discolouration of milk only	11	10
2	Flakes in milk with discolouration	55	50
3	Serous or clotty milk	36	32.73
4	Haemagalactia	7	6.36
	Total	110	100

Conclusion

The epidemiological study in recurrent and unresponsive cases of clinical mastitis revealed that the occurrence of such cases increases with age and parity. The occurrence was also associated with highest milk production period. This indicates the need for proper health care and hygienic measures in high producing animals. Majority of the animals showed only changes in milk colour and consistency without major changes in the udder. Staphylococci being the predominant isolate, the study reveals it's potential role in recurrent and chronic mastitis.

References

1. Abril AG, Villa T, Barros-Velázquez J, Cañas B, Sánchez-Pérez A, Calo-Mata P *et al. Staphylococcus aureus* exotoxins and their detection in the dairy industry and mastitis. Toxins. 2020;12(9).537.
2. Cheng WN, Han SG. Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments-A review. Asian-Australasian J Anim. Sci. 2020;33(11).1699-1713.
3. Contreras GA, Rodríguez JM. Mastitis: comparative etiology and epidemiology. J Mammary Gland Biol Neoplasia. 2011;16(4):339-356.
4. George LW, Divers TJ, Ducharme N, Welcome FL. Diseases of the teats and udder. In: Rebhun's Diseases of dairy cattle. (2nd Ed.) Divers TJ and SF Peek (eds.). Saunders-Elsevier Publishers, St. Louis, USA. 2008, 335.
5. Hogeveen H, Huijps K, Lam TJGM. Economic aspects of mastitis: new developments. New Zealand Vet J. 2011;59(1):16-23.
6. Islam MA, Rahman AKMA, Rony SA, Islam MS. Prevalence and risk factors of mastitis in lactating dairy cows at Baghabari milk shed area of Sirajganj. Bangladesh J Vet. Med 2010;8:157.
7. Jingar SC, Mehla RK, Singh M, Kumar A, Kantwa SC, Singh N. Comparative study on the incidence of mastitis during different parities in cows and buffaloes. Indian J Anim. Res. 2014;48(2):194-197.
8. Kurjogi MM, Kaliwal BB. Epidemiology of bovine mastitis in cows of Dharwad district. Int. Sch. Res. Notices. 2014;1:1-9.
9. Mourya A, Shukla PC, Gupta DK, Sharma RK, Nayak A, Singh B, *et al*. Prevalence of subclinical mastitis in Cows in and around Jabalpur, Madhya Pradesh. J Entomol. Zool. Studies. 2020;8(4):40-44.
10. Pyorala S, Kaartinen L, Käck H, Rainio V. Efficacy of two therapy regimens for treatment of experimentally induced Escherichia coli mastitis in cows. J Dairy Sci. 1994;77(2):453-461.
11. Quinn P, Markey B, Carter M, Carter GR. Clinical Veterinary Microbiology. (2nd Ed.) Mosby, St. Louis: 2013, 514.
12. Radostits OM, Gay CC, Hinchcliff KW, Constable PD. Veterinary Medicine: A Text Book of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. (11th Ed.), Saunders Elsevier, London. 2010, 2162.
13. Rainard P, Foucras G, Fitzgerald JR, Watts JL, Koop G, Middleton JR. Knowledge gaps and research priorities in *Staphylococcus aureus* mastitis control. Transbound Emerg Dis. 2018;65:149-165.
14. Salih RRM, Ahmed FAM. Isolation of Klebsiella spp. from gangrenous mastitis in cattle in Khartoum state, Sudan. J Anim. Fed. Res. 2015;5(6):165-168.
15. Shaikh SR, Digraskar SU, Siddiqui MF, Borikar ST, Rajurkar SR, Suryawanshi PR. Epidemiological studies of mastitis in cows reared under different managemental system in and around Parbhani. J Pharm Innov. 2019;8(2):01-05.
16. Sharma N, Singh SG, Sharma S, Gupta SK, Hussain K. Mastitis occurrence pattern in dairy cows and importance of related risk factors in the occurrence of mastitis. J Anim. Res. 2018;8(2):315-326.
17. Sinha R, Sinha B, Kumari R, MR V, Verma A, Gupta ID. Effect of season, stage of lactation, parity and level of milk production on incidence of clinical mastitis in Karan Fries and Sahiwal cows. Biol Rhythm Res. 2021;52(4):593-602.
18. Suojala L, Kaartinen L, Pyorala S. Treatment for bovine *Escherichia coli* mastitis–an evidence-based approach. J Vet. Pharmacol. Therapeutics. 2013;36(6):521-531.
19. Zigo F, Vasil M, Ondrasovicova S, Výrostkova J, Bujok J, Pecka-Kielb E. Maintaining optimal mammary gland health and prevention of mastitis. Front. Vet. Sci. 2021;8:607311.