RESEARCH ARTICLE
JIVA 20 (3) December 2022
https://doi.org/10.55296/JIVA/20.3.2022.25-37

Received: 14-09-2022
Accepted: 15-10-2022

IDENTIFICATION OF THE ETIOLOGY OF SUBCLINICAL MASTITIS IN AN ORGANISED DAIRY HERD

Krupa Rose Jose*[1], Vijayakumar K.[2], Justin Davis K.[3] and Shyma V. H.[4]

[1]Ph.D. Scholar, [2]Dean, [3,4]Assistant Professor
[1,3,4]Department of Veterinary Epidemiology and Preventive Medicine
College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala
Kerala Veterinary and Animal Sciences University
**Corresponding author: krupaputhuparampil@gmail.com*

ABSTRACT

The study describes the identification of the etiological agents of subclinical mastitis (SCM) among lactating dairy cows under different phases of lactation in an organised dairy farm of Thrissur district, Kerala. The preliminary diagnosis of subclinical mastitis (SCM) was made using California Mastitis test (CMT), a cow side field test. The somatic cell count (SCC) was estimated and the milk samples of affected quarters were subjected to bacterial isolation and identification. The predominant causative agents isolated from milk of cows with SCM were coagulase negative staphylococci (56.25 per cent) followed by *S. aureus* (25 per cent) and *Micrococci* spp. (18.75 per cent). Further species identification of the coagulase negative staphylococci revealed that the majority were *S. xylosus* (28.23 per cent) followed by *S. scuiri* (15.63 per cent), *S. hominis* (9.38 per cent) and *S. schleiferi* (3.13 per cent).

Keywords: California mastitis test, Coagulase negative staphylococci, Somatic cell count, *Staphylococcus aureus*, Subclinical mastitis

INTRODUCTION

India being the largest producer and consumer of milk and milk products holds a large potential for the export of dairy products. However, there are some areas where a major thrust is required, the most important of which relates to food safety and quality assurance of the exported milk and milk products. Animal diseases is an important factor that contribute to these limitations in the milk production and export. As per reports of disease occurrence in dairy animals, mastitis stands at the fore front due to its prevalence being reported from more than 90 per

cent of high yielding crossbred dairy cows (Sudhan and Sharma, 2010).

Udder is a perfect habitat for microbial growth and the milk provides nutrient rich ecosystem for a diverse range of commensal and pathogenic bacteria. The optimum udder conditions such as temperature and nutrition along with the direct link of mammary gland to the external environment via the teat opening allows infringement and reckless multiplication of pathogenic microorganisms to initiate a series of inflammatory response that is regarded as mastitis (Stelwagen *et al*., 2009). It leads to a drastic reduction in milk yield and thereby the potential weight gain by the offspring, ultimately results in compromising the dairy farm economics. Even though most infections resulted in relatively mild clinical or subclinical local inflammation, more serious cases may lead to agalactia or extensive systemic involvement ultimately resulting in death (Hamadani *et al*., 2013). Infected milk also poses a potential health risk to consumers as it may be a major source of many enterotoxigenic pathogens that are responsible for food borne illness (FAO, 2014).

Even though there were spatial, temporal and herd wise variations in the incidence and patterns of mastitis and the causative agents, one of the major reasons behind its continued high prevalence could be the delay in detection of subclinical mastitis (Sharma *et al*. 2012). Subclinical mastitis accounts for 15 to 40 times more than the clinical mastitis and is an important feature in the epidemiology of different forms of bovine mastitis (Bakken and Gudding, 1982). It is difficult to diagnose and adversely affects the milk production and quality (Reshi *et al*., 2015). The affected animal secretes apparently normal milk for a long time and often remains elusive in a herd during which the infection can be transmitted to other cattle as well as susceptible individuals creating a contaminated environment. Therefore, routine screening and early identification of SCM is of paramount importance as it helps to reduce production losses and enhances the prospects of an uneventful recovery (Jadhav *et al*., 2018). Hence, the current study was conducted with the objectives of estimating the prevalence of subclinical mastitis followed by isolation and identification of the organisms responsible for SCM.

MATERIALS AND METHODS

Study area and study population

The study was undertaken among 98 apparently healthy cross bred lactating dairy cows of varying phases of lactation, parity and different age groups managed

in an organised dairy farm. All the animals were kept under uniform management conditions and were machine milked twice daily.

Screening for subclinical mastitis

Individual quarter samples from each lactating cow were screened separately for SCM with the help of California mastitis test (CMT) and SCC.

California Mastitis Test

The CMT was conducted besides the cow as per Schalm and Noorlander (1957). The results were graded as zero (negative), trace, one (weakly positive), two (distinctly positive), and three (strongly positive). California mastitis test values of 'zero', 'trace' and 'one' were regarded as negative, however CMT scores of 'two' and above were regarded as an indication of SCM.

Somatic cell count

Somatic cell count was performed using the DeLaval® cell counter as per manufacturers guidelines. The SCC value of more than 2,00,000 cells per ml of milk was used as a criterion for considering the animal to be sub clinically infected.

Microbial isolation and identification

The milk samples with a CMT score above 'two' and having SCC greater than 2,00,000 were further investigated for bacterial isolation and identification. Milk samples were subjected to bacterial isolation and identification on the basis of morphological, cultural and biochemical characteristics. For this a loopful of milk samples was cultured on to Brain Heart Infusion Broth and incubated aerobically at 37°C for 24 hours. Broth culture was examined by Gram's staining and the size, shape, arrangement and staining character were recorded. Further, the broth culture was inoculated on different selective and differential media like Brain heart infusion agar and Mannitol salt agar, and incubated aerobically at 37°C for 24 hours. On the basis of selective growth, the isolates were confirmed as *Staphylococcus* spp. and colonies showing golden or yellow colour on Mannitol salt agar was identified as pathogenic *Staphylococci* spp. The pure cultures of isolated organisms were also subjected to biochemical characterisation *viz.* catalase, oxidase, coagulase, Voges Proskaeur, nitrate reduction and sugar fermentation tests using appropriate biochemical media and reagents.

RESULTS AND DISCUSSION

Screening for subclinical mastitis

In the present study, 98 apparently healthy cross bred lactating cows belonging to different age groups (3 to 12 years), parity (1 to 5) and stages of lactation (early,

mid or late) maintained under uniform management conditions at an organised dairy farm were screened for SCM using CMT and SCC. Out of the 392 quarters from 98 animals, 11 quarters (2.8 per cent) from 11 different animals were blind and not included in the study. Hence the results of 381 quarters were included in the study. The SCM in cows was detected through tests like CMT and SCC followed by isolation and identification of the bacteria.

California Mastitis Test

The California Mastitis Test (CMT), also known as the rapid mastitis test, the Schalm test or the mastitis N-K test (Whyte *et al*., 2005), is an inexpensive rapid cow-side test that was developed in 1957 (Schalm and Noorlander, 1957) by modification of the Whiteside Test (Whiteside, 1939). The test is based on the principle that the changes in precipitation and gel formation produced by the mixture of reagent and milk reflects the cell count of milk, the number of which increases sharply in response to inflammation (Barnum and Newbould, 1961). California mastitis test could have a useful role in herd monitoring programmes as screening test to detect intramammary infection caused by major pathogens in early lactation.

In the present study, out of the 381 quarters subjected to CMT the occurrence of SCM based on the CMT score was 50.23 per cent. Out of the 50.23 per cent of affected quarters, 37.27 per cent were graded as 'two' and 16.53 per cent were graded as 'three' respectively (Figure 1). This occurrence pertains to a herd from an organised cattle farm where in the management practices followed are uniform. But the overall prevalence is well within the range that has been reported by different researchers (Abrahemsen *et al*., 2014; Dar *et al*., 2014).

Somatic Cell Count

Somatic cell count is an indicator of the udder health status and therefore plays an important role in the assessment of milk quality, hygiene and control of mastitis at the cow, herd and population level (Schukken *et al*., 2003). The cut-off value for SCC to declare SCM is not uniform across the globe, and each country has its own regulatory SCC limits. According to the FDA regulations, the legal milk SCC limit for cows in United states is 7.5 lakh cells per millilitre. However, in European countries, New Zealand, Australia and Canada, the cut off values is four lakh cells per millilitre. In India, there are no such regulatory SCC standards for milk production (Jadhav *et al*., 2018). A detailed review of literature (Ghosh *et al*., 2004; Samanta *et al*., 2006; De and Mukherjee,

2009; Singh and Garg, 2012; Tarate *et al*., 2012; Gera and Guha, 2012) revealed that a wide variation of SCC limits ranging from 1.22 lakh cells to 15.51 lakh cells per millilitre of milk are being used to describe the health status of cows in India. However, the majority of the observations range from two lakhs to more than or equal to 4 lakh cells per millilitre. Hence, in the present study, a SCC value of more than 2 lakh cells per millilitre was considered to declare SCM. Out of the 381 quarters examined for SCC, 245 (64.3 per cent) quarters had a SCC above two lakhs when examined using DeLaval® cell counter (Figure 2). In the present study, the SCC ranged from 1 to 1.56 X 10^6 cells per milliliter in milk from healthy cows while in CMT positive milk samples, ranged between 1.68 to 4.32 X 10^6 cells per milliliter, respectively.

Isolation and identification of bacteria from subclinical mastitis

Bacteriological culture of milk samples is the standard approach for determination of the causative pathogens (Wallace *et al*., 2004). In the present study, isolation of bacteria from bovine mastitic milk were achieved by standard microbiological culture techniques. The milk samples with a CMT score above 'two' and SCC above two lakh cells per millilitres were enriched in nutrient broth (MM244, Himedia, Mumbai) and plated on to brain heart infusion agar (BHIA; M211, Himedia, Mumbai). Upon initial inoculation of 187 milk samples which were found to be positive on both the CMT and SCC, 146 samples did not produce any colonies on BHI agar. Whereas, 41 samples yielded bacterial growth, among which nine samples were mixed cultures of more than three different colonies and were considered as contaminated. Hence, a total of 32 bacterial isolates were obtained

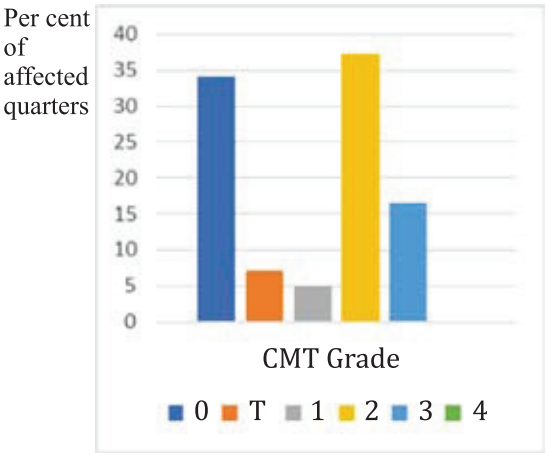


Fig.1: Results of CMT

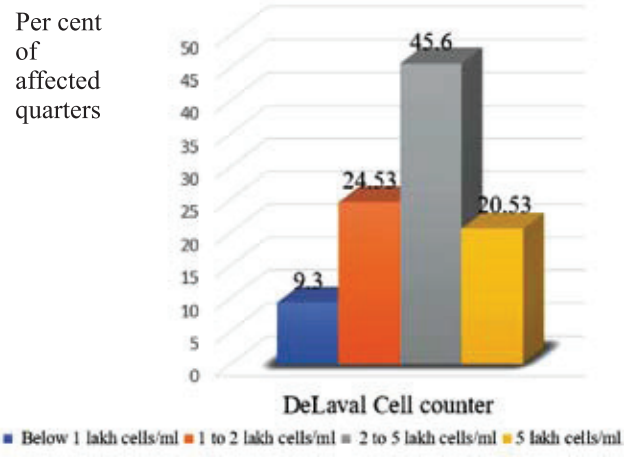


Fig.2: Results of SCC obtained by DeLaval® cell counter

on pure culture. The higher proportion of culture negative samples might be due to the inhibitory action of the endogenous antibacterial substances present in milk (Yazdankhah and Olsen, 1998). It could also be attributed to the non-bacterial etiology that could not be isolated by streaking on to a bacteriological medium (Wellenberg *et al.*, 2002).

Morphological characterisation of the isolates

In the present study, among the 32 pure bacterial isolates, all were Gram positive cocci, out of which 26 were suggestive of staphylococci, whereas six isolates were arranged in tetrads suggestive of Micrococci indicating a higher prevalence of contagious pathogens. The findings of the present study corroborate with Birhanu *et al*. (2017) and Persson *et al*. (2011) who observed a higher prevalence of Gram-positive isolates like staphylococci and streptococci compared with coliforms. However, Perez *et al*. (2015) reported that 95.2 per cent of the isolates were Gram negative, implying a higher prevalence of environmental pathogens rather than contagious pathogens. These results are rather reasonable due to the variation in the agro-climatic condition, management practises and the pathogen ecology in the herd environment. Thus, it can be concluded that, apart from being the basis for routine diagnosis, treatment or culling decisions, bacteriological culture guides in formulating strategic interventions in mastitis control programmes based on the epidemiological situation of the herd (Perez *et al*., 2015).

Colony characterisation of the isolates

A presumptive identification of the bacterial isolates was done by subculturing them on to selective media and recording their colony characteristics. All the 32 Gram positive cocci were inoculated on to mannitol salt agar (MSA), a media with high salt concentration (7.5 per cent) that allows the selective proliferation of salt tolerant bacteria (Chapman, 1945). It selectively differentiates *S. aureus* from CNS and *Micrococci* spp. *Staphylococcus aureus* has the potential to ferment mannitol and changed the media acidic producing yellow colonies with yellow zones whereas other CNS and Micrococci being mannitol non fermenters, produced small pink or red colonies with no colour change in the media (Davies *et al*., 2005). The present study revealed that among the 32 isolates streaked on to MSA, eight isolates (25 per cent) developed yellow colonies with yellowish discolouration of media suggesting the presence of *S. aureus*, and 24 isolates showed red colonies suggestive of Micrococci or other *Staphylococcus* spp.

Biochemical characterisation of the isolates

Biochemical characterisation of the 32 Gram positive bacterial isolates were performed using various conventional culture techniques *viz*., catalase, oxidase, coagulase, nitrate reduction, Voges Proskauer (VP) and sugar fermentation tests (Barrow and Feltham, 1993; Quinn *et al*., 2013) as shown in figure 3. Based on the preliminary biochemical tests, 26 isolates were found to be oxidase negative, catalase positive and reduced nitrate indicative of *Staphylococcus* spp. The remaining six isolates were presumptively identified as micrococci based on positive catalase and oxidase tests as well as negative nitrate reduction test respectively.

Second stage identification of the putative Staphylococci isolates were aided by further biochemical tests like coagulase, VP and sugar fermentation tests. Tube coagulase test is a standard

Table 1. Biochemical characterisation of bacteria from SCM

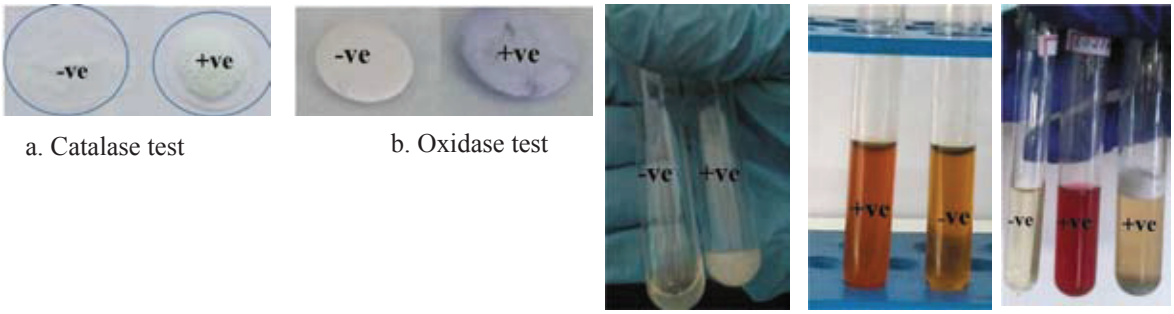
Sl. No.	Particulars of identification tests	Observations	Number of positive samples	Presumptive bacterial isolate
1.	Catalase test	Appearance of an effervescence	32 (100%)	*Staphylococci/ Micrococci*
2.	Oxidase test	Absence of bluish discolouration of disc	26 (81.25%)	*Staphylococci* spp.
		Bluish discolouration of the disc	6 (18.75%)	*Micrococci* spp.
3.	Coagulase test	Clot formation	8 (25%)	*Coagulase +ve Staphylococci*
		No clot formation	18 (56.25%)	*Coagulase –ve Staphylococci*
4.	Indole test	No reaction	32 (100%)	*Staphylococci/ Micrococci*
5.	Methyl red test	Red colour	32 (100%)	*Staphylococci/ Micrococci*
6.	Voges Proskauer test	Deep red colour noticed	12 (37.5%)	*Staphylococci*
		No reaction	20 (62.5%)	*Staphylococci/ Micrococci*
7.	Citrate utilisation test	Growth of organism along streak line and blue colouration of media	32 (100%)	*Staphylococci/ Micrococci*
8.	Nitrate reduction test	Cherry red colour of media	32 (100%)	*Staphylococci/ Micrococci*

(Values in the parenthesis indicates the per cent of positive samples)

means of distinguishing *S. aureus* from CNS (Sperber and Tatini, 1975). It detected the secreted fibrinogen binding protein-coagulase, an essential virulence factor of *S. aureus,* that interacted with fibrinogen to form a clot which helped bacteria to protect from host phagocytic clearance (Ko *et al*., 2016). In the present study, out of the total 32 bacterial isolates, eight isolates (25 per cent) gave a positive coagulase reaction and 18 isolates (56.25 percent) were identified as coagulase negative since they do not exhibit any degree of coagulation on incubation with rabbit coagulase plasma for 18 h (Table 1). Meanwhile, variable reactions were given by different *Staphylococci* spp. for the remaining tests like VP and sugar fermentation tests (lactose, maltose, mannitol, sucrose, trehalose, raffinose, xylose and cellobiose).

HiStaph identification test kit (KB004, Himedia, Mumbai) is a commercial test kit recommended for the identification and differentiation of the organisms belonging to the *Staphylococcus* spp (Figure 4). According to Cunha *et al*. (2004), the differentiation of CNS using ready to use commercial test kits that employed a battery of selected biochemical tests, were simple, inexpensive and highly efficient for routine use especially in places with limited resources. In the present study, differentiation of *Staphylococcus* spp. was in perfect agreement both by commercially available biochemical kit as well as using standard biochemical tests. However, contradictory findings have been documented by Kateete *et al*. (2010) and Fernandes *et al*. (2021) claimed that phenotypic identification of *Staphylococcus* spp. based on morphological and biochemical properties are inherently weak owing to the variation in expression and interpretation of the phenotypic characteristics there by limiting the reproducibility, accuracy and typeability of these tests.

In the current study, it could



a. Catalase test b. Oxidase test c. Coagulase test d. Voges Proskaeur test test e. Nitrate reduction test

Fig. 3: Biochemical characterisation of the isolates

Fig. 4: Identification of *S. xylosus* using HiStaph test kit

be concluded that among the total 32 isolates, 25 per cent were identified as *S. aureus*, 18.75 per cent were *Micrococci* spp., and 56.25 per cent were CNS based on the morphological and biochemical characterisation of the isolates, indicating a considerable rise in the occurrence of Micrococci and CNS. Similar findings were made by Piepers *et al*. (2007), Sampimon *et al*. (2011) and El- Diasty *et al*. (2019) who reported a higher prevalence of CNS from bovine SCM. But contradictory findings were reported by Sebastian (2001) and Rathish (2014) who isolated 6.4 per cent and 23.81 per cent CNS respectively among all the isolates. Further, species identification of the CNS revealed that the majority were *S. xylosus* (28.23 per cent) followed by *S. scuiri* (15.63 per cent), *S. hominis* (9.38 per cent) and *S. schleiferi* (3.13 per cent) (Figure 5). This was in line

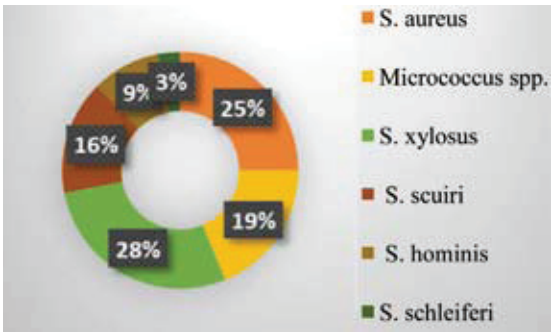


Fig. 5. Frequency of bacteria isolated from bovine subclinical mastitis

with the findings of Rathish (2014) who reported *S. xylosus* as the most dominant udder pathogen among the CNS isolates. However, in Swedish dairy herds (Thorberg *et al*., 2009) with a high prevalence of CNS, *S. chromogens*, *S. epidermidis* and *S. simulans* were the most prevalent species followed by *S. xylosus, S. haemolyticus, S. saprophyticus, S. scuiri* and *S. cohini*. The reasons that some CNS species are more associated with persistent IMI are not known, but may indicate differences in virulence, adaptation to the environment of the udder, or both and further study is warranted on this.

CONCLUSION

The results of the study suggest a higher prevalence of subclinical mastitis among the study population. The coagulase negative Staphylococci is being incriminated as a major emerging pathogen followed by *Staphylococcus aureus*. Strategic interventions encompassing adequate housing with proper sanitation, regular screening for early detection and appropriate treatment of subclinical cases, dry cow therapy and application of pre and post-dipping practices must be enforced at the grass root level to reduce the incidence of SCM. However, the accurate measurement of the impact, sources, transmission mechanisms and control options for individual CNS species requires accurate

species identification based on molecular techniques.

CONFLICTS OF INTEREST

There were no conflicts of interest reported by the author (s).

ACKNOWLEDGEMENT

The authors are thankful to the Kerala Veterinary and Animal Sciences University for providing the facilities needed for carrying out the research.

REFERENCES

Abrahmsen, M., Persson, Y., Kanyima, B. M. and Bage, R. 2014. Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda. *Tropical Anim. Health Prod.* **46**: 99-105.

Bakken, G. and Gudding, R. 1982. The interdependence between clinical and subclinical mastitis. *Acta Agric. Scand*. **32**: 17-22.

Barnum, D. A. and Newbould, F. H. S. 1961. The use of the California mastitis test for the detection of bovine mastitis. *Canadian Vet. J.* **2**: 80-83.

Barrow, C. I. and Feltham, R. K. A. 1993. Cowan and Steel's Manual for the Identification of Medical Bacteria. (3rd Ed.). Cambridge University Press, Great Britain, pp. 331.

Birhanu, M., Leta, S., Mamo, G. and Tesfaye, S. 2017. Prevalence of bovine subclinical mastitis and isolation of its major causes in Bishoftu Town, Ethiopia. *BMC Res. Notes*. **10**: 764-767.

Chapman, G.H. 1945. The significance of sodium chloride in studies of staphylococci. *J. Bacteriol*. **50**: 201-205.

Cunha, Maria de Lourdes R. S, Yuri K. Sinzato, and Liciana V. A. Silveira. 2004. Comparison of methods for the identification of coagulase-negative staphylococci. *Mem. Inst. Oswaldo Cruz*. **99**: 855-860.

Dar, K. H., Ansari, M. M., Dar, S.H., Tantary, H. A., Baba, M. A. and Mehraj-ud-Din, N. 2014. Studies on subclinical mastitis in dairy cows of Jammu and Kashmir. *Intern. J. Vet. Sci.* **3**: 95-99.

Davies, F. R., Devligers F. C. and Chambers, H. F. 2005. Re-emergence of antibiotic resistant *Staphylococcus aureus* in the genomics era. *J. Clin. Invest*. **119**:2464–2474.

De, U.K. and Mukherjee, R. 2009. Prevalence of mastitis in crossbred cows. *Indian Vet. J.* **86**:858-859.

El-Diasty, M., Talaat, H., Atwa, S., Elbaz, E. and Eissa, M. 2019. Occurrence of Coagulase- negative Staphylococcal mastitis in dairy cows. Mansoura. *Vet. Med. J*. **23**: 35-39.

Fernandes Queiroga Moraes, G., Cordeiro, L. V. and de Andrade Júnior, F. P. 2021. Main laboratory methods used for the isolation and identification of Staphylococcus spp. *Rev. Colomb. Cienc. Quim. Farm.* **50**: 5-28.

Food and Agriculture Organisation (FAO). 2014. Impact of mastitis in small scale dairy production systems. Animal Production and Health Working Paper. No. 13. Rome.

Gera, S. and Guha, A. 2012. Effect of subclinical mastitis on milk biochemical constituents in crossbred cows. *Indian Vet. J*. **89**: 33-34.

Ghosh, C.P., Nagpaul, P.K. and Shiv Prasad. 2004. Subclinical mastitis in cattle and buffalo and its impact on somatic cell count and milk composition. *Indian J. Dairy Sci*. **57**: 329- 333.

Hamadani, H., Khan, A. A., Banday, M. T., Ashraf, I., Handoo, N., Bashir, A. and Hamadani, A. 2013. Bovine mastitis-A disease of serious concern for dairy farmers. *Int. J. Livest. Res.* **3**: 42-55.

Jadhav, P. V., Das, D. N., Suresh, K. P. and Shome, B. R. 2018. Threshold somatic cell count for delineation of subclinical mastitis cases. *Vet. World.* **11**: 789.

Kateete, D. P., Kimani, C. N., Katabazi, F. A., Okeng, A., Okee, M. S., Nanteza, A., Joloba, M. L. and Najjuka, F. C. 2010. Identification of *Staphylococcus aureus*: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. *Annals Clin. Microbiol. Antimicrob.* **9***:*1-7.

Ko, Y. P., Kang, M., Ganesh, V. K., Ravirajan, D., Li, B. and Hook, M. 2016. Coagulase and Efb of Staphylococcus aureus have a common fibrinogen binding motif. *M. Bio*. **7**: 45- 49.

Perez, S., De Meulemeester, L., de Kruif, A., Opsomer, G., Barkema, H.W. and De Vliegher, S. 2015. Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. *J. Dairy Res*. **74**: 474-.478.

Persson, Y., Nyman, A. K. J. and Grönlund-Andersson, U. 2011. Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden. *Acta Vet. Scandinavica*. **53**: 36- 39.

Piepers, S., De Meulemeester, L., de Kruif, A., Opsomer, G., Barkema, H.W. and De Vliegher, S. 2007. Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. *J. Dairy Res.* **74**: 474-478.

Quinn, P., Markey, B., Carter, M. and Carter, G. R. 2013. In: Clinical Veterinary Microbiology. (2nd Ed) Mosby, St. Louis, pp. 514.

Rathish, R. L. 2014. Clinico- therapeutic studies and experimental evaluation of a bacterin against common bacterial isolate of bovine mastitis. Ph.D. thesis, Kerala Veterinary and Animal Sciences University, Pookode, pp. 177.

Reshi, A. A., Husain, I., Bhat, S. A., Rehman, M. U., Razak, R., Bilal, S. and Mir, M. R. 2015. Bovine mastitis as an evolving disease and its impact on the dairy industry. *Int. J. Curr. Res. Rev.***7**: 48-55.

Samanta, A., Prasad, S. and Ghosh, C. P. 2006. Incidence of subclinical mastitis in Karan Swiss and Karan Fries crossbred cows. *Indian J Dairy Sci*. **57**: 329-333.

Sampimon, O. C., Lam, T. J. G. M., Mevius, D. J., Schukken, Y. H. and Zadoks, R. N. 2011. Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine milk samples. *Vet. Microbiol*. **150**: 173-179.

Schalm O. W. and Noorlander D. O. 1957 Experiments and observations leading to development of the California mastitis test. *J. American Vet. Med. Assn*. **130**: 199–204.

Schukken, Y. H., Wilson, D. J., Welcome, F., Garrison-Tikofsky, L. and Gonzalez, R. N. 2003. Monitoring udder health and milk quality using somatic cell counts. *Vet. Res*. **34** :579- 596.

Sebastian, S. R. 2001. Clinicotherapeutic studies on bacterial mastitis in bovines. M.V.Sc. thesis, Kerala Agriculture University, Vellanikkara, Thrissur. pp 66.

Sharma, N., Rho, G. J., Hong, Y. H., Kang, T. Y., Lee, H. K., Hur, T. Y. and Jeong, D. K. 2012. Bovine mastitis: An Asian perspective. *Asian J. Anim. Vet. Adv*.**7**: 454-476.

Singh H. M. and Garg, H. M. 2012. Effects of antibiotic dry cow therapy and internal teat sealant (Teatseal) on milk somatic cell counts, clinical, and subclinical mastitis in early lactation. *J. Anim. Sci*. **94**: 76-77.

Sperber, W. Z. and Tatini, S. R. 1975. Interpretation of the tube coagulase test for identification of *Staphylococcus*

aureus. *Applied Microbiol*. **29**: 502-505.

Stelwagen, K., Carpenter, E., Haigh, B., Hodgkinson, A. and Wheeler, T. T. 2009. Immune components of bovine colostrum and milk. *J. Anim. Sci*. **87**: 3-9.

Sudhan, N. A. and Sharma, N. 2010. Mastitis–An important production disease of dairy animals, SMVS.

Tarate, S. B., Jadhav, P. V., Ghadhigaonkar, D. D., Khade, P., Bhatt, V., Khan, A., Joshi, C. G. and Rank, D. N. 2012. Evaluation of breed- dependent variation in microbial load during subclinical mastitis in cattle using Real Time PCR. *Indian J. Dairy Sci*. **65**: 60- 65.

Thorberg, B. M., Danielsson-Tham, M. L., Emanuelson, U. and Waller, K. P. 2009. Bovine subclinical mastitis caused by different types of coagulase-negative staphylococci. *J. Dairy Sci*. **92**: 4962-4970.

Wallace, J. A., Stipetic, K., Schukken, Y. H., Dingwell, R.T., Baillargeon, P., Bacic, G. and Leslie, K. E. 2004. An evaluation of a treatment protocol for intramammary infections in early postpartum dairy cows based on a positive California mastitis test result. *Bovine Practitioner*. pp. 72-78.

Wellenberg, G. J., van der Poel, W. H. and Van Oirschot, J. T. 2002. Viral infections and bovine mastitis: A review. *Vet. Microbiol*. **88**: 27-45.

Whiteside W. H. 1939 Observations on a new test for the presence of mastitis in milk. *Canadian Pub. Health J*. **30**: 44- 48.

Whyte, D., Walmsley, M., Liew, A., Claycomb, R. and Mein, G. 2005. Chemical and rheological aspects of gel formation in the California Mastitis Test. *J. Dairy Res*. **72**: 115-121.

Yazdankhah, S. P. and Olsen, E. 1998. Simple and direct detection of *Staphylococcus aureus* in milk by a tube coagulase test. *Lett. Appl. Microbiol*. **27**:111-115.