Haryana Vet. (Dec., 2017) **56 (2),** 189-193 **Research Article**

PREVALENCE AND RISK FACTORS OF SUBCLINICAL MASTITIS IN BUFFALOES AT AN ORGANIZED DAIRY FARM IN WESTERN HARYANA

R. JHAMBH[1*], U. DIMRI[1], A. GOPALAKRISHNAN[1], M. SINGH[2] and R. CHHABRA[2]
[1]Division of Medicine, ICAR–Indian Veterinary Research Institute, Izatnagar-243 122, Uttar Pradesh, India
[2]College Central Laboratory, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004, Haryana, India

ABSTRACT

The present investigation was undertaken to study the prevalence and risk factors of subclinical mastitis (SCM) in dairy buffaloes at an organized dairy farm in western zone of Haryana. A total of 864 quarters from 217 animals were screened for SCM on the basis of California mastitis test (CMT), somatic cell count (SCC) and differential cell count (DCC). Out of which, 148 quarters from 69 animals showing CMT reaction $\geq$ 2, SCC $\geq$ 0.5 million/ml and neutrophils percentage $\geq$ 40% of total counts per quarter's milk were subjected to cultural examination for identification of major mastitis pathogens which revealed 68 milk samples from 44 animals positive for either single or mixed infection, indicating the 7.87% quarter-wise prevalence of SCM. A total of 75 isolates were identified based upon culture and biochemical characteristics which revealed predominance of coagulase-negative *Staphylococci* (CNS; 48%) followed by *Streptococci* other than *S. agalactiae* (29.33%) and a low prevalence of major contagious pathogens, *S. aureus* (13.33%) and *S. agalactiae* (5.33%) in SCM. Both the hind quarters were affected most, followed by right-fore and left-fore quarters respectively. With respect to lactation, the highest occurrence of SCM was recorded in buffaloes in their 4th to 6th lactation, followed by 1st to 3rd lactation predisposed to early stage of lactation, followed by mid-lactation, owing to physiological stress of high milk yield and alterations in homeostatic mechanisms. High yielding buffaloes were more affected. Therefore, the animals in the period of high physiological demands need to be maintained on high plane of nutrition and husbandry practices to prevent the disease.

Key words: Buffaloes, dairy farm, organized, prevalence, risk factors, subclinical mastitis

Mastitis, the inflammation of the parenchyma of the mammary gland, presents a significant health problem in dairy animals (Sordillo, 2011). In dairy animals, it is the highly prevalent disease not only in cattle but also affects buffaloes (Fagiolo and Lai, 2007) even though they have been traditionally considered less susceptible to mastitis than cattle (Wanasinghe, 1985). However, in comparison to cattle, buffaloes have more pendulous udder and longer teats that may contribute to greater risk of mastitis (Fagiolo and Lai, 2007). Based upon severity of the inflammation, it can be subclinical, clinical and chronic. The subclinical form is difficult to detect due to the absence of any visible indications and has major cost implications associated with decreased milk production (Viguier *et al.,* 2009). In India, total annual economic loss to dairy industry due to mastitis is estimated to be Rs. 71655 million out of which Rs. 41512 million (approx. 60%) is due to subclinical form (Bansal and Gupta, 2009). In buffaloes, mastitis is mostly due to bacterial infections and majority of infections are caused by the contagious pathogens *Staphylococcus aureus, Streptococcus agalactiae* and by the environmental pathogens *Streptococcus uberis, Streptococcus dysgalactiae*, coliforms (*E. coli, Klebsiella, Enterobacter*), *Pseudomonas* etc. (Fagiolo and Lai, 2007). In an extensive study on occurrence of mastitis in buffaloes in Haryana, the prevalence of mastitis on quarter basis has been implicated to be 51.65% out of which 32.9% has been due to subclinical form (Sharma and Sindhu, 2007). However, this prevalence may vary depending upon the hygiene and management practices at the dairy farm. Therefore, the present investigation was undertaken to study the prevalence and associated risk factors of subclinical mastitis (SCM) in dairy buffaloes at an organized dairy farm in western zone of Haryana.

*Corresponding author: jhambricky@gmail.com

MATERIALS AND METHODS

Study Area: The study was conducted on lactating Murrah buffaloes maintained at an organized dairy farm, Hi-tech Dairy Farm, Sirsa which comes under the western zone of Haryana having the tropical climate with intensively hot summer and cool winter. All the animals at the farm were maintained under identical feeding and management practices. All the animals were supplemented with mineral mixture in their feed as per milk potential. The milking of all the animals was practiced by hand only twice a day, following the pre and post-milking teat disinfection as spray using commercial teat disinfectant (Liq. Kohrsolin containing Gluteraldehyde, Virbac India). The milk yield of the lactating animals ranged from 6 to 16 kg/day with an average wet milk yield of 9.8 kg/day/animal.

Selection of Animals: The study was conducted in the month of March, 2017. The animals were screened for subclinical mastitis on the basis of physical examination of udder, California mastitis test (CMT), somatic cell count (SCC) and differential cell count (DCC) on quarter's milk samples. The physical examination of all lactating animals was carried as per Schalm *et al.* (1971) for exclusion of abnormal size, shape, consistency, blind teats, indurations etc. The CMT was conducted at animal-side as per Schlam and Noorlander (1957). The SCC and DCC in quarter's milk were done as per Schlam *et al*. (1971) and Dulin *et al.* (1982) respectively. The quarter's milk samples showing CMT reaction $\geq$ 2 *viz.* distinct slime/gel formations, SCC $\geq$ 0.5 million/ml and neutrophils percentage $\geq$ 40% of total counts were subjected to cultural examination of milk for major mastitis pathogens at the College Central Laboratory of the University. Subsequently, the registration of such animals with their identification number, parity, stage of lactation and average milk yield were recorded.

Cultural Examination of Milk: Cultural examination of quarter's milk samples was done as per Quinn *et al.* (2004). Milk samples aseptically collected in sterile glass tubes were thoroughly mixed and streaked primarily on 5% ovine blood agar and MacConkey's lactose agar (MLA) plates with a sterile platinum loop under strict sterile environment. The inoculated plates were incubated at 37 ^{0}C for 24 to 48 h. The causative organisms were identified initially by colony characteristics, gram staining and biochemical characteristics for presence of catalase, cytochrome C oxidase and coagulase. Further, the organisms grown on blood agar plates were streaked on selective media e.g. Mannitol Salt Agar (*Staphylococcus aureus*), Edward's media (Streptococci spp.). Hotis test was done to identify *Streptococcus agalactiae*.

RESULTS AND DISCUSSION

The quarter's milk samples positive on basis of CMT, SCC, DCC and culturally positive for intramammary infection were classified into SCM as per International Dairy Federation (IDF) criteria while those with inflammatory response but negative on culture examination were classified into non-specific mastitis.

Prevalence of SCM: The prevalence of SCM in dairy buffaloes at organized dairy farm is depicted in Table 1. A total of 864 quarters from 217 animals were screened for subclinical mastitis on the basis of CMT, SCC and DCC. Of these, 148 quarters from 69 animals were found positive viz. CMT reaction > 2, SCC > 0.5 million/ml and neutrophils percentage > 40% of total counts per quarter's milk which were subjected to cultural examination for identification of major mastitis pathogens. Out of 148 quarter's milk samples, 68 milk samples from 44 animals were found culturally positive for either single or mixed infection, indicating the 7.87% quarter-wise prevalence of subclinical mastitis while 80 samples showed no growth on culture medium and classified into non-specific mastitis. Singh (2015) reported 5.83% prevalence of SCM at the same farm; however, the study did not include all the lactating buffaloes maintained at the farm. Similar prevalence of SCM ranging from 4.58 to 7.05% has been reported earlier by other workers in buffaloes from organized herds in Haryana (Charaya *et al.,* 2013; Pankaj *et al.,* 2013). In contrast, Sindhu *et al.* (2009) reported a very low prevalence of SCM (0.88%) in buffaloes from an organized farm. On the other hand, a high prevalence of SCM (32.9%) has been reported in traditionally managed buffaloes in Haryana (Sharma and Sindhu, 2007). Low prevalence rate of SCM in the present investigation might be attributed to proper management practices at the farm such as pre- and post-milking teat disinfection (Oliver *et al.,* 2001), supplementation of micronutrients in feed (Heinrichs *et al.,* 2009) etc. Such practices have been shown to reduce the risk factors and occurrence of mastitis in dairy animals during lactation.

Table 1
Prevalence of subclinical mastitis in dairy buffaloes at organized dairy farm

Total animals/ quarters screened	Animals/quarterspositive on basis of CMT / SCC / DCC	Animals/quarters diagnosed with subclinical mastitis	Quarter-wise prevalence (%) – subclinical mastitis
217/864	69/148	44/68	7.87%

Relative Distribution of Isolates: A total of 75 isolates were identified based upon culture and biochemical characteristics which revealed coagulase-negative *Staphylococci* (CNS) (48%) followed by *Streptococci* other than *S. agalactiae* (29.33%) to be the predominant bacterial isolates of SCM while the major contagious pathogens, *S. aureus* (13.33%) and *S. agalactiae* (5.33%) were found in low frequency as depicted in Table 2. Overall staphylococci followed by *Streptococci* were the most prevalent mastitogens, accounting for 61.33% and 34.66% of infections respectively. Singh (2015) also reported staphylococci, followed by streptococci as the predominant bacterial isolates of SCM at the same farm. Similar pattern of distribution has been reported earlier by other workers in Haryana (Charaya *et al.,* 2013; Pankaj *et al.,* 2013) as well as other part of India (Preethirani *et al.,* 2015). In contrast, Hegde *et al.* (2013) reported an equivalent high prevalence of both *S. aureus* and coagulase negative *Staphylococci* as pathogens of bovine SCM at organized and unorganized dairy sectors. Lower prevalence of contagious pathogens *viz. S. aureus* and *S. agalactiae* in the present investigation might be attributed to improved milking practices at the farm such as pre-milking teat disinfection in association with good udder preparation and post-milking teat disinfection. Numerous studies have reported the practice of disinfecting teats following milking to reduce the incidence of mastitis and new intramammary infections for contagious mastitis pathogens such as *S. aureus* and *S. agalactiae* (Pankey *et al.,* 1985; Nickerson *et al.,* 1986; Boddie and Nickerson, 1997). Typically, pre-milking teat disinfection is recommended for the prevention of environmental infections. However, a recent Canadian study found it to be associated with a lower prevalence of *S. aureus* also (Dufour *et al.,* 2012). Lower prevalence of contagious pathogens of SCM might also be due to successful treatment of clinical cases or culling of chronically infected animals at the farm which is the recommended protocol for control of contagious mastitis pathogens (Keefe, 2012). Further, the present investigation reported the highest prevalence of coagulase negative *Staphylococci* (CNS) as mastitis pathogens of SCM which have traditionally been considered to be minor mastitis pathogens as the infections are very mild or mostly remains subclinical (Taponen *et al.,* 2006). However, CNS can cause persistent infections, resulting in increased milk SCC and decreased milk quality. These now have become the most common bovine mastitis isolates in many countries and therefore have been described as emerging mastitis pathogens (Pyorala and Taponen, 2009). It is difficult to establish whether CNS species behave as contagious or environmental pathogens, but traditionally been considered normal skin microbiota that can cause mastitis as opportunistic pathogens (Taponen and Pyorala, 2009). Control measures against contagious pathogens such as post-milking teat disinfection are less effective against these pathogens as exposure of teats to environmental mastitis pathogens continues between milkings when most teat disinfectants have lost their effectiveness (Oliver *et al.,* 2001). However, it can be controlled by further improving the hygienic and sanitation practices at the farm.

Occurrence of SCM with Respect to Quarter Involved: The occurrence of SCM with respect to

Table 2
Relative frequency of distribution of bacterial isolates of subclinical mastitis in dairy buffaloes

Quarters culturally positive	Total bacterial isolates recovered	*Staphylococcus aureus*	Coagulase--negative staphylococci	*Streptococcus agalactiae*	Other streptococci	*Diplococci* spp.	Yeast
68	75	10 (13.33%)	36 (48%)	4 (5.33%)	22 (29.33%)	2 (2.67%)	1 (1.33%)

quarter involved revealed both the hind quarters (26.5%) to be the most affected followed by right-fore (25%) and left-fore (22.1%) quarters respectively. Khan and Muhammad (2005) and Sharma *et al.* (2007) also reported higher incidence of mastitis in hind quarters than that of fore-quarters in dairy buffaloes with more predisposition towards the left side quarters. A greater predisposition to infection in hind quarters might be attributed to more exposure to the dung and urine, excessive forward and side way pulling, while walking undue stress, milking management and other husbandry practices (Joshi and Gokhle, 2006).

Occurrence of SCM with Respect to Lactation and Milk Yield: Lactation-wise occurrence of SCM revealed the highest occurrence in buffaloes in their 4th to 6th lactation (59.1%), followed by 1st to 3rd lactation (27.3%) and lowest in more than 6th lactation buffaloes (13.6%). Our findings are accordance with Joshi and Gokhle (2006) who described an increase in prevalence of SCM in dairy animals with increase in lactation number with highest prevalence in 4th lactation and thereafter a decrease above 5th lactation. Likewise, Sharma *et al.* (2007) reported the higher prevalence of SCM in buffaloes in their 3rd to 4th lactation. Higher prevalence of mastitis in 4th to 6th parity might be related to stress of peak milk yield in this period. Further, the breakdown of streak canal barrier with advancing age might also responsible for increased susceptibility to intramammary infection with successive lactation (Joshi and Gokhle, 2006). Lowest occurrence in more than 6th parity buffaloes might also be due to less number of higher parity buffaloes, owing to annual culling at the farm.

With respect to stage of lactation, the highest occurrence was recorded in buffaloes in early stage of lactation (45.4%), followed by mid-lactation (40.9%) and the lowest in late stage of lactation (13.6%). Similar findings of highest incidence in early stage of lactation in buffaloes and indigenous cows have reported by other workers (Rasool *et al.*, 1985; Jinger *et al.,* 2014). A greater susceptibility to mastitis in early lactation might be related to high oxidative stress and low antioxidant defense during this period (Sharma *et al.,* 2011). However, Joshi and Gokhle (2006) reported cross-bred cows in their 4th to 5th month of lactation to be most susceptible to SCM, followed by 1st to 3rd month, explained on the basis of physiological stress of high milk yield and alterations in homeostatic mechanisms. On the contrary, Sharma *et al.* (2007) reported the maximum prevalence of SCM in buffaloes in late lactation, followed by early and mid-lactation. Further, number of cases of SCM were recorded more in high producing animals (8-16 kg/day) than low producing animals (<8 kg/day). Likewise, Jinger *et al.,* (2014) reported a high incidence of clinical mastitis in high yielder Murrah buffaloes and indigenous cows. There is a probable correlation between incidence of mastitis and high milk yield in preceding lactation in dairy cows (Fleischer *et al.,* 2010), owing to high metabolic demands. However, Grohn *et al.* (1995) demonstrated high yielding cows not necessarily more susceptible to disease as long as husbandry and nutrition meet their increased biological needs. Thereby, the high yielding animals needs to be maintained on high plane of nutrition and husbandry practices to avoid metabolic disturbances and prevent the disease.

The present investigation revealed a low quarter-wise prevalence of subclinical (7.87%) mastitis in buffaloes with a low prevalence of major contagious pathogens, *S. aureus* and *S. agalactiae,* whilst the emergence of coagulase-negative *Staphylococci* (CNS) and *Streptococci* other than *S. agalactiae*. Further, it was directly associated with periods of high physiological demands such as peak milk yield, early stage of lactation. Accordingly, the present findings might be utilized for strategic control of mastitis at the organized dairy farm.

ACKNOWLEDGEMENT

The authors are highly grateful to the Incharge Veterinarian and Para-veterinary staffs of Hi-tech Dairy Farm, Sirsa for their co-operation for conducting the study.

REFERENCES

Bansal, B.K. and Gupta, D.K. (2009). Economic analysis of bovine mastitis in India and Punjab-A Review. *Indian J. Dairy Sci.* **62(3):** 337-45.

Boddie, R.L. and Nickerson, S.C. (1997). Evaluation of two iodophor teat germicides: activity against *Staphylococcus aureus* and *Streptococcus agalactiae*. *J. Dairy Sci.* **80:** 1846-1850.

Charaya, G., Sharma, A., Singh, M., Tiwari, S., Pankaj and Kumar, A. (2013). Subclinical mastitis at an organized farm: prevalence, etiology and antibiogram. *Haryana Vet.* **52**: 30-32.

Dufour, S., Dohoo, I.R., Barkema, H.W., Descôteaux, L., Devries, T.J., Reyher, K.K., Roy, J.P. and Scholl, D.T. (2012).

Manageable risk factors associated with the lactational incidence, elimination, and prevalence of *Staphylococcus aureus* intramammary infections in dairy cows. *J. Dairy Sci.* **95:** 1283-1300.

Dulin, A.M., Paappe, M.J. and Weinland, B.T. (1982). Cytospin centrifuge in differential counts of milk somatic cells. *J. Dairy Sci.* **65:** 1247-1251.

Fagiolo, A. and Lai, O. (2007). Mastitis in buffalo. *Ital. J. Anim. Sci.* **6(2)**: 200-206.

Fleischer, P., Metzner, M., Beyerbach, M., Hoedemaker, M. and Klee, W. (2010). The relationship between milk yield and the incidence of some diseases in dairy cows. *J. Dairy Sci.* **84**: 2025-2035.

Grohn, Y.T., Eicker, S.W. and Hertl. J.A. (1995). The association between previous 305-day milk yield and disease in New York state dairy cows. *J. Dairy Sci.* **78**: 1693-1702.

Hegde, R., Isloor, S., Prabhu, N.K., Shome, B.R., Rathnamma, D., Suryanarayana, V.V. (2013). Incidence of subclinical mastitis and prevalence of major mastitis pathogens in organized farms and unorganized sectors. *Indian J. Microbiol.* **53**: 315-320.

Heinrichs, A.J., Costello, S.S. and Jones, C.M. (2009). Control of heifer mastitis by nutrition. *Vet. Microbiol.* **134**: 172-176.

Jingar, S.C., Mehla, R.K., Singh, M. and Singh, P.K. (2014). Effect of stages and level of milk production on mastitis incidence in cows and Murrah buffaloes. *J. Bio. Innov.* **3(3)**: 117-123.

Joshi, S. and Gokhale, S. (2006). Status of mastitis as an emerging disease in improved and periurban dairy farms in India. *Ann. N. Y. Acad. Sci.* **1081**: 74-83.

Keefe, G. (2012). Update on control of *Staphylococcus aureus* and *Streptococcus agalactiae* for management of mastitis. *Vet. Clin. Food Anim.* **28**: 203-216.

Khan, A.Z. and Muhammad G. (2005). Quarter-wise comparative prevalence of mastitis in buffaloes and crossbred cows. *Pakistan Vet. J.* **25(1)**: 9-12.

Nickerson, S.C., Watts, J.L., Boddie, R.L. and Pankey, J.W. (1986). Evaluation of 0.5% and 1% iodophor teat dips on commercial dairies. *J. Dairy Sci.* **69**: 1693-1698.

Oliver, S.P., Gillespie, B.E., Lewis, M.J., Ivey, S.J., Almeida, R.A., Luther, D.A., Johnson, D.L., Lamar, K.C., Moorehead, H.D. and Dowlen, H.H. (2001). Efficacy of a new pre-milking teat disinfectant containing a phenolic combination for the prevention of mastitis. *J. Dairy Sci.* **84**: 1545-1549.

Pankaj, Sharma, A., Chhabra, R. and Sindhu, N. (2013). Subclinical mastitis in Murrah buffaloes with special reference to prevalence, etiology and antibiogram. *Buffalo Bull.* **32(2)**: 107-115.

Pankey, J.W., Boddie, R.L. and Nickerson, S.C. (1985). Efficacy evaluation of two new teat dip formulations under experimental challenge. *J. Dairy Sci.* **68**: 462-465.

Preethirani, P.L., Isloor, S., Sundareshan, S., Nuthanalakshmi, V., Deepthikiran, K., Sinha, A.Y., Rathnamma, D., Prabhu, K.N., Sharada, R., Mukkur, T.K. and Hegde, N.R. (2015). Isolation, biochemical and molecular identification, and *in-vitro* antimicrobial resistance patterns of bacteria isolated from bubaline subclinical mastitis in south India. *PLoS One* **10(11)**: e0142717. DOI:10.1371/journal.pone.0142717.

Pyorala, S. and Taponen, S (2009). Coagulase-negative staphylococci - Emerging mastitis pathogens. *Vet. Microbiol.* **134**: 3-8.

Quinn, P.J., Carter, M.E., Markey, B. and Carter, G.R. (2004). Clinical Veterinary Microbiology, Mosby, Elsevier Limited, Philadelphia, USA.

Rasool, G., Jabbar, M.A., Kazmi, S.E. and Ahmed, A. (1985). Incidence of subclinical mastitis in Nilli-Ravi buffaloes and Sahiwal cows. *Pakistan Vet. J.* **5**: 76-78.

Schalm, O.W. and Noorlander, D.O. (1957). Experiments and observations leading to development of the California mastitis test. *J. Am. Vet. Med. Assoc.* **130**: 199-204.

Schlam, O.W., Carrol, E.J. and Jain, N.C. (1971). Bovine Mastitis. Lea and Febiger, Philladelphia.

Sharma, A. and Sindhu, N. (2007). Occurrence of clinical and subclinical mastitis in buffaloes in the State of Haryana (India). *Ital. J. Anim. Sci.* **6(2)**: 965-967.

Sharma, N., Maiti, S.K. and Sharma, K.K. (2007). Prevalence, Etiology and Antibiogram of Microorganisms Associated with Sub-clinical Mastitis in Buffaloes in Durg, Chhattisgarh State (India). *International J. Dairy Sci.* **2(2)**: 145-151.

Sharma, N., Singh, N.K., Singh, O.P. Pandey, V. and Verma, P.K. (2011). Oxidative stress and antioxidant status during transition period in dairy cows. *Asian-Aust. J. Anim. Sci.* **24(4)**: 479-484.

Sindhu, N, Sharma, A., Nehra, V. and Jain, V.K. (2009). Occurrence of subclinical mastitis in cows and buffaloes at an organized farm. *Haryana Vet.* **48**: 85-87.

Singh, M. (2015). Microbiological and immunological studies on buffalo subclinical mastitis. Ph.D. thesis, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar.

Sordillo, S.M. (2011). New concepts in the causes and control of mastitis. *J. Mammary Gland Biol. Neoplasia* **16**: 271-273.

Taponen, S. and Pyorala, S. (2009). Coagulase-negative staphylococci as cause of bovine mastitis—Not so different from *Staphylococcus aureus*? *Vet. Microbiol.* **134**: 29-36.

Taponen, S., Simojoki, H., Haveri, M., Larsen, H.D. and Pyorala, S. (2006). Clinical characteristics and persistence of bovine mastitis caused by different species of coagulase-negative *Staphylococci* identified with API or AFLP. *Vet. Microbiol.* **115**: 199-207.

Viguier, C., Arora, S., Gilmartin, N., Welbeck, K. and O'Kennedy, R. (2009). Mastitis detection: current trends and future perspectives. *Trends Biotechnol.* **27(8)**: 486-493.

Wanasinghe, D.D. (1985). Mastitis among buffaloes in Sri Lanka. Proc. First World Buffalo Congr. Cairo, Egypt. **4**: 1331-1333.