Original Article

Buffalo Bulletin (June 2013) Vol.32 No.2

SUB-CLINICAL MASTITIS IN MURRAH BUFFALOES WITH SPECIAL REFERENCE TO PREVALENCE, ETIOLOGY AND ANTIBIOGRAM

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ABSTRACT

This study was carried out to determine the prevalence of sub-clinical mastitis, its etiological agents and their antibiogram in Murrah buffaloes at an organized farm. A total of 326 quarter milk samples were screened from 82 apparently healthy buffaloes. The percent prevalence of sub-clinical mastitis was found to be lower on the basis of SCC (>5x10^5/ml) alone (23.17) as compared to cultural examination (29.26). However, the quarter-wise percent prevalence on the basis of SCC (11.04) was similar to bacteriological examination (11.65). On the basis of International Dairy Federation criteria, 7.05% of the quarters (SCC above 500,000/ml of milk and culturally positive), 4.60% quarters (SCC below 500,000/ml of milk but culturally positive) and 3.98% (culturally negative and SCC above 500,000/ml) were found to suffer from sub-clinical, latent and non-specific mastitis, respectively. Out of 38 culturally positive quarters, a total of 44 organisms were recovered. Of these, 15.90% were coagulase positive staphylococci and 47.72% were coagulase negative staphylococci followed by *Streptococcus dysgalactiae* 25%, *Streptococcus agalactiae* 9.09% and *Streptococcus uberis* 2.27%. and 13.63% of the quarters revealed mixed infections with *Staphylococcus* spp. + *Streptococcus* spp. Among Staphylococci, *Staphylococcus aureus* and *Staphylococcus haemolyticus* were the main isolates followed by *Staphylococcus epidermidis*, *Staphylococcus simulans*, *Staphylococcus hyicus*, *Staphylococcus pasteuri*, *Staphylococcus saprophyticus* subsp. *saprophyticus*, *Staphylococcus arlettae* and *Staphylococcus gallinarum*. All the strains of staphylococci and streptococci were found sensitive to cloxacillin, ceftriaxone and cefoperazone. Streptococci revealed 100 percent sensitivity towards penicillin, enrofloxacin, ciprofloxacin, lincomycin and cephalexin.

Keywords: sub-clinical mastitis, Murrah buffalo, prevalence, etiology, antibiogram

INTRODUCTION

In India, the buffalo population is approximately 94 million head. Of the total production of milk, about 53 percent comes from buffaloes and 43 percent from cows. Haryana has the world's best dairy type buffalo, the Murrah, capable of milk yields as high as 35 kg

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a day. In a review on present status of mastitis in buffaloes at periurban dairy farms in India, Joshi and Gokhale (2006) stated mastitis was one of the most important factors in dairy development in the tropics. Sub-clinical mastitis has been reported to be more important (5-20% in buffaloes) than clinical mastitis (1-10) because it is 15-40 times more prevalent than the clinical form**,** it drastically reduces milk yield, and it usually precedes the clinical form and is usually the basis of herd problems when mastitis outbreaks occur. In India, Dua (2001) has reported annual losses due to mastitis to the tune of Rs 60.5321 billion of which, Rs. 43.6532 billion has been attributed to sub-clinical mastitis. Therefore, the present study was planned to determine the prevalence of sub-clinical mastitis in Murrah buffaloes at an organized farm, to determine the type of organisms responsible for its causation and to determine their antimicrobial sensitivity towards antimicrobials in common use and some of the newer antimicrobials.

MATERIALS AND METHODS

Source of milk samples: A total of 326 quarter buffalo milk samples were collected from 82 apparently healthy buffaloes of Murrah breed located at an organized farm. Animals which had calved recently (less than two weeks) or those in late lactation (more than nine months) were not included in the study.

Collection of milk samples: Milk was collected under aseptic conditions. The udders of cows were cleaned thoroughly with a cloth containing dilute potassium permanganate solution. Hands were properly washed with soap and water and teat apices disinfected with 70 percent alcohol. The first few milk strippings were discarded and 15-20 ml milk sample of each quarter was collected separately in a sterilized test tube. These test tubes were marked as right fore (RF), right hind (RH), left fore (LF) and left hind (LH) and the collection was done first from the near side and then from the off side to avoid contamination of teat apices. Each test tube was given the number possessed by the animal.

Bacteriological examination: The prevalence of sub-clinical mastitis was determined following International Dairy Federation Criteria based on bacteriological examination of milk and somatic cell count (SCC).

For bacteriological examination, the milk samples were shaken thoroughly and 0.01 ml of the milk sample was streaked on 5% sheep blood agar and MacConkey's lactose agar plates. The plates were incubated aerobically at 37^{o}C for 24 to 48 h. Sub-cultures of the resulting growth were made on blood agar for purification of isolates and identified on the basis of Gram's reaction, morphology and colony characteristics. All the isolates were characterized up to species level following standard bacteriological procedures.

Somatic cell count: The SCC on milk samples was performed as described by Schalm *et al*. (1971) and the milk smears were stained with Newman-Lampert stain (methylene blue-1.2 gm, ethyl alcohol (95%) 54 ml, tetra chloro ethane 40 ml, glacial acetic acid 6 ml).

Antimicrobial sensitivity testing: Different strains of various organisms isolated from udder infections were subjected to *in-vitro* drug sensitivity testing, using 20 antimicrobials by a disc-diffusion method as suggested by Bauer *et al.* (1966). The sensitivity was observed on the basis of a zone size interpretation chart provided by the manufacturer. The results were recorded as sensitive, intermediate and resistant.

RESULTS AND DISCUSSION

Results of cultural examination and somatic cell count (SCC) on 326 quarters of 82 buffaloes are presented in Table 1.

Figures in parentheses indicate percentage

The percent prevalence of sub-clinical mastitis was found to be lower on the basis of SCC (>5x10^5/ml) alone (23.17) as compared to cultural examination (29.26). However, the quarter-wise percent prevalence on the basis of SCC (11.04) was similar to bacteriological examination (11.65).

On the basis of IDF criteria 7.05% of the quarters (SCC above 500,000/ml of milk and culturally positive), 4.60% quarters (SCC below 500,000/ml of milk but culturally positive) and 3.98% (culturally negative and SCC above 500,000/ml) were found to suffer sub-clinical, latent and non-specific mastitis, respectively. Similar prevalence of SCM has been reported by Bansal *et al*., 1995; In contrast to our study, several workers (Kalorey *et al*., 1983; Rahman *et al.,* 1983; Tuteja *et al.,* 1999; Maiti *et al.,* 2003 and Chavan *et al*., 2007) reported high animal-wise and quarter-wise prevalence of SCM. These differences in the prevalence rates of SCM as reported by different workers are perhaps due to difference in managemental and hygienic practices adopted in different dairy herds. The incidence of mastitis varied among farms and the risk increased with increasing parity (Sargeant *et al.,* 1998). Thirunavukarasu and Prabaharan (1998) reported that the incidence of mastitis was significantly associated with animal factors such as breed (Dego and Tareke, 2003), milk yield, stage of lactation (Sharma *et al*., 2007) and udder morphology, besides farm practices and sanitation. The climatic conditions also affect the prevalence of mastitis (Schultze, 1985). The lower rate of prevalence in the present investigation in comparison to previous studies on the same farm might be attributed to adoption of proper management, hygienic and control measures at the farm.

Tuteja *et al.* (1999) reported high percent prevalence (26.67) of latent mastitis in comparison to our study. Serieys (1985) and Roder and Gedek (1986) reported that the SCC could be influenced by the type of infecting organisms and season. Thus, a low cell count does not reflect the true bacteriological status of the udder. The significance of latent mastitis cannot be undermined since some of these cases are likely to convert into the sub-clinical form and subsequently into clinical mastitis, particularly under unfavorable environmental conditions. Moreover, latent infection also reflects the possibility of teat canal infections serving as a potential source of infection to the milk secretory tissue. Even mammary parenchyma may be damaged due to liberation of bacterial toxins in the infected teat canal (Nickerson *et al*., 1986). In comparison to our study, on the same farm Tuteja *et al.* (1999) observed a high percentage (7.3) of quarters suffering from non-specific mastitis whereas Sindhu *et al*. (2009) reported a lower percentage (2.19) of quarters having non-specific mastitis. Failure to detect pathogens in such cases might be due to intermittent excretion of the organisms or their disappearance because of spontaneous recovery. Salsberg *et al.* (1984) observed that somatic cell counts increased more during summer months from June to August in Holstein cows than in cooler months. The possibility of mycoplasmal mastitis cannot be ruled out in such cases, since the organism cannot be cultivated on common bacteriological media.

The relative frequency of various micro-organisms from the apparently healthy milk quarters

including 11 quarters harboring mixed infection is given in Table 2.

Out of 38 culturally positive quarters, a total 44 organisms were recovered. Of these, 15.90 % were coagulase positive staphylococci and 47.72% were coagulase negative staphylococci followed by *Streptococcus dysgalactiae* 25% *Streptococcus agalactiae* 9.09%, and *Streptococcus uberis* 2.27% and 13.63% quarters revealed mixed infections with *Staphylococcus* spp. + *Streptococcus* spp.

All the CPS and CNS were further characterized to species level. Details are given in Table 3. *Staphylococcus aureus* and *Staphylococcus haemolyticus* were the main isolates followed by *Staphylococcus epidermidis, Staphylococcus simulans*, *Staphylococcus hyicus, Staphylococcus pasteuri, Staphylococcus saprophyticus* subsp. *saprophyticus*, *Staphylococcus arlettae* and *Staphylococcus gallinarum*. Results indicated substantial differences in the prevalence of pathogens among different herds. In our study contagious bacteria like staphylococci and *Streptococcus agalactiae* caused most of the infections. Such infections are usually spread from animal to animal at the time of milking. The mastitis situation can be improved by improving milking practices and hygiene. Our findings are in close agreement with those of Bansal *et al.* (1995) and Petzer *et al*. (2009) who reported isolation of CNS, *Streptococcus agalactiae* and *Streptococcus uberis*. Rani *et al*., 2008 reported that the prevalence of mastitis varies with breed, age, lactation and season. Amongst various mastitogenic bacteria isolated, staphylococci were the most prevalent, accounting for 63.62 percent of the infections, followed by streptococci (36.36 percent), respectively. Similar findings were reported in India by Babu *et al.* (1983), Sharma and Kapur (2000) and Bulla (2002). The high prevalence of staphylococci has been reported by several workers in India (Kalorey *et al.,* 1983; Javed and Siddique, 1999; Tijare *et al.,* 1999; Tuteja, 1999; Kaya *et al.,* 2000; Sharma *et al*., 2007) and abroad (Hawari and Dabas, 2008; Tenhagen *et al*., 2009 and Nickerson and Stephen, 2009). The prevalence of a pathogen is influenced by parity, type of sample and season (Sharma *et al*., 2007; Hagnestan *et al*., 2009). Distribution of pathogens changes over time; therefore, bacteriological examination at the herd level must be taken regularly to monitor udder health.

Similar to our findings, other workers from India have also reported staphylococci and streptococci to be the main etiological agents of mastitis in different parts of the country (Chavan *et al.,* 2007; Sharma and Sindhu, 2007; Behera *et al.,* 2008; Palanivel *et al*., 2008; Roychoudhary and Dutta, 2009; Sindhu *et al*., 2010). Among staphylococci, *Staphylococcus aureus* and *Staphylococcus haemolyticus* were found to be the most prevalent followed by *Staphylococcus epidermidis*. Many workers have found *Staphylococcus aureus* to be more prevalent than *Staphylococcus epidermidis* (Char *et al.,* 1983; Saini *et al.,* 1994; Armenteros *et al*., 2006; Unnerstad *et al*., 2009). Contrary to this, several workers (Chavan *et al*., 2007; Ferguson *et al*., 2008; Petzer *et al*., 2009; Tenhagen *et al*., 2009; Sampimon *et al*., 2009) reported high prevalence of coagulase negative staphylococci (CNS). These findings show the increasing importance of CNS, which were formally described as a minor pathogen in the case of mastitis. Since, in the veterinary field, our attention has been mainly directed towards coagulase-positive staphylococci, the potential of pathogenicity of CNS remains un-elucidated. Moreover, *Staphylococcus epidermidis* is supposed to be normal flora of teat skin, its higher prevalence, as observed in this study, might

Table 1. Prevalence of sub-clinical mastitis in 326 quarters of 82 buffaloes at an organized farm.

Buffaloes culturally positive	Buffaloes showing SCC > 5 lac/ml	Quarters culturally positive	Quarters showing			
			SCC > 5 lac/ml	SCC > 5 lac/ml and culturally positive	SCC < 5 lac/ml and culturally positive	SCC > 5 lac/ml and culturally negative
24 (29.26)	19 (23.17)	38 (11.65)	36 (11.04)	23 (7.05)	15 (4.60)	13 (3.98)

Table 2. Frequency of isolation of different organisms.

Organisms	Number (percent)
Coagulase positive staphylococci	7 (15.90)
Coagulase negative staphylococci	21 (47.72)
Streptococcus dysgalactiae	11 (25.00)
Streptococcus agalactiae	4 (9.09)
Streptococcus uberis	1 (2.27)

Table 3. Characterization of staphylococci isolated from buffalo milk.

Coagulase test	Sr. No.	Organisms	Number
Coagulase positive staphylococci	1.	*Staphylococcus aureus*	5
	2.	*Staphylococcus hyicus*	2
Coagulase negative staphylococci	1.	*Staphylococcus hyicus*	2
	2.	*Staphylococcus epidermidis*	4
	3.	*Staphylococcus hominis* subsp. *hominis*	1
	4.	*Staphylococcus pasteuri*	2
	5.	*Staphylococcus arlettae*	1
	6.	*Staphylococcus haemolyticus*	5
	7.	*Staphylococcus saprophyticus* subsp. *saprophyticus*	2
	8.	*Staphylococcus gallinarum*	1
	9.	*Staphylococcus simulans*	3
		TOTAL	**28**

be a consequence of unhygienic milking practice, due to which the organisms gained access into mammary gland through milkers' hands, causing an increase in SCC and inflicting pathogenicity in the alveolar tissue. Further studies are required on role of *Staphylococcus haemolyticus* in causing mastitis.

In the current study, amongst streptococcal isolates, *Streptococcus dysgalactiae* were the predominating organisms 25 percent) followed by *Streptococcus agalactiae* (9.09 percent) and *Streptococcus uberis* (2.27 percent). The higher prevalence of *Streptococcus dysgalactiae* than *Streptococcus agalactiae* was reported by Kalra and Dhanda (1964) and Tuteja (1999). In contrast to our study Hameed *et al*., 2007; Chavan *et al*., 2007; Getahun *et al*., 2008; Ferguson *et al*., 2008, reported higher prevalence of *Streptococcus agalactiae* than *Streptococcus dysgalactiae,* whereas other workers (Javed and Siddique, 1999; Sampimon *et al*., 2009) found higher prevalence of *Streptococcus uberis* than that recorded in this study. Our findings are in close agreement with Petzer *et al*. (2009) who reported isolation of CNS, *Streptococcus agalactiae* and *Streptococcus uberis*.

A good amount of literature is available on the antibiogram of different mastitogens. It is not possible to compare our results with their

Table 4. Antibiogram of different organisms isolated.

Antimicrobials	**Percent sensitivity**	
	Staphylococci (28)	**Streptococci (16)**
Erythromycin	72.2	90.9
Penicillin	88.8	100
Streptomycin	50	72.7
Tetracycline	100	72.7
Chloramphenicol	83.3	81.6
Ampicillin	88.8	36.6
Neomycin	66.6	63.6
Cloxacillin	100	100
Enrofloxacin	94.4	100
Gentamicin	83.3	100
Amikacin	50	81.8
Amoxycillin	94.4	81.6
Ceftrioxone	100	100
Cefoperazone	100	100
Ciprofloxacin	83.3	100
Colistin	44.4	27.2
Co-Triamoxazole	94.4	63.6
Nitrofurantion	94.4	90.9
Lincomycin	94.4	100
Cephalexin	94.4	100

findings. While considering overall sensitivity, all the strains of staphylococci and streptococci were found sensitive to cloxacillin, ceftriaxone and cefoperazone. Streptococci revealed 100 percent sensitivity towards penicillin, enrofloxacin, ciprofloxacin, lincomycin and cephalexin. Similar to our study, Ranjan *et al.* 2010 also found high sensitivity towards Enrofloxacin (91.67%) whereas they observed lower sensitivity towards Ceftriaxone (84.10%). It was interesting to note that staphylococci isolates revealed 100 percent sensitivity towards tetracycline. Studies conducted by several workers (Sharma *et al*., 2007; Chavan *et al*., 2007; Roychoudhury and Dutta, 2009; Sharma *et al.,* 2010) have showed increased resistance towards different traditional and newly introduced antibiotics. In contrast to these studies, the antibiogram obtained in the current study indicated high sensitivity towards newer and older antibiotics, showing rational use of these antibiotics at farms under study. Antibiotic resistance patterns vary among different farms, regions, states and countries depending upon the type of organisms and use of antibiotics in a particular area; therefore, antimicrobial sensitivity is suggested before institution of treatment. The information obtained by this study will also be of useful to the dairy industry and individual farmers. It will be helpful in prioritizing mastitis control efforts.

ACKNOWLEDGEMENT

The technical help rendered by Sh. Bhupinder Singh and Sh. Randhir Singh of the Veterinary College Central Laboratory, COVS, LLRUVAS, is gratefully acknowledged.

REFERENCES

Armenteros, M., P. Ponce, J. Capdevila, V. Zaldivar and R. Hernandez. 2006. Prevalence of mastitis in first lactation dairy cows and sensitivity pattern of the bacteria isolated in a specialized dairy. *Revi. De Salud Anim.,* **28**: 8-12.

Babu, Y.H., Sudhakar, C.S. Srinivas and M.A. Khan. 1983. Studies on incidence and in vitro sensitivity of isolates of antimicrobials from sub-clinical mastitis in Jersey cows. *Livestock Advisor,* **8**: 47-49.

Bansal, B.K., K.B. Singh, R. Rohan, D.V. Joshi, D.C. Nauriyal and Rajesh Mohan. 1995. Incidence of sub clinical mastitis in some cow and buffalo herds in Punjab. *J. Res. Punjab Agri. Uni.,* **32**: 79-81.

Bauer, A.W., W.W.M. Kirby, J.C. Sherris and M. Turch. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.,* **45**: 493-496.

Bulla, T.R. 2002. Studies on diagnosis and treatment of sub-clinical mastitis in buffaloes. M.V.Sc. Thesis, Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), Hisar, Haryana, India.

Char, N.L., P. Rao and P.V.R. Rao. 1983. Studies on mastitis in buffaloes. *Livestock Advisor,* **8**: 19-22.

Chavan, V.V., S.U. Digraskar, S.N. Dhonde and P.B. Hase. 2007. Observation on bubaline subclinical mastitis in and around Parbhani. *Indian J. Field Vet.,* **3**: 50.

Dego, O.K. and F. Tareke. 2003. Bovine mastitis in selected areas of southern Ethiopia. *Trop. Anim. Health Prod.,* **35**: 197-205.

Dua, K. 2001. Studies on incidence, etiology and estimated economic losses due to mastitis

in Punjab and India - An update. *Indian Dairyman,* **53**: 41-48.

Ferguson, J.D., G. Azzaro, M. Gambina and G. Licitra. 2008. Prevalence of mastitis pathogens in Ragusa, Sicily, from 2000 to 2006. *J. Dairy Sci.,* **90**: 5798-5813.

Getahun, K., B. Kelay, M. Bekana and F. Lobago. 2008. Bovine mastitis and antibiotic resistance patterns in Selalle smallholder dairy farms, central Ethiopia. *Trop. Anim. Health Prod.,* **40**: 261-268.

Hagnestan, N.C., U. Emanuelson, B. Berglund and E. Strandberg. 2009. Relationship between somatic cell count and milk yield in different stages of lactation. *J. Dairy Sci.,* **92**: 3124-3133.

Hameed, K., A. Galal, G. Sender and K.A. Korwin. 2007. Public health hazard due to mastitis in dairy cows. *Anim. Sci. Pap. Rep.*, **25**: 73-85.

Hawari, A.D. and F. Al Dabbas. 2008. Prevalence and distribution of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Jordan. *Am. J. Anim. Vet. Sci.,* **3**: 36-39.

Javed, I. and M. Siddique. 1999. Some epidemiological aspects of mastitis in cows and the characterization of the isolates. *Pak. Vet. J.,* **19**: 149-154.

Joshi, S. and S. Gokhale. 2006. Status of mastitis as an emerging disease in improved and periurban dairy farms in India. *Ann. N. Y. Acad. Sci.,* **108**: 4-83.

Kalorey, D.R, J.H. Purohit and P.M. Dholakia. 1983. Studies on incidence of sub-clinical mastitis, its etiology and in vitro sensitivity of isolates. *Indian J. Anim. Sci.,* **53**: 961-963.

Kalra, D.S. and M.R. Dhanda. 1964. Incidence of mastitis in cows and buffaloes in North West India. *Vet. Rec.,* **76**: 219-222.

Kaya, O., S. Kirkan, M. Gulal and B. Unal. 2000. Identification and antibiotic susceptibility of microbes causing mastitis in dairy cows. *Vet. Bull.,* **70**: 290.

Maiti, S.K., N. Sharma and B. Awasthi. 2003. Studies on incidence of sub-clinical mastitis in cattle and buffaloes of Chattisgarh. *Vet. Pract.,* **4**(2): 90.

Nagal, KB., S. Mandeep and R.C. Katoch. 1999. Incidence and etiology of bovine mastitis. *Indian J. Anim. Sci.,* **69**: 150-152.

Nickerson, S.C. and C. Stephen. 2009. Control of heifer mastitis: Antimicrobial treatment-An overview. *Vet. Microbiol.,* **134**: 128-135.

Nickerson, S.C., M.J. Paape and G. Ziv. 1986. Mammary leukocyte response to drug therapy. *J. Dairy Sci.,* **69**: 1733.

Petzer, I.M., J. Karzis, J.C. Watermeyer, T.J. Van der Schans and R. Van Reenen. 2009. Trends in udder health and emerging mastitogenic pathogens in South African dairy herds. *J. S. Afr. Vet. Assoc.,* **80**: 17-22.

Rahman, H., D.S. Sambyal and K.K. Baxi.1983. Incidence and etiology of sub-clinical mastitis in cows and buffaloes in Punjab. *Punjab Agri. Uni. J. Res.,* **20**: 208-212.

Rani, N.L., S. Manda, K. Suresh and Makkenasreenu. 2008. An epidemiological study of mastitis in buffaloes. *Indian Vet. J.,* **85**: 1350-1351.

Ranjan, R., M.K. Gupta, S. Singh and S. Kumar. 2010. Current trend of drug sensitivity in bovine mastitis. *Vet. World,* **3**: 17-20.

Roder, R and W. Gedek. 1986. Milk cell counts associated with different mastitis pathogens. *Berl. Munch Tierarztl.,* **99**(3): 73-76.

Roychoudhury, P. and T.K. Dutta. 2009. Prevalence

and antibiotic sensitivity pattern of bacteria from bovine mastitis in Mizoram. *Indian J. Anim. Sci.,* **79**: 483-484.

Saini, S.S., J.K Sharma and M.S. Kwatia. 1994. Prevalence and etiology of sub-clinical mastitis among crossbred cows and buffaloes in Punjab. *Indian J. Dairy Sci.,* **47**: 103-106.

Salsberg, E., A.H. Meek and S.W. Marlin. 1984. Somatic cell counts, associated factors and relationship to production. *Can. J. Comparat. Med.,* **40**: 251-257.

Sampimon, O., H.W. Barkema, I. Berends, J. Sol and T. Lam. 2009. Prevalence of intramammary infection in Dutch dairy herds. *J. Dairy Res.* **76**: 129-136.

Sargeant, J.M., H.M. Scott, K.E. Leslie, M. Ireland and A. Bashiri. 1998. Clinical mastitis in dairy cattle in Ontario: Frequency of occurrence and bacterial isolates. *J. Dairy Sci.,* **39**: 33-38.

Schalm, O.W., E.J. Carol and N.C. Jain. 1971. Bovine mastitis; Lea and Febiger, Philadelphia.

Schultze, M. 1985. Factors that influence bovine mastitis. *J. Dairy Res.,* **30**: 320-325.

Serieys, F. 1985. Cell counts in milk from individual cows, influence of mammary infection parity, stage of lactation and milk yield. *Annal. De Res. Vet.,* **16**: 255-261.

Sharma, A. and M.P. Kapur. 2000. Prevalence of sub clinical mastitis in cows at an organized farm. *Haryana Vet.,* **39**: 13-16.

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

Sharma, D.K., P.K. Jallewar and K.K. Sharma. 2010. Antibiogram of bacteria isolated from bovine subclinical mastitis. *Indian Vet. J.,* **87**: 407.

Sharma, N., S.K. Maiti and K.K. Sharma. 2007. Prevalence, Etiology and Antibiogram of Microorganisms associated with subclinical mastitis in buffaloes in Durg, Chhatisgarh State (India). *Int. J. Dairy Sci.,* **2**: 2.

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

Sindhu, N., A. Sharma and V.K Jain. 2010. Coagulase gene based molecular detection of Staphylococcus aureus directly from mastitic milk samples of Murrah buffalo. *Buffalo Bull.,* **29**: 62-69.

Tenhagen, B.A., I. Hansen, A. Reinecke and W. Heuwieser. 2009. Prevalence of pathogens in milk samples of dairy cows with clinical mastitis and in heifers at first parturition. *J. Dairy Res.,* **76**: 179-187.

Thirunavukarasu, M. and R. Prabharan. 1998. Factors influencing mastitis in bovines. *Indian J. Anim. Prod. Manag.,* **14**: 110-114.

Tijare, D.B., A.K. Singh, V.K. Chaturvedi and N.S. Dhanesar. 1999. Sensitivity of indirect tests in detection of sub-clinical mastitis in buffaloes. *Indian Vet. J.,* **76**: 912-915.

Tuteja, F.C. 1999. *Studies on mastitis in buffaloes with reference to serum selenium status and control by treating teat canal infections*. Ph.D. Thesis, CCS HAU, Hisar, Haryana, India.

Unnerstad, H., L.A. Ericsson, K.P. Waller, T. Ekman, K. Artursson, O.M. Nilsson and B. Bengtsson. 2009. Microbial etiology of acute clinical mastitis and agent-specific risk factors. *Vet. Microbiol.,* **137**: 90-97.