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Prevalence, Etiology and Antibiogram of Microorganisms Associated with Sub-clinical Mastitis in Buffaloes in Durg, Chhattisgarh State (India)

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Abstract: A study was carried out in 2000 quarters milk samples of 500 lactating buffaloes in Durg district of Chhattisgarh State, India. 330 (66.00%) animals were found to be positive for sub clinical mastitis (SCM) by Modified White Side Test (MWST), 343 (68.60%) by Modified California Mastitis Test (MCMT) and 360 (72.00%) by Somatic Cell Count (SCC). The overall quarter-wise prevalence of SCM was 38.99% by MWST, 42.00% by MCMT and 45.00% by SCC. 1.9% quarters were found blind. Single quarters and hind quarters involvement was maximum. In regard to stage of lactation infection rate was higher during the late lactation followed by early and mid stage of lactation. The prevalence was highest during third and fourth lactation and at 3 to 9 years of age. Among the microorganisms isolated the *Staphylococcus* sp. occupied prime position. According to microbial sensitivity test, maximum number of isolates (27.27%) showed sensitivity to cefotaxime antibiotic whereas most of the isolates were resistant to ampicillin.

Key words: Prevalence, etiology, antibiogram, mastitis

INTRODUCTION

Mastitis is a multietiolgical complex disease which is characterized by physical, chemical and bacteriological changes in milk and pathological changes in glandular tissue (Radostits *et al*., 1995). Bovine mastitis is a global problem as it adversely affects animal health, quality of milk and economics of milk production and every country including developed ones suffer huge financial losses (Sharma *et al*., 2004). According to National Mastitis Council (1996) the total economic losses due to mastitis include value of reduced milk production (70%), premature culling (14%), veterinary expenses (9%) and milk discarded or low graded (7%). The relative importance of mastitis varies in different areas and countries (Stableforth, 1959). It has been estimated that a financial loss per year due to mastitis amounts 35 billions $ world over. In India, economic loss due to Sub Clinical Mastitis (SCM) in buffaloes has been estimated to be Rs. 1723.32 crore as compared to Rs. 696.29 crore due to of clinical type of the disease (Dua, 2001). Apart from its economic importance, mastitis also carries public health significance, more importantly in relation to drug residue in milk and passage of pathogenic organisms to humans. Today, it stands second to Foot and Mouth disease as the most challenging disease in dairy animals. India is endowed with the largest livestock population in the word.

The proportion of contribution of livestock sector (4.8 to 6.6%) to total Gross Domestic Product has remained steady over the years. It was 6.61% (5.5% from livestock and 1.1% from fisheries) during 1999 to 2000 at current prices (Tiwari, 2002). Despite all scientific progress, mastitis remains

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prevalent in most of the dairy herds. Today it can be estimated that nearly half of the dairy cow and buffalo population is suffering from clinical and sub clinical mastitis. Considering such high prevalence and its economic importance, the veterinarians especially those who are engaged in animal practice should be well acquainted with the knowledge about mastitis. In this paper the prevalence, etiology and antibiogram of SCM in Durg District of Chhattisgarh State (India) by 3 different indirect mastitis tests (viz., Modified White Side Test, Modified California Mastitis Test and Somatic Cell Count) has been reported. In addition, susceptibility of isolates from animals reacting positive in Modified California Mastitis Test was also documented.

MATERIALS AND METHODS

A total of 500 lactating buffaloes of different age, parities and stage of lactation belonging to different organized/unorganized/Government/private dairy farms, Chhattisgarh State (India) were investigated. These animals were screened for the detection of sub clinical mastitis by Modified White Side Test (MWST) as per the method of Schalm *et al.* (1971) Modified California Mastitis Test (MCMT) as per the method of Devi (1989) and Somatic Cell Count (SCC) as per the method of Schalm *et al.* (1971).

A total of 75 milk samples positive to MCMT were subjected to isolation and identification of bacteria on the basis of morphological, cultural and biochemical characteristics as per method of Buchnan and Gibbons (1984). Susceptibility of individual bacterial isolates to six antibiotics were determined by disc diffusion method (Bauer *et al*., 1996) using commercially available antibiotic discs (Ampicillin (A)-10 mcg, Cefotaxime (Ce)-30 mcg, chloramphenicol (C)-30 mcg, Erythromycin (E)-15 mcg, Gentamicin (G)-10 mcg and Tetracycline-30 mcg) marketed by Hi-media Laboratory, Mumbai, India.

After proper sanitization of teat orifice with 70% ethyl alcohol, 10-20 mL of milk samples from all four quarters viz. Left Fore (LF) Left Hind (LH) Right Fore (RF) and Right Hind (RH) were collected aseptically following squirting first few streams, in sterile polyethylene screw caped wide mouth vials (Buswell, 1995). The milk samples were kept in an ice box and carried to the laboratory of College of Veterinary Science and Animal Husbandry, Anjora, Durg (Chhattisgarh State) India, where the milk samples were kept at 4-8°C in refrigeratr for further laboratory investigation.

RESULTS

A total of 500 lactating buffaloes, were screened by Modified White Side Test, Modified California Mastitis Test and Somatic Cell Count for detection of sub clinical mastitis. Out of 500 animals, 330 (66.00%) animals by MWST, 343 (68.60%) by MCMT and 360 (72.00%) by SCC were found positive for sub clinical mastitis. In this study the overall quarter-wise prevalence of SCM in buffaloes was 38.99% by MWST, 42.00% by MCMT and 45.00% by SCC. The study showed no marked difference in infection rate between individual quarters. However, infection rate was somewhat higher in hind quarters and it was comparable between left and right side quarters. Out of 2000 quarters, 38 (1.9%) quarters were found blind and could not be subjected to any mastitis test. The details of quarter-wise prevalence of mastitis on individual and combination basis are presented in Table 1. On individual quarter basis, occurrence of SCM was somewhat higher in left quarters in comparison to other quarters and on combination basis, prevalence rate was slightly higher in hind quarters than fore quarters. During the present study, of the infected animals prevalence of infection in single, double, triple and quadruple quarters were 13.25, 21.30, 24.15 and 51.30% by MWST, respectively, 14.35, 21.76, 24.30 and 39.58% by MCMT, respectively and 14.93, 22.08, 24.24 and

38.74% by SCC, respectively. It appeared that single quarter involvement was maximum, followed by double and triple quarters; the percentage involvement of quadruple quarters was the lowest.

When the prevalence of SCM in buffaloes in regard to intensity of reactions to indirect mastitis tests was considered maximum percentage of illness of was of mild degree and minimum percentage was of severe nature (Table 2). In this study, SCC ranged from 0.33 to 104 ($\times 10^5$) mL^{-1} of milk sample.

In the present study, the overall prevalence of SCM was maximum in the late (79.08% by MWST, 81.69% by MCMT and 86.27% by SCC) lactation followed by early (63.53% by MWST,

Table 1: Prevalence of SCM in buffaloes in durg, Chhattisgarh State (animal-wise and quarter-wise) (As detected by different indirect mastitis tests)

				Quarter-wise prevalence							
				Individual basis				Combination basis			
S. No.	Tests	No. of buffaloes tested, reacting positive and percent positive	Total No. of functional quarters	LF	LH	RF	RH	Left side	Right side	Fore quarter	Hind quarter
1.	MWST	500	1962 (38)	494	485	496	487	979	983	990	972
		330	765	195	206	180	184	401	364	375	390
		66.00	38.99	25.49	26.93	23.53	24.05	52.42	47.58	49.01	50.98
2.	MCMT	500	1962 (38)	494	485	496	487	979	983	990	972
		343	824	169	232	206	217	401	423	375	449
		68.60	42.00	20.50	28.16	25.00	26.33	48.66	51.33	45.50	54.49
3.	SCC	500	1962 (38)	494	485	496	487	979	983	990	972
		360	883	194	274	229	186	468	415	423	460
		72.00	45.00	21.97	31.03	25.93	21.06	53.00	47.00	47.90	52.09

Figures in the parentheses indicate total number of blind teats

Table 2: Frequency distribution of different grades (Trace, +, ++ and +++) of three different indirect mastitis tests (MWST, MCMT and SCC) as a function of different udder quarters in 500 buffaloes tested for SCM in Durg, Chhattisgarh State, India

Tests	MWST				MCMT				
Grade	Trace	+	++	Total	Trace	+	++	+++	Total
LF	20	110	65	195	28	113	20	8	169
(494)*	(4.05)	(22.27)	(13.16)	(39.47)	(5.67)	(22.87)	(4.05)	(1.60)	
LH	45	115	46	206	55	121	32	24	232
(485)*	(9.28)	(23.71)	(9.48)	(42.47)	(11.34)	(24.95)	(6.59)	(4.59)	(47.83)
RF	28	112	40	180	31	119	36	20	206
(496)*	(5.64)	(22.58)	(8.06)	(36.29)	(6.25)	(23.99)	(7.26)	(4.03)	(41.53)
RH	30	120	34	184	33	125	45	14	217
(487)*	(6.16)	(24.64)	(6.98)	(37.78)	(6.78)	(25.67)	(9.24)	(2.87)	(44.45)
Total	123	457	185	765	147	478	133	66	824
(1962)*	(6.26)	(23.29)	(9.43)	(38.99)	(7.49)	(24.36)	(6.78)	(3.36)	(42.00)

Tests	SCC ($\times 10^5$ p mL^{-1})				
Grade	1-3	3-9	9-27	27-81or>81	Total
LF	35	112	27	20	194
(494)*	(7.08)	(22.67)	(5.46)	(4.05)	(39.27)
LH	66	126	45	37	247
(485)*	(13.61)	(25.98)	(9.28)	(7.63)	(56.49)
RF	34	117	43	35	229
(496)*	(6.85)	(23.59)	(8.67)	(5.13)	(46.17)
RH	30	97	34	25	186
(487)*	(6.61)	(19.92)	(6.98)	(5.13)	(38.19)
Total	165	452	149	117	883
(1962)*	(8.41)	(23.04)	(7.59)	(5.69)	(45.00)

Figures in the parenthesis indicate percentage values, *Indicate number of quarters tested

Table 3: *In vitro* antibiotic susceptibility profiles of isolates of different species recovered from 75 quarters of sub clinically mastitic buffaloes in Durg, Chhattisgarh State (India)

Name of organisms	Total No. of isolates tested	Antibiotics tested					
		C	Ce	G	E	T	A
Staphylococcus sp.	59	10 (16.95)	15 (25.42)	8 (13.56)	12 (20.34)	12 (20.34)	2 (16.95)
Streptococcus sp.	17	3 (15.00)	7 (35.00)	2 (10.00)	5 (25.00)	3 (15.00)	0 (00.00)
Escherichia coli	14	2 (12.50)	3 (18.75)	5 (31.25)	4 (25.00)	2 (12.50)	0 (00.00)
Corynebacterium sp.	11	0 (00.00)	5 (55.55)	0 (00.00)	2 (22.22)	2 (22.22)	0 (00.00)
Diplococcus	5	0 (00.00)	1 (16.67)	2 (33.33)	0 (00.00)	3 (50.00)	0 (00.00)

Figures in parentheses indicate percentage values

65.88% by MCMT and 69.41% by SCC) and mid stage (57.06% by MWST, 59.32% by MCMT and 62.15% by SCC) of lactation.

In the present study, the prevalence of SCM was recorded from 1st to 11th lactations in buffaloes. The prevalence rate varied from 20 to 88.37% by MWST, 23 to 89% by MCMT and 30.77 to 91.86% by SCC in different lactations, but maximum prevalence (88.37% by MWST, 89.53% by MCMT and 96.86% by SCC) was during 3rd and 4th lactations, respectively and minimum (20% by MWST, 40% by MCMT and 53.33% by SCC) during 9th lactations onwards.

Analysis of age wise infection frequency of SCM in buffaloes revealed that infection rate varied from 33.33 to 77.96% by MWST, 33.33 to 79.66% by MCMT and 42.86 to 83.05% by SCC. The higher prevalence of SCM in buffaloes was recorded in 5 to 9 years old animals. A total of 110 isolates were recovered from 75 quarters positive for SCM. Of 110 isolates from the buffaloes, 59 (53.64%) isolates belonged to *Staphylococcus* sp. which was found to be the chief etiological agent causing SCM. Hence hygiene at milking is of paramount important in the control of this infection. *Sreptococcus* sp. were the second largest mastitogen group accounting for 18.18% of isolates followed by *Escherichia coli* (14.54%) *Corynebacterium* sp. (8.18%) and *Diplococcus* sp. (5.95%).

Out of 110 isolates obtained from cases of SCM from buffaloes tested for their antibiogram, maximum number of isolates were sensitive to cefotaxime (28.18%). It was followed by in order by erythromycin (20.91%) tetracycline (20%) gentamicin (15.45%) and chloramphenicol (13.64%). Detailed results of *in-vitro* antibiotic sensitivity pattern of 110 isolates of SCM from buffaloes are shown in Table 3.

Out of 59 isolates of *Staphylococcus* sp. 15 (25.42%) were sensitive to cefotaxime, 12 (20.34%) to erythromycin, 12 (20.34%) to tetracycline, 10 (16.95%) to chloramphenicol and 8 (13.56%) to gentamicin. The least sensitivity was shown to ampicillin. However, *E. coli* isolates showed highest (31.25%) sensitivity to gentamicin. Out of 9 isolates of *Corynebacterium* sp. 5 (55.55%) were sensitive to cefotaxime.

DISCUSSION

Out of 500 lactating buffaloes examined during present investigation 66.00% were positive for SCM by MWST, 68.60% by MCMT and 72.00% by SCC. The figures on animal basis closely proximated with the observations of Kumar and Sharma (2002) who recorded 66.27% occurrence of SCM in buffaloes in Haryana, India and 70.32% prevalence of SCM in buffaloes in Rajnandgaon District of Chhattisgarh State, India, also recorded by Sharma *et al*. (2004).

The present findings of quarter-wise prevalence are in agreement with the earlier report of Sharma *et al.* (2004), who recorded 43.53% quarter-wise prevalence of SCM in buffaloes. The finding of present study on individual and quarter basis did not agree with the observations of Tijare (1997) who reported low prevalence of SCM on quarter basis and higher prevalence of SCM in RH and LF quarters. It might be due to fact that no treatment of one quarter, being unnoticed, could be undertaken or owner's ignorance towards marginal disease in milk production. The higher prevalence of SCM in hind quarters may due to higher chances of contamination of hind quarters with feces, urine and uterine discharges.

In the present study, the overall prevalence of SCM was maximum in the late lactation followed by early and mid stage of lactation. Patil *et al.* (1995), also reported that prevalence of SCM was highest during late lactation period as compared to early and mid lactation periods. Higher prevalence during late lactation might be due to fact that this period is more vulnerable to usher infection.

In the present study, maximum prevalence was during 3rd and 4th lactations, respectively and minimum during 9th lactations onwards. Kumar and Sharma (2002) also recorded majority of SCM cases during 3rd lactation. The initial increase and then decrease in infection rate in respect to lactation may be correlated to production performance of the animal.

The higher prevalence of SCM in buffaloes was recorded in 5 to 9 years old animals. Kumar and Sharma (2002) reported higher prevalence of SCM in buffaloes between 5 to 7 years of age. Whereas, Sharma and Prasad (2002) recorded maximum prevalence of SCM in the age group of 7 years and above. Hence, the data obtained during present study were in accordance with the observations of above workers and a little variation might be related to different geographical attributes for the buffaloes and varying susceptibility.

On cultural examination the *Staphylococcus* sp. was found to be the chief etiological agent causing SCM. This finding is in agreement with the earlier report of Sharma and Prasad (2002) who recorded 54.50% occurrence due to of *Staphylococcus* sp. While Shrirame *et al.* (2002) recorded 72.35% of incidence due to *Staphylococci.* Usually the infections are spread during the milking process (Harmon, 1993). Hence hygiene at milking is of paramount important in the control of this infection. *Sreptococcus* sp. was the second largest mastitogen group of isolates recovered from buffalo. This was in accordance with reports of Prasad (2000) and Sharma and Kapur (2000) who, respectively recorded 16.88 and 20.99% occurrence of *Streptococcus* sp. *Escherichia coli* isolates in the present study accounted for 14.54% share among different isolates of mastitis milk. Kader *et al.* (2002) also recorded 11.11% occurrence of *E. coli* in mastitic milk. *Streptococcus* sp. is a contagious pathogen and its major reservoir is the infected udder. Whereas, *Escherichia coli* is the environmental pathogen. The mastitogens are adoptive organisms, which show a marked degree of variation in their biological characters.

The emergence of drug resistant organisms causing mastitis due to indiscriminate use of antibiotics is well known. Moreover, due to lack of prophylactic agents, chemotherapy continues to play a major role in the therapeutic management of the disease. For success of the treatment, sensitivity testing plays a pivotal role. Recently newer antibiotics have been introduced for the treatment of both sub clinical and clinical mastitis. Thus, it has become imperative to control this dreaded disease with most effective antibiotic therapy. Hence the present study was also designed to probe into in-vitro sensitivity of isolated bacterial strains from cases of SCM against a range of traditional as well as newly introduced antibiotics potentially useful for the treatment and control programme.

Out of 110 isolates obtained from cases of SCM from buffaloes tested for their antibiogram, maximum number of isolates were sensitive to cefotaxime, which is in agreement with the Nath and Dutta (2003). It was followed by in order by erythromycin, tetracycline, gentamicin and

chloramphenicol. These results are closely related with the findings of Choudhuri (2000) and Kader *et al*. (2002). The present findings are in agreement with the reports of Tijare (1997) *Streptococcus* sp. (35%) isolates were sensitive to cefotaxime. However, *E. coli* isolates showed highest sensitivity to gentamicin. Out of 9 isolates of *Corynebacterium* sp. 5 (55.55%) were sensitive to cefotaxime. Taking both gram positive and gram negative isolates into consideration cefotaxime showed maximum sensitivity.

REFERENCES

Bauer, A.W., W.M.M. Kieby, J.C. Shrenis and M. Turck, 1996. Antibiotic susceptibity testing by a standardized single disc diffusion method. Am. J. Clin. Pathol., 45: 453-496.

Buchnan, R.E. and N.E. Gibbon, 1984. Bergeys Manual of Determinative Bacteriology. 8th Edn., The Williams and Wilkins Company Baltimore, USA.

Buswell, J., 1995. Simple mastitis bacteriology for the practice. In: Practice, pp: 426-432.

Choudhuri, P.C., 2000. Status paper on mastitis in Andhra Pradesh. In: Proceeding of Round Table Conf. (IAAVR) on Mastitis held at Izzatnagar, 18th-19th Feb., pp: 84-92.

Devi, B.K., 1989. Studies on sub clinical mastitis in cows with special reference to incidence, etiology, sensitivity and treatment in Malwa Region Madhya Pradesh. M.V.S.C. and A.H. Thesis submitted to J.N.K.V.V., Jabalpur, M.P., India.

Dua, K., 2001. Incidence, etiology and estimated economic losses due to mastitis in Punjab and in India-An update. Indian Dairyman, 53: 41-48.

Harmon, R.J., 1993. Symposium mastitis and genetic evaluation for somatic cell count. J. Dairy Sci., 77: 2103-2112.

Kader, M.A., M.A. Samad, S. Saha and M.A. Taleb, 2002. Prevalence and etiology of sub clinical mastitis with antibiotic sensitivity to isolated organisms among milch cows in Bangladesh. Ind. J. Dairy Sci., 55: 218-223.

Kumar, R. and A. Sharma, 2002. Prevalence, etiology and antibiogram of mastitis in cows and buffaloes in Hissar, Haryana. Ind. J. Anim. Sci., 72: 361-363.

Nath, A.K. and J.B. Dutta, 2003. Antimicrobial sensitivity of isolates from bovine sub clinical mastitis. In: Compendium of XXI Indian Society of Veterinary Medicine. Anand, Gujarat, India, pp: 237.

National Mastitis Council, 1996. Current concepts in bovine mastitis. www.cowmastitis.com.

Patil, N.A., M.D. Harapanahalli, J.A. Mulia, S.V. Hosmani and B.K. Pugasheddi, 1995. Comparative study on prevalence and diagnosis of sub clinical mastitis in cows and buffaloes. Ind. J. Dairy Sci., 48: 478-479.

Prasad, B., 2000. Mastitis in dairy animals of Himachal Pradesh. In: Proceeding of Round Table Conference on Mastitis, Izatnagar, India, pp: 16-22.

Radostits, O.M., D.C. Blood and C.C. Gay, 1995. Veterinary Medicine. 8th Edn. ELBS-Bailliere Tindal, London.

Schalm, O.W., E.G. Carriol and N.C. Jain, 1971. Bovine Mastitis. Lea and Febiger, Philabelphia, USA.

Sharma, A. and M.P. Kapoor, 2000. Prevalence of sub clinical mastitis in buffaloes. In: Proceeding of Round Table Conference on Mastitis, Izatnagar, India. 18-19 Feb.2000, pp: 8-11.

Sharma, A.K. and B. Prasad, 2002. Microbial profile and antibiogram studies in mastitic dairy animals of Palampur of H.P. In proceeding of XX Indian Society Of Veterinary Medicine. Bikaner, Rajasthan, India. 14th-16th Feb., pp: 93.

Sharma, Neelesh, S.K. Maiti and K.M. Koley, 2004. Studies on the incidence of sub clinical mastitis in buffaloes of Rajnandgaon District of Chhattisgarh State. Vet. Practitioner, 5: 123-124.

Sharma, Neelesh, S.K. Maiti and R. Singh, 2004. Mastitis in dairy animals-A global problem. Dairy Planner, 1: 40-41.

Shrirame, K.R., S.S. Bhardwaj, D.R. Kalory and S.D. Harne, 2002. Study of pathogenicity markers of *Staphylococci* isolated from sub clinical mastitis: In Proceeding of Round Table Conference on Mastitis, Nagpur, India. 4-5 Feb. 2002, pp:18-22.

Stableforth, A.W., 1959. Streptococcal Diseases. Diseases Due to Bacteria. Stableforth A.W. and I.A. Galloway (Eds.), Butterworths Scientific Publication London, 2: 589-650.

Tijare, D.B., 1997. Bacteriological studies on bovine sub clinical mastitis. M.V.Sc. and A.H. Thesis submitted to J.N.K.V.V., Jabalpur (M.P.), India.

Tiwari, D.N., 2002. Development of livestock economy. 3IDFT Exposure, 2002, Tech. Sym. on dairy Mastitis and Milk Quality New Delhi, India Sept. 5, 2002.