

EFFECT OF IMMUNOPOTENTIATING AGENTS ON SUBCLINICAL MASTITIS IN CATTLE AND BUFFALOES

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Summary

Different parameters of subclinical mastitis were compared in 327 cattle and 493 buffaloes and the effect of immunopotentiating agents on subclinical mastitis in these animals was studied. Subclinical mastitis was detected in 8.2 percent buffalo and 24.0 percent cow quarter by modified whiteside test (MWT). In both the species there was decrease in lactose contents with increase in the degree of MWT reactivity i.e. 4.8 ± 1.14 to 2.31 ± 0.82 in cattle and 5.01 ± 1.47 to 2.36 ± 1.02 in buffaloes. While the chloride contents of the milk increased with increase in the MWT reactivity i.e. 0.19 ± 0.4 to 0.36 ± 0.06 in cattle and 0.20 ± 0.04 to 0.31 ± 0.13 in buffaloes. Micro-organisms belonging to *Staphylococcus*, *Micrococcus*, *Bacillus*, *Streptococcus*, *Enterobacteria*, *Corynebacterium* groups and yeasts were isolated from subclinical mastitis cases. Vitamin E and Levamisole cured 64.5 and 60.0 percent cases of subclinical mastitis in buffaloes but only 32.0 and 24.0 percent cases in cattle. Cure was not affected by the degree of MWT and the type of organisms involved.

(Key Words: Subclinical Mastitis, Buffalo, Cattle, Immunopotentiating Agent, Chemical Determination, Somatic Cell Count)

Introduction

Mastitis is economically the most important disease in dairy industry. It causes economic losses in terms of reduced milk production, permanent loss of quarter (s) and cost of treatment. The need to detect the udder infection at subclinical stage and its cure has been recognized as essentials to the success of mastitis control programme. Methods like modified whiteside test (MWT), California mastitis test, lactose and chloride estimations and somatic cell count in milk have been used and evaluated to assess the incidence of subclinical mastitis in the dairy cattle (Schalm et al., 1971). Occasional studies on the use of some of these tests in buffalo have also been reported (Mohammad et al., 1980).

Approaches to cure the mastitis mostly rely on the use of antibiotics. In the antibiotic therapy, the success depends among other things upon degree and stage of infection and antibiotic

susceptibility of the causative organism. Moreover, the antibiotic residues pass into the milk and meat products and may lead to serious public health problems (Hutchinson and Erbel, 1987). Therefore, non antibiotic approaches to control the bovine mastitis needs attention.

The animals with subclinical mastitis may be treated by increasing the resistance against infections by immunopotentiating agents. A few studies have indicated the use of levamisole in the treatment of subclinical mastitis (Ziv et al., 1981; Ishikawa and Shimizu, 1982; Shimada, 1983; Buddle and Pulford, 1985). The present study compares the different parameters of subclinical mastitis in cattle and buffaloes and the effect of different immunopotentiating agents on the treatment of subclinical mastitis.

Materials and Methods

Experimental protocol

Milk samples were collected in sterile test tubes from each quarter separately of 327 lactating Jersey cows and 493 Nili Ravi buffaloes. Each sample was tested by modified Whiteside test (MWT) for the detection of subclinical mastitis (Schalm et al., 1971). MWT positive samples were

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Received September 25, 1990

Accepted August 13, 1992

processed for bacteriological examination, lactose and chloride determination and somatic cell counts. MWT negative quarter (s) from the same animal were also processed similarly. MWT positive animals were treated as given in the later section.

Bacteriological examination

A loopful of milk was streaked on blood agar plates. The plates were incubated aerobically and anaerobically at 37°C for 24 hours. The isolated colonies were identified on the basis of morphological, cultural and biochemical characteristics.

Somatic cell count

Somatic cell count in the milk was carried out microscopically as described by Schalm et al. (1971).

Chemical determinations

Lactose concentration in the milk was estimated as described by Sharma and Misra (1966). Chloride contents were determined following Brumbly (1962).

Treatment schedule

MWT positive animals were randomly divided into five groups (1 through 4). Group 1 consisted of 31 buffaloes and 25 cattle and was given orally 20 grams of Rovimix E (Vitamin E 50 SD, kindly gifted by Messers F. Hoffmann La Roche and Co. Switzerland) for five days. Group 2 consisted of 25 buffaloes and 25 cattle and was treated with 40 ml of levamisole subcutaneously (Jansen Pharmaceutical, Belgium).

Group 3 consisted of five buffaloes and 16 cattle given orally 80 grams of Lasoni Herbal powder (Almuslim Herbal Research Corporation,

Faisalabad). Group 4 with eight cattle and seven buffaloes served as untreated control.

Efficacy of the treatment was judged by MWT seven days post treatment.

Results

Subclinical mastitis was detected in 8.2 percent buffalo and 24.0 percent cow quarters by MWT (table 1). Prevalence of subclinical mastitis was lower in buffaloes maintained on organized dairy farms. Lactose and chloride contents and somatic cell count seem to correlate with degree of infection in both cattle and buffaloes (table 2). As the MWT became strongly positive, lactose contents of the milk decreased, chloride contents increased and somatic cell counts also increased significantly. Pattern of change in lactose and chloride contents and somatic cell count was similar in cattle and buffaloes. Bacteria of genera *Staphylococcus* (135 isolates), *Micrococcus* (67 isolates), *Bacillus* (48 isolates), *Streptococcus* (45 isolates), *Enterobacteriaceae* family (44 isolates), *Corynebacterium* (29 isolates) and yeast spp. (4 isolates) were isolated from samples obtained from animals with subclinical mastitis. The type and frequency of organism was similar in cattle and buffaloes. Severity of infection as indicated by strong MWT reactivity could not be correlated with type and number of isolated organisms. A few isolated colonies of bacteria were also cultured from some milk samples with MWT negative status.

Vitamin-E was the most effective immunopotentiating agent for the treatment of subclinical mastitis (table 3). Vitamin E and Levamisole cured 64.5 and 60.0 percent cases of subclinical mastitis in buffaloes but only 32.0 and 24.0

TABLE 1. PREVALENCE OF SUBCLINICAL MASTITIS IN CATTLE AND BUFFALOES

Source	Number of udder quarters					
	Tested	Cattle		Tested	Buffaloes	
		MWT positive No.	% age		MWT positive No.	% age
Animal sciences institute farm	1240	291	23.5	960	78	8.1
Military dairy farm	—	—	—	928	62	6.7
Villages	68	23	33.8	84	21	25.0
Total	1308	314	24.0	1972	161	8.2

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TABLE 2. MEAN±S.D. OF LACTOSE AND CHLORIDE CONTENTS AND SOMATIC CELL COUNTS OF MILK SAMPLES FROM CATTLE AND BUFFALOES WITH DIFFERENT MWT REACTIVITY

MWT reaction	No. of samples	Cattle			Buffaloes			Somatic cell count
		Lactose	Chloride	Somatic cell count	No. of samples	Lactose	Chloride	
-	97	4.8 ±1.14	0.19±0.04	3.3±2.66	77	5.01±1.47	0.20±0.04	1.19±2.09
+	46	3.74±1.10	0.21±0.06	27.0±3.38	40	3.48±1.37	0.25±0.05	11.30±1.72
++	38	3.14±1.19	0.25±0.06	28.6±3.40	50	3.02±0.94	0.24±0.07	20.20±2.66
+++	23	2.31±0.82	0.30±0.06	65.4±3.15	8	2.36±1.02	0.31±0.13	187.00±3.19

TABLE 3. EFFECT OF IMMUNOPOTENTIATING AGENTS ON THE SUB CLINICAL MASTITIS IN CATTLE AND BUFFALOES

Treatment	Cattle			Buffaloes		
	Treated	Cured	% Recovery	Treated	Cured	% Recovery
Vitamin E	25	8	32.0	31	20	64.5
Levamisole	25	6	24.0	25	15	60.0
Lasoni powder	16	2	12.5	5	0	0.0
Untreated control	8	0	0.0	7	0	0.0

percent cases in cattle. Lasoni powder (a local herbal powder) was not effective in the treatment of subclinical mastitis. Cure rate was not affected by the degree of MWT reactivity and the type of organisms involved. Untreated control cases did not show any spontaneous improvement.

Discussion

Prevalence of subclinical mastitis was found to be higher in cattle than buffaloes. This could be attributed to the inclusion of large number of cow samples milked by milking machine which can significantly increase the rate of subclinical mastitis if not properly used. Furthermore, there might be species differences in the teat and udder anatomy of these two species. Similar findings have also been reported by (Rehman et al., 1984 and Rasool et al., 1985). Prevalence of subclinical mastitis was significantly less in buffaloes maintained at organized farm than those kept by the villagers. Poor hygienic and husbandry conditions under which buffaloes are kept in the villages are expected to increase the subclinical mastitis.

Severity of infection could not be correlated with any specific organisms. Most of the isolated organisms were common contaminants and specific mastitis pathogen like *St. agalactiae* was not isolated. This suggested the mastitis was basically a hygienic problem and transmission was from environment to animal rather than from animal to animal.

The pattern of change in lactose and chloride contents of the milk and somatic cell counts was similar in cattle and buffaloes. It suggests that the disease process is probably similar in cattle and buffaloes. MWT seems to be working well in buffaloes. Significant differences were seen in lactose and chloride contents and somatic cells count in MWT negative and positive samples. Somatic cell count in positive samples was at least five to ten times higher in MWT positive samples as compared to negative samples. Furthermore, as MWT severity increased, number of somatic cell count also increased proportionally.

Immunopotentiating agents i.e. vitamin E and levamisole gave a better response in the treatment of subclinical mastitis cases in buffaloes than cattle. Our previous study has also shown that

effect of vitamin E supplementation was better in buffaloes than cattle calves (Afzal et al., 1988). Levamisole increases proportion of B lymphocytes in milk and thus may enhance the activity of bovine mammary immune system (Ishikawa, and Shimizu, 1983). Levamisole has previously also been reported to be useful in the treatment of subclinical mastitis (Ishikawa and Shimizu, 1982; Shimada et al., 1983). Vitamin E has been reported to increase resistance against bacteria by increasing the antibody levels and T and B cell cooperation and macrophage function (Tauaka et al., 1979; Tengerdy et al., 1980; Tengerdy et al., 1984). Although effect of vitamin E on the treatment of subclinical mastitis has not previously been reported, vitamin E supplementation has been observed to reduce incidence of clinical mastitis by 37% and also reduce duration of clinical symptoms (Smith et al., 1984).

Although the cost of treatment with Vitamin E and levamisole is generally similar to the one incurred on antibiotic treatment but no withdrawal period is needed as required for antibiotic therapy.

Literature Cited

- Afzal, M., M. Hussain, K. N. M. Khan and R. Muner. 1988. Effect of vitamin E and selenium on immunity in newborn Jersey and buffalo calves. *AJAS*, 1:13-19.
- Brumblay, R. U. 1962. *Quantative Analysis*. Barnes and Noble, Inc. New York.
- Buddle, B. M. and H. D. Pulford. 1985. Evaluation of levamisole for use in control of bovine *Staphylococcus aureus* mastitis. *N. Z. Vet. J.* 33:177-180.
- Hutchinson, L. J. and C. Y. Erbel. 1987. Residue avoidance in food-producing animals. In (D. E. Johnston, Ed.) *Bristol Veterinary Handbook of Antimicrobial Therapy*. 2nd Edition Veterinary Learning System Co., Inc. Publishers, U. S. A. pp. 17-22.
- Ishikawa, H. and T. Shimizu. 1982. Protein composition of whey from subclinical mastitis and effect of treatment with levamisole. *J. Dairy Sci.* 65:653-658.
- Ishikawa, H. and T. Shimizu. 1983. Depression of B lymphocytes by mastitis and treatment with levamisole. *J. Dairy Sci.* 66:556-561.
- Mohammed, A. H., M. Abdel-Ghani, A. A. Mohammed and M. I. Dessouky. 1980. A study on somatic cells and some milk enzymes in subclinical mastitis in buffaloes and cows. *Egyptian J. Dairy Sci.* 8: 177-188.
- Rahman, H., D. S. Sambyal and K. K. Baxi. 1984. Incidence and etiology of subclinical mastitis in cows and buffaloes in Punjab (India). *J. Res. Punjab Agric. Univ.* 20:208-212.
- Rasool, G., M. A. Jabbar, S. E. Kazmi and A. Ahmad. 1985. Incidence of subclinical mastitis in Nili-Ravi buffaloes and Sahiwal cows. *Pak. Vet. J.* 5:76-78.
- Schlam, O. W., F. J. Carroll and N. C. Jain. 1971. *Bovine mastitis*. Lea and Febiger, Philadelphia.
- Sharma, R. S. and D. S. Misra. 1966. Milk lactose and its importance in diagnosis of mastitis. *Indian Vet. J.* 43:154-159.
- Shimada, Y. 1983. Efficiency of levamisole on bovine subclinical mastitis. *Jour. Japan Vet. Med Assoc.* 36:382-387.
- Smith, K. L., J. H. Harrison, D. D. Hancock, D. A. Todhunter and H. R. Conard. 1984. Effect of Vitamin E and Selenium supplementation on incidence of clinical Mastitis and duration of clinical symptoms. *J. Dairy Sci.* 67:1293-1300.
- Tanaka, T., H. Fujiwara and M. Torisir. 1979. Vitamin E and immunity. I. Enhancement of helper T cell activity. *Immunology*, 38:727-730.
- Tengerdy, R. P. 1980. Effect of vitamin E on immune response. In L. J. Machlin (Ed.) *Vitamin E: A Comprehensive Treatise*. Marcel Dekker, Inc., New York. pp. 429-444.
- Tengerdy, R. P., M. M. Mathias, and C. F. Nockels. 1984. Effect of vitamin E on immunity and disease resistance. In Prasad (Ed.) *Vitamins, Nutrition and Cancer*. Karger, Basel. pp. 123-133.
- Ziv, G., M. Storper and A. Saran. 1981. The effect of levamisole therapy during the dry period on clinical and subclinical bovine mastitis. *Refuah Vet.* 38:108-113.