

IMPACT OF POST MILKING TEAT DIPPING AND *Staphylococcus aureus* VACCINATION ON SOMATIC CELL COUNT AND SERUM ANTIBODY TITRE IN SAHIWAL COWS

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The study was carried out at the Livestock Experimental Station, Department of Livestock Management, University of Agriculture, Faisalabad to investigate the effect of Iodophore post milking teat dip (Germ IOD™ Cenavisa S.A. Laboratories, Fair International Trading Co., Karachi, Pakistan) and Dextran sulphate + aluminium hydroxide (DXS+AL (OH)₃) adjuvanted *Staphylococcus aureus* mastitis vaccine on somatic cell count (SCC) and serum antibody titre during a study period of 12 weeks. Twenty Sahiwal cows apparently free of mastitis were selected and put on 4 treatments such as: A = control; B = teat dipping only; C = Vaccination only; D = teat dipping + vaccination. Post milking teat dipping was done for a contact time of 30 seconds/teat. Mastitis vaccine prepared by the Department of Clinical Medicine and Surgery was administered IM @ 5ml /cow at neck region twice in 4 weeks interval. Milk and blood samples were collected on the day of primary vaccination (day 0) and then on monthly basis. These samples were collected aseptically and transported to Mastitis Research Lab, Dept. of Clinical Medicine & Surgery University of Agriculture, Faisalabad, Pakistan for the determination of SCC and serum antibody titres using Indirect Haemagglutination Inhibition Test. Maximum reduction in SCC was found due to teat dipping plus vaccination (62.71%; Treatment D), followed by dipping only (53.49%; Treatment B) and vaccination only (52.94%; Treatment C). However, a significant increase of 59.91% in SCC was found in control cows (Treatment A) at the end of trial. Serum antibody titre (Geometric Mean Titre) increased due to teat dipping /vaccination but a gradual decline was found in control cows. The increase in GMT was more prominent in D group and maintained up to the end of the trial.

Keywords: Mastitis, teat dipping, *Staphylococcus aureus* vaccine, somatic cell counts, serum antibody titre

INTRODUCTION

Mastitis, an important disease of dairy animals influences the quality and quantity of milk and culling of animals at an unacceptable age. The penetration of pathogenic microorganisms in the teat canal irritate and invade the delicate mammary tissue causing an inflammatory response and consequent changes occur in the milk. Degree of changes depends on the infecting agent and the inflammatory response. Mastitis, the inflammation of the udder regardless of the cause, is the most costly disease of the dairy cattle, which results in severe economic losses from reduced milk production, treatment cost, increased labor, milk withheld following treatment and premature culling (Miller *et al.*, 1983). Among infectious agents, bacterial pathogens are the major threat to mammary gland. These microorganisms are often contagious, are widely distributed in the environment of dairy animals and thus increase prevalence rate of Intramammary infections (IMI). Field surveys of major livestock diseases in Pakistan have ranked mastitis as number one disease of the dairy animals (Candy *et al.*, 1983). Every dairy buffalo/cow becomes the victim of mastitis before she dies Muhammad (2008). Mastitis affects the

milk quality and quantity and may spread to other cows in the herd. Mastitis also changes the composition of the milk and the extent to which various compositional changes occur depends on the inflammatory response (Kitchen, 1981). The degree of changes depends on the pathogenicity of the mastitis causing bacteria and amount of affected glandular tissue of the udder, especially the epithelial area. The main changes in the udder include; leaking of ions, protein and enzymes from the blood into the milk due to an increased permeability, invasions of phagocytising cells into the milk compartment, and a decrease of synthetic capacity of the gland, resulting in decreased concentration of certain milk constituents (Korhonen & Kaartinen, 1995).

Bacterial antigens elicit a specific cell mediated immunity response in the mammary gland of the bovines, resulting in the production of lymphokines and an increase in neutrophils in the gland upon subsequent exposure of the gland to the homologous antigen. This influx of neutrophils could have a significant impact on the resistance of the mammary gland to invading pathogens (Guidry, 1985). Somatic cell count has been reported to be an index of mammary health for detection of sub-clinical mastitis in

cows and buffaloes (Singh and Ludri, 2001). These cells are secreted during the normal course of lactation and influenced by a variety of factors like season, management, stage of lactation, parity, vaccination and teat dipping (Grosse-Westhues, 1975; Singh and Ludri 2000).

In order to evaluate the vaccine, estimation of serum antibody titre for certain duration is mandatory to establish its effectiveness for that period. Worldwide, antibody titration is being conducted through antigen-specific Enzyme Linked Immunosorbant Assay (ELISA) (Giraud *et al.*, 1997). In a country like Pakistan, where facilities to conduct such tests are lacking, and other methods yet need to be evaluated and standardized. Currently, tests like Direct Bacterial Agglutination, Haemagglutination, Haemagglutination Inhibition and Indirect Haemagglutination are being used to fulfill the requirement. In the present study, the method of indirect Haemagglutination (IHA) was standardized with certain modifications to assess antibody titres. In Pakistan, very limited work has been conducted on the evaluation of iodophor and mastitis vaccine. The present study was therefore carried out to see the impact of post milking teat dipping and *staphylococcus aureus* mastitis vaccine on the milk somatic cell count and serum antibody titre in lactating Sahiwal cows.

MATERIAL AND METHODS

Selection of animals

The study was conducted at the Livestock Experiment Station (LES), Department of Livestock Management, University of Agriculture, Faisalabad, on 20 lactating Sahiwal cows apparently free of mastitis. All animals were hand milked and no mastitis control program was in place at this farm. Animals with one or more blind, non functional quarters were not included. Similarly, animals which have had an episode of mastitis from calving to the start of trial were excluded. The cows of the same parity and stage of lactation were divided randomly into following four groups, each comprising five cows, C=Control; D=Postmilking teat dipping only; V=*Staphylococcus aureus* vaccination only; DV=Teat dipping and *Staphylococcus aureus* vaccination. An Iodophor (Germ IOD, Cenavisa S.A. Laboratories, Fair International Trading Co., Karachi, Pakistan) was used as a teat dip. Teat dipping was done after each milking for a study period of three months. The dip solution was prepared @ 150ml/L of water immediately before use, providing 0.27% available iodine. Each teat was dipped separately in a dip cup, especially made for this purpose, for a contact time of 30 seconds (Nickerson, 1994). *Staphylococcus aureus* mastitis vaccine (DXS+ Al (OH₃) adjuvant) prepared by the Department of

Clinical Medicine and Surgery (CMS Deptt.), was administered intramuscularly @ 5ml/animal in the neck region twice at four weeks interval and data were recorded at day 0 (Pre-trial) and then on monthly basis up to 90 days.

Collection of milk and blood samples

Milk samples were collected from all 20 cows following the procedure described by (NMC, 1990). Sterile vials of 15 ml capacity were used. Each teat end was scrubbed vigorously with a separate pledget of cotton moistened with 70 % ethyl alcohol. While holding the vials as horizontal as possible, the cap was removed without touching the inner surface and held with the inner surface facing downwards. After discarding the first few streams, about 5 ml milk was collected aseptically. Immediately after collection, all samples were placed on crushed ice and brought to the Mastitis Research Lab., (CMS Deptt.) where somatic cell count was performed with a little modification of the original method of Schalm *et al.* (1971). Blood samples were collected from all 20 cows at day 0 and then with an interval of 30 days up to the end of the trial following the procedure described by NMC, 1990. These samples were collected aseptically and shifted to the above said Mastitis Research Lab., where serum antibody titres were determined by using indirect haemagglutination test (IHI) according to procedure described by Zia (1989). Data thus collected was analyzed in terms of percent change and presented in tabulated form.

RESULTS AND DISCUSSION

Somatic cell count (SCC)

The somatic cell count decreased due to teat dipping/vaccination, whereas an increase was found in control group. Maximum reduction in SCC was found following teat dipping plus vaccination (62.71%) followed by dipping only (53.49%) and vaccination only (52.94 %). However, an increase of 59.91 % in SCC was found in control cows at the end of trial (Table 1). Present finding are in line with those of Calzolari *et al.* (1997), Leitner *et al.* (2003), Laglois and Pyles (1975) who reported significant decrease in SCC due to teat dipping. Tenhagen *et al.* (2001) also reported a decrease in SCC in vaccinated animals.

In control cows, the antibody titre (Geometric mean titre; GMT) decreased from 6.1 to 4.0 (34.42 %) at the end of the study. The GMT increased in the serum of cows following teat dipping. The highest titre against *staphylococcus aureus* was recorded at day 30 and day 90 with GMT value of 6.1 whereas this value was 2 at day 60 and 5.3 pre-trial. The percent increase in serum antibody titre was 15.09 (5.3 to 6.1).

Table 1. Effect of teat dipping/*Staphylococcus aureus* vaccination on mean somatic cell Count (x100000) at different sampling days

Groups	Sampling days				% change
	0	30	30	90	
C	2.47	2.90	3.0	3.95	59.91**
D	2.86	2.63	2.54	1.33	53.49*
V	2.89	2.77	2.61	1.36	52.94*
DV	2.95	2.24	1.98	1.10	62.71*

C = Control

V = *Staphylococcus aureus* vaccination

D = Teat dipping only

DV = Teat dipping plus *Staphylococcus aureus* vaccination

* = % decrease

** = % increase

Table 2. Geometric mean antibody titres against *Staphylococcus aureus* at each sampling interval in Sahiwal cows

ps	Sampling days				% change
	0	30	30	90	
C	6.1	3.5	2.0	4.0	34.42*
D	5.3	6.1	2	6.1	15.09**
V	6.1	10.4	42.2	30.00	391.80**
DV	4.6	21.5	48.5	48	943.47**

C = Control

V = *Staphylococcus aureus* vaccination

D = Teat dipping only

DV = Teat dipping plus *Staphylococcus aureus* vaccination

* = % decrease

** = % increase

Serum IHA antibody titre against *staphylococcus aureus* was the highest at day 60 (42.2) followed by a steady decline at day 90(30.0) in vaccinated cows. The titre started to increase after first vaccine dose but an abrupt increase was recorded following booster dose. The percent increase in IHA titre in the serum of vaccinated cows was 391.80. The increase in titre was more evident in cows of DV group and after first vaccine dose; the GMT value increased from 4.6 to 21.10(378 %) and after booster increased from 21.10 to 48.50 (127 %). The increase was maintained up to the end of trial. The findings of the present study are in line with those of Watson and Lee (1978), Leitner *et al.* (2003). Higher IHA antibody titres in the vaccinated cows may be attributed to the incorporation of two adjuvants (DXS and aluminium hydroxide). DXS has a specific affinity for triggering the IgG2, response whereas aluminium hydroxide specially enhances the IgG1 antibody response. The highest titre in DV group may be attributed to active immune system and synergistic effect of teat dipping/ vaccination. Teat dipping reduced the entrance of new infection and vaccine developed the antibodies. This was not true in case of D and V groups because only a single practice was adopted at a time.

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