

EFFECT OF DIFFERENT *STAPHYLOCOCCUS AUREUS* MASTITIS VACCINES ON THE MILK YIELD, FAT, PROTEIN AND SOMATIC CELL COUNT IN BUFFALOES

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ABSTRACT

Four *Staph. aureus* mastitis vaccines were evaluated in five different groups of non-mastitic healthy pregnant buffaloes. These vaccines [live attenuated (Group B1), simple bacterin (Group B2), dextran sulphate adjuvanted (Group B3) and oil adjuvanted (Group B4)] were administered to 20 non-mastitic healthy pregnant buffaloes, while the group 5 was kept as unvaccinated control. Each vaccine was administered twice @ 5 ml IM at 60 and 30 days pre-partum. The effect of these vaccines on milk yield, fat, protein, and somatic cell count was studied till 4th month post partum. There was a significant difference in the milk yield, fat and protein percentage between the vaccinated and non-vaccinated groups, while difference of these parameters among the vaccinated groups of buffaloes was non significant. All the vaccines reduced the somatic cell count significantly as compared to control group. Linear somatic cell count scouring method indicated the highest milk loss/lactation in the control group (1086 litres), followed by bacterin (703 litres), dextran sulphate (549 litres), live attenuated (526 litres) and oil based (577 litres) vaccines.

Key words: *Staph. aureus* vaccine, mastitis, milk quality, quantity and somatic cell count.

INTRODUCTION

Bovine mastitis is one of the most problematic disease and continues to have major economic impact on the dairy industry throughout the world (Dodd, 1983). Numerous agents can cause mastitis in dairy cows but *Staph. aureus* is the most common etiological agent of bovine mastitis (Bramely and Dodd, 1984; Schukken *et al.*, 1993). Although various management practices for decreasing the prevalence of *Staph. aureus* have been adopted under modern dairying but many dairies still have some level of infection with *Staph. aureus* (Leslie and Dechukken, 1993; Schukken *et al.*, 1993). Similar scenario is prevailing in Pakistan (Bachaya *et al.*, 2005). To cope with this problem, vaccination to prevent mastitis has been the subject of concern for many researchers (Foster, 1991). These vaccines have been employed as live (Watson, 1984) or killed *Staph. aureus* (Brock *et al.*, 1975), isolated capsular materials (Guidry *et al.*, 1994), toxoids (Adlam *et al.*, 1977) or combined preparations of killed cells and toxoids (Nickerson *et al.*, 1993; Nordhang *et al.*, 1994a; Nordhaug *et al.*, 1994b). These vaccines have been reported to increase specific antibody of *Staph. aureus* antigens in serum and improve the milk production (Opdebeek and Norcross, 1983; Nordhaug *et al.*, 1994a; Watson *et al.*, 1996) and quality by lowering the milk somatic cell count.

Most of the studies conducted to control the mastitis have been done under modern dairying but unfortunately no such system exists in Pakistan.

Therefore, the present study was designed to see the effect of *Staph. aureus* vaccines on the milk quality and quantity in terms of milk yield, fat, protein, and somatic cell count in dairy buffaloes.

MATERIALS AND METHODS

A typical alpha-beta haemolytic *Staph. aureus* isolated from the mastitic buffalo was used to prepare 4 different types of vaccines. This selected typical alpha-beta isolate of *Staph. aureus* showed 7 digit API-*Staph* biochemical profiles 6336153 on biotyping with *Staph*-track system.

Preparation of vaccines

For incorporation of crude extract, supernatant fluid was collected from 48-hour broth culture of *Staph. aureus*. The supernatant was separately autoclaved at 121°C for 20 minutes. This preparation was then centrifuged at 6000 x g for 30 minutes at 4°C and the supernatant was added to the vaccine preparation at a concentration of approximately 5 mg of dry weight per five ml dose (Giraud *et al.*, 1997). Finally, different *Staph. aureus* vaccines were prepared as under:

To prepare the plain *Staph. aureus* vaccine (PSAV), formaline inactivated *Staph. aureus* was adjusted to 10¹⁰ cells/ml spectrophotometrically. Sodium azide was added as a preservative at a final concentration of 0.001% (w/v) and stored at 4°C for future use (Watson and Davies, 1993). To prepare the dextran sulphate-adjuvanted *Staph. aureus* vaccine

(DSAV), dextran sulphate (DXS, Sigma-Aldrich Co., USA) was added as an adjuvant in the plain vaccine at a final concentration of 50 mg dextran sulphate per ml for 5 ml dose (Watson and Davies, 1993).

To prepare the oil-adjuvanted *Staph. aureus* vaccine (OSAV), liquid paraffin was used as an adjuvant, whereas Tween-80 and Span-80 were used as emulsifiers. Emulsification of oil and Span-80 was done at low speed (12000 rpm) with the help of ultra homogenizer. Antigen suspension was slowly added and continuously stirred oil phase at 18000 rpm in the ultrahomogenizer at 4°C. Final oil emulsion vaccine was dispersed in sterilized glass vials with automatic cap seal system and stored at 4°C for future use (Shaukat *et al.*, 1998).

For the preparation of live attenuated *Staph. aureus* vaccine (LSAV), an α - β haemolytic selected isolate of *Staph. aureus* was repeatedly passaged through culture on 5% sheep blood agar for 18 passages until it lost its haemolytic activity and then maintained in trypticase soy broth. This live attenuated isolate was grown for 24 hours in nutrient broth (NB). Organisms were deposited by centrifugation (3000 \times g; 15 min.), washed ($\times 2$) with phosphate buffered saline (PBS; pH 7.2) and resuspended in sterile PBS. The concentration of bacteria was finally adjusted to 10^{10} cells/ml using spectrophotometric method (Watson and Lee, 1978).

Evaluation of vaccines

A total of 25 pregnant non-mastitic buffaloes in their last trimester were randomly assigned to 5 groups. Animals of each group were respectively vaccinated with live attenuated *Staph. aureus* vaccine (LSAV), plain bacterin *Staph. aureus* vaccine (PSAV), dextran sulphate-adjuvanted *Staph. aureus* vaccine (DSAV) and oil adjuvanted *Staph. aureus* vaccine (OSAV), whereas buffaloes in group B5 were kept as unvaccinated control (UC). Five ml of the respective vaccine was administered IM 8 and 4 weeks prepartum.

Evaluation criteria were milk yield, butter fat and protein concentrations and somatic cell count, determined at monthly intervals starting from calving to four months post partum. In addition, linear somatic cell count scoring method was applied to calculate the milk loss/lactation of all groups keeping in view the somatic cell count during four months post partum study.

Gerber's fat test was followed for determination of fat percentage in milk (Aggrawala and Sharma, 1961). Milk protein was determined by formal titration method (Davide, 1977). Modified somatic cell count technique was used to determine the effect on milk quality in terms of somatic cell count (Schalm *et al.*, 1971). A 10 μ l of fresh milk was spread over a glass slide having a marked area of 10 mm \times 10 mm using a micropipette. The fine milk smear so prepared was dried in an oven at

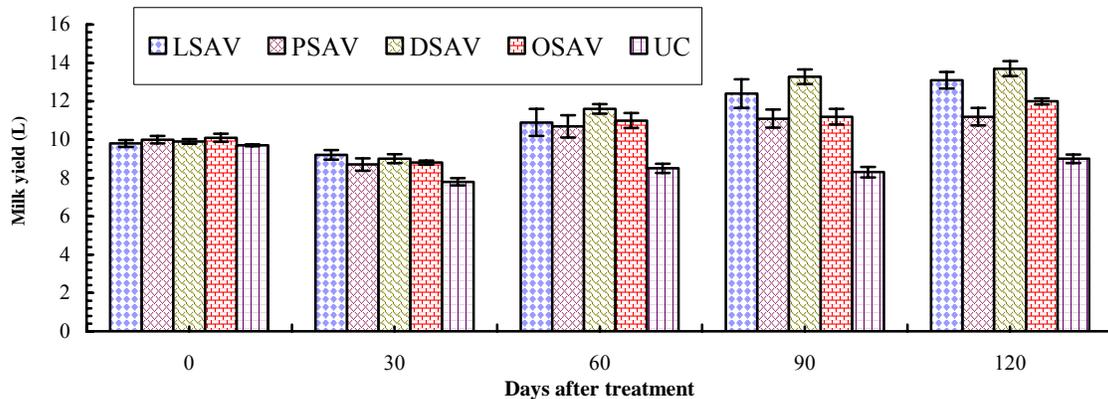
30-40°C. The slides were then dipped in xylene for 1 to 2 minutes to remove the fat globules and dried subsequently. The slides were then stained using Newman-Lampert's stain for 15 minutes and dried at room temperature. The excess of stain was removed from the smears with tap water and the slides were again dried at room temperature. These poorly stained smears were further stained with blue (basis) aliquot of Dip-Quik stain (J-332-A3, blue portion, Jorgensen Labs. Inc. Loveland, Colorado, 80538, USA) for 10-15 seconds, followed by tap water rinsing and drying. This significantly enhanced the differentiation among cells and the substrate. The somatic cell counts were measured under microscope with a magnification of 15 \times 40 in 50 fields and were multiplied by the microscopic factor to get the cells per ml of milk. Linear somatic cell counts securing method was applied on the data of somatic cell count to assess the milk loss (Shook, 1993). The data thus generated were subjected to statistical analysis using SAS-2000 computer programme.

RESULTS

The effect of vaccination on milk yield of buffaloes vaccinated with 4 different *Staph. aureus* vaccines is presented graphically in Fig. 1. A decrease in the milk yield vis-à-vis at parturition was recorded in all groups, which differed non-significantly ($P < 0.05$) till day 30 postpartum (PP). The difference was significant ($P < 0.05$) between vaccinated and control animals at day 60 and day 120 PP. When compared on the basis of overall milk yield for 4 months, a non-significant difference was found among vaccinated groups, which differed significantly ($P < 0.05$) with the control group.

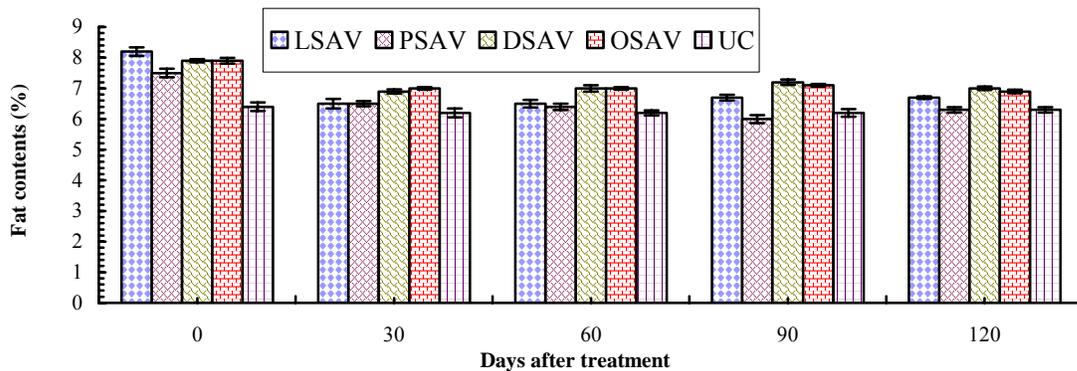
Milk fat contents were the highest (range 6.48–8.18%) at parturition, followed by a decrease (range 6.21–7.18%) for the remaining study period. The differences among various groups at different sampling days were non-significant. When compared on the basis of 120 days study period, the groups B1 and B2 did not differ significantly. The mean fat percentage also remained significantly higher ($P < 0.05$) in groups B1 and B2 as compared to that of control group (Fig. 2).

Milk protein contents were the highest in the colostrum (range 15.26–17.14%), followed by a sharp decrease (range 3.44–4.30%) and then remained plateau throughout the remaining part of the study period. When compared for 4 months, mean protein concentration of group B3 was significantly ($P < 0.05$) higher as compared to those of groups B1 and B2, the latter two groups differed non-significantly from each other. The difference in protein concentration was highly significant ($P < 0.01$) when compared to that of group B4 and B5, which differed non-significantly from each other. The mean protein concentration was also statistically higher in groups B1 and B2 as compared to those of groups B4 and B5 (Fig. 3).



LSAV = Live-attenuated *Staph. aureus* vaccine group, PSAV = Plain bacterin *Staph. aureus* vaccine group, DSAV = Dextran sulphate-adjuvanted *Staph. aureus* vaccine group, PSAV = Oil-adjuvanted *Staph. aureus* vaccine group, UC = Unvaccinated control group.

Fig. 1: Comparative milk yield (liter/hour) of 4 groups of buffaloes vaccinated twice during the dry period with four different *Staph. aureus* vaccines.



LSAV = Live-attenuated *Staph. aureus* vaccine group, PSAV = Plain bacterin *Staph. aureus* vaccine group, DSAV = Dextran sulphate-adjuvanted *Staph. aureus* vaccine group, PSAV = Oil-adjuvanted *Staph. aureus* vaccine group, UC = Unvaccinated control group.

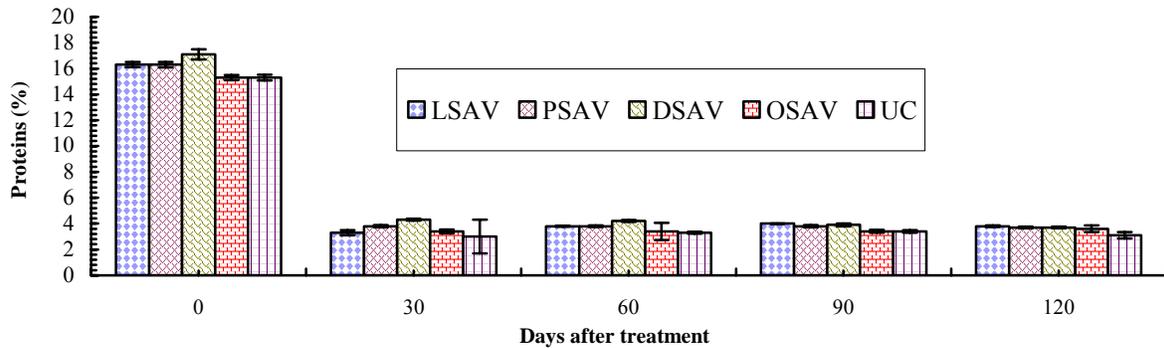
Fig. 2: Comparative fat (%) recorded at monthly intervals among different groups of buffaloes vaccinated twice during dry period with four different *Staph. aureus* vaccines.

Highest values of somatic cell count (SCC) were recorded at parturition in all groups (7.55×10^5 to 7.94×10^5 /ml). A sharp decrease in SCC was registered at day 30 PP in all groups (Table 1). At this sampling time point, SCC was the highest ($4.365 \pm 0.592 \times 10^5$ /ml) in placebo control animals (group B5), followed by B2, B1, B3 and B4 in the given order. The difference among the four vaccinal groups was non-significant. At day 60 PP, a further decrease in SCC was registered in all vaccinal groups.

On the other hand, in placebo control group B5, at this sampling point the count almost quadrupled to that recorded at previous sampling point (day 30 PP). The difference among the vaccinal groups was non-significant. From day 60 PP onward till the end of the

study (day 120), SCC remained almost steady in all vaccinal groups, which differed non-significantly from each other. From day 60 onward, differences in SCC between vaccinal groups and placebo control were highly significant ($P < 0.01$).

Linear somatic cell count scoring method showed that there was 526 liter/lactation mean loss of milk in group B1 vaccinated with live attenuated *Staph. aureus* vaccine, followed by 703 liters loss in plain bacterin *Staph. aureus* vaccine (B2). It was 549 liters in dextran sulphate adjuvanted *Staph. aureus* vaccinated group (B3), while it increased slightly to 577 liters in oil adjuvanted *Staph. aureus* vaccinated group (B4), followed by the highest loss of 1086 liters per lactation in unvaccinated control group.



LSAV = Live-attenuated *Staph. aureus* vaccine group, PSAV = Plain bacterin *Staph. aureus* vaccine group, DSAV = Dextran sulphate-adjuvanted *Staph. aureus* vaccine group, PSAV = Oil-adjuvanted *Staph. aureus* vaccine group, UC = Unvaccinated control group.

Fig. 3: Comparative proteins (%) recorded at monthly intervals among different groups of buffaloes vaccinated twice during dry period with four different *Staph. aureus* vaccines.

Table 1: Somatic cell count (mean \pm SD; $\times 10^5$ /ml) in quarter milk samples of 4 groups of buffaloes vaccinated twice during the dry period with 4 different *Staph. aureus* vaccines

Groups	Somatic cell count at different post-partum days				
	0	30	60	90	120
B1	7.77 \pm 0.81	2.53 \pm 0.80	1.53 \pm 0.99 ^a	1.48 \pm 0.85 ^a	1.19 \pm 0.75 ^a
B2	7.83 \pm 0.83	3.44 \pm 3.38	2.89 \pm 3.59 ^a	2.28 \pm 2.61 ^a	2.39 \pm 2.45 ^a
B3	7.70 \pm 0.80	2.43 \pm 0.88	1.57 \pm 0.91 ^a	1.54 \pm 0.91 ^a	1.4 \pm 0.89 ^a
B4	7.94 \pm 0.52	2.29 \pm 0.73	1.71 \pm 0.74 ^a	1.92 \pm 1.18 ^a	1.4 \pm 1.15 ^a
B5	7.55 \pm 0.71	4.36 \pm 0.59	16.91 \pm 1.42 ^b	11.61 \pm 1.01 ^b	11.32 \pm 1.13 ^b

Values in columns with different superscripts differ highly significantly ($P < 0.01$).

B1 = Live-attenuated *Staph. aureus* vaccine group,

B2 = *Staph. aureus* plain bacterin group,

B3 = Dextran sulphate-adjuvanted *Staph. aureus* bacterin group,

B4 = Oil-adjuvanted *Staph. aureus* bacterin group,

B5 = Unvaccinated (placebo) control group.

DISCUSSION

The mean milk yield for a period of 4 months was the highest in B3 (DSAV), followed by B1 (SLAV), B4 (OSAV), B2 (PSAV) and B5 (UC). While the difference among vaccinates was non-significant, it was significant when comparing the control group (B5) with all the vaccinate groups. Amorena *et al.* (1996) observed that in the five months following vaccination, vaccinated cows had a lower SCC (20.6% reduction) and higher milk production (6.1% increment) in relation to the control group, suggesting

that the economic benefit of the vaccine may be 13 times higher than the cost.

Many physiological and environmental factors influence the yield and composition of milk. Factors that can increase milk yield are increased body weight, advancing age, intensive nutrition, fall and winter calving and cool to moderate environmental temperatures. Factors that tend to decrease the milk yield are infection especially of udder (Uallah *et al.*, 2005), advanced lactation, advanced stage of gestation, short dry period, spring and summer calving, high environmental temperatures and humidity (Rosenthal, 1991).

Milk fat and protein contents in the vaccinated and control buffaloes (B5) differed significantly. There was a very high protein concentration among all groups in the colostrum. This is in line with the findings of many workers (Waite *et al.*, 1965; Qazi and Manus, 1966; Khan, 1967; Anwar, 1975), who analyzed colostrum of cows and buffaloes and found a very high protein concentration, followed by a decrease to a minimum and thereafter rise steadily until the end of lactation. It is worth mentioning that all these studies were conducted in normal animals. At the same time, fat percentage was high at the time of parturition in all the groups, followed by a slight decrease at the following monthly intervals. This is also in line with the findings of Anwar (1975). This increase may be ascribed to the normal physiological process that goes on at the time of parturition. Jenness (1985) described that cows and buffaloes colostrum contained more mineral salts (ash) and protein and less lactose than milk; fat content is often, but not always, higher than that of milk.

In the present study, mean SCC in B1, B3 and B4 groups was significantly lower than that of the groups B2 and B5. This rise of SCC in B2 and B5 groups may be ascribed to the presence of infection in some quarters of the buffaloes of these groups. This is in line with the findings of Sheldrake *et al.* (1983), who also found that a higher elevation in SCC is an indication of

inflammation in the udder. The major pathogens cause the greatest SCC increase and include *Staph. aureus*, *Strep. agalactiae*, coliforms and other streptococcus species other than *Strep. agalactiae*, whereas minor pathogens (*C. bovis* and coagulase negative staphylococci) usually cause only a moderate increase in SCC (Harmon and Langlois, 1986). Nickerson (1997) was also of this view that cell counts were higher during the first 2 weeks after calving due to the presence of colostrum and the stress associated with onset of lactation. The present finding is also in line with the results reported by Khan (1997) that SCC was high during the early stages of lactation and then decreased gradually, followed by slight rise at the end of lactation.

An increase in somatic cell count in milk leads to the release of lipolytic (lipases) and proteolytic (plasmin) enzymes which can degrade the triglycerides of milk fat and casein contents of the milk (Saeman *et al.*, 1988; Barbano, 1989). This leads to poor quality milk in the mastitis-affected animals.

In conclusion, it may be said that all *Staph. aureus* mastitis vaccines are helpful in improving the quality and quantity of milk in buffaloes.

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