Efficiency of Polyvalant Mastitis Vaccine in Lactating Dairy Cows

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ABSTRACT

The aim of the study was to investigate the efficacy of a polyvalent mastitis vaccine under field conditions. Dairy cows (n=218) that had not been previously received a mastitis vaccine were separated into two treatment groups; vaccine (n=111) and control (n=107) in four different dairy farms. Initially, individual Somatic Cell Count (SCC), California Mastitis Tests (CMT) scores and clinical mastitis (CM) cases were detected. Two doses of the vaccine, 4 weeks apart, were administered in vaccine group by intramuscular injection. Cows in control group were received physiological saline. After the treatments, animals were examined monthly for SCC, CMT and CM during six months. In addition, milk samples were also taken from udder quarters of the cows with CMT +3 scores and CM for bacteriological examination. SCC of vaccine group was lower ($P<0.056$) than the control at the end of the study. CM rates were not significant in the vaccine (26.1%) and control (18.7%) groups. *Staphylococcus aureus* (*S. aureus*) isolations from milk samples of udder quarters with CMT (+3) scores were not different in the vaccine (25.8%) and control (34.8%) groups. *Streptecoccus uberis* (*S. uberis*), *Coagulase Negative Staphylococcus* (CNS) and *Streptecoccus spp*. intamammary infection (IMI) isolation rates was not different between groups. Thus, polyvalent mastitis vaccine did not have enough protective effect against mastitis, nevertheless CM that occured in vaccine group seemed to have fewer inflamation degrees.

Key Words: Cow, mastitis, polyvalent vaccine

INTRODUCTION

Mastitis is one of the most serious and costly diseases affecting dairy cows production (Heringstad et al 2000). A large number of control measures have been developed and combined in mastitis control programs including hygienic cleaning procedures, disinfection, antibiotic therapies and culling (Oliver and Mitchell 1984). These practises have reduced occurrence of intramammary infection, but failed to prevent it. Recently, the enhancement of host defence mechanisms is an important measure to control new intramammary infections and influence the clinical outcome in cases where mastitis pathogens invade the udder and establish an infection. Vaccination is a tool to improve the spesific immunity against infectious agents. Vaccination against mastitis has been attempted to increase antibody titres in blood and milk to a specific bacterium, thereby promoting immunity by enhancing phagocytosis of bacterium and neutralizing its toxins (Colditz and Watson 1985).

Although there are several studies (Nickerson et al 1985; Pankey et al 1985; Hogan et al 1992; Nourhaug et al 1994; Finch et al 1997) on the mastitis vaccines consisting of only one bacterium strain, its toxin, virulens factor or the cell component we observed limited number of trials (Giraudo et al 1997; Calzolari et al 1997; Küçük and Alaçam 2003) on polyvalent (multivalent) mastitis vaccine containing more than one bacterium strains. The purpose of this study is to observe the clinical efficacy of polyvalant mastitis vaccine under field conditions.

MATERIAL AND METHODS

Herds

This study was performed in four different dairy farms located in Bursa region, Turkey, for a 7 months period from December to June. Animals in these farms had not been received vaccination against mastitis before. Lactating Holstein dairy cows (n=218) were divided into two groups, vaccinated (n=111) and control (n=107) in each herd. These groups were seperated equally according to lactation number, stage of lactation and milk production.

Mean milk production of the cows in these herds at the beginning of the study was 20 kg/d/cow. Mean individual SCC of dairy cows were 4.17×10^5 (farm 1), 7.11×10^5 (farm 2), 9.76×10^5 (farm 3), and 3.81×10^5 (farm 4) cells/ml. Post milking teat-dipping and antibiotic treatments for the dry cows were applied only in the first and the fourth farms. During the trial, none of the working conditions of the herds were modified.

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Vaccine and Placebo

The vaccine material of this study was an inactivated commercial mastitis vaccine (Hipramastivac®, Hipra Laboratorios S.A., Spain) containing *S. aureus* (TC5 ve TC8 strain)*, E. coli* (J5 strain)*, S. agalactiae, S. uberis, S. dysgalactia, S. pyogenes, P. aeruginosa,* and *A. pyogenes* bacterins. Physiological saline had been used in control cows for placebo.

Experimental Design

Cows in the vaccinated group were received two doses of the vaccine, 4 weeks apart, by intramuscular injection to the brachiocephalic muscle of the neck, regardless of their stage of lactation.

Each cow was examined for CM at the initial examination and in all monthly visits. CM was defined by the diagnosis of abnormal changes of udder (eg. pain, swelling, warmth) and milk (e.g. watery, clots, flakes or blood).

Milk samples from lactating dairy cows were evaluated for individual SCC and CMT. Routine monthly examinations of the herds upto 7 months were performed. SCC was detected by Direct Microscopic Cell Count Methods. Briefly, 0.01 ml of milk was spread on a 1 cm^2 (1x1 cm) area of a microscopic slide and left to air-dry. The slides were treated with the solution of 52 ml absolute alcohol, 44 ml xylol and 4 ml glacial asetic acid to fixation cells and remove milk fat for 7 min. The slides were then stained with Giemsa stain for 15 minutes and were counted by light microscope with immersion objective. The number of counted field's on each slide and working factor for detecting SCC in milk was determined by International Dairy Foundation (IDF 1981).

California Mastitis Test was carried out using the method described by National Mastitis Council Guidelines (NMC 1999). Milk samples of the cows with CMT (+3) score and CM were determined for bacteriological identification as diagnosed. Bacteria were cultured from milk samples and identified. Complete identification was carried out with the BBL CRYSTAL (Becton-Dickinson, Sparks, USA).

Examinations for the diagnosis of CM and evaluations of SCC and CMT were done without knowledge of the cow's treatment group. All animal handling and care procedure were approved by the Ethic Committee of Faculty of Veterinary Medicine, Uludag University.

Statical Analyses

The regression analysis was used to test the efficiency of vaccination on SCC. The model was developed to determine the effects of farm factors (housing, nutrition, milking procedure, etc.,) and the time factors (month) on the SCC at udder quarters. Farm factors were taken into account in comparison to the most efficient fourth farm. The estimated model is

$$log (SCC) = \beta_1 + \beta_2 VC + \beta_3 F1 + \beta_4 F2 + \beta_5 F3 + \beta_6 SM + \beta_7 TIME$$

where *VC* is a treatment-effect dummy varible taking the value of 1 when a cow is vaccinated and 0 when she is a control animal; *F1*, *F2* and *F3* are farm dummies representing Farm 1, Farm 2 and Farm 3, respectively, when an observation is from the first farm *F1*=1 and *F1*=0 otherwise and v.s., the forth farm was represented by the constant term. *TIME* was indicate following time periods after the vaccination, before the vaccination was *TIME* = 0, during the vaccination was *TIME*=1, following examinations were *TIME*=2, 3, 4, 5 and 6.

CM and bacteriological results was evaluated by chi-square test.

RESULTS

At the initial clinical examination in herds, 6 cases of CM were detected in herds and these cows were not included in the study. After the vaccination during the six month, 22.4% (49/218) of the cows had CM. Percentage of CM was 26.1 (29/111) in vaccinated and 18.7 (20/107) control groups in all herds after the treatments and this difference was not statistically important. CM in vaccinated groups was lower than control groups in second and third farms, but controversially CM was higher in vaccinated groups in the first and fourth farms (Figure 1). Due to the insufficient number of bacteriolojical samples of CM, they are not evaluated. Eighteen of the 49 CM cases (n=15 vaccinated, n=3 control) monitored during the study. In vaccinated group, none of the cows with CM were systemically affected. Only 5 of 15 vaccinated udder quarters had clinical findings such as swelling, warmth, redness, pain and their milk was waterly. Other vaccinated udder quarters with CM (10/15), the apperience of milk was normal but have clot in.
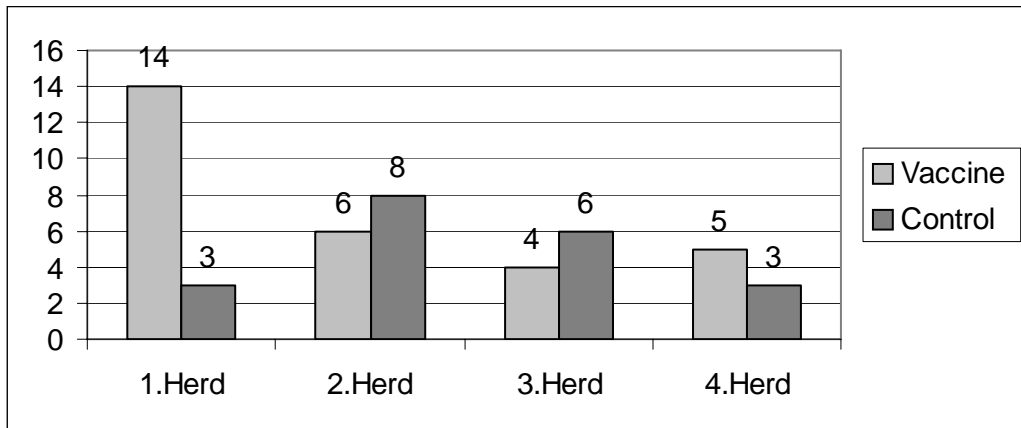
Figure 1. Clinical mastitis cases in the vaccinated and control groups in herds.

Figure 2 shows the geometric mean of the SCC in milk samples of both groups at 7 sampling points in the lactation. In the regression model, individual SCC was significantly ($P< 0.056$) lower in vaccinated than control group. After the treatments, SCC in two groups was decrease toward the end of the six month ($P< 0,000$). In addition, SCC in second and third farm was detected higher than ($P< 0,000$) other farms.
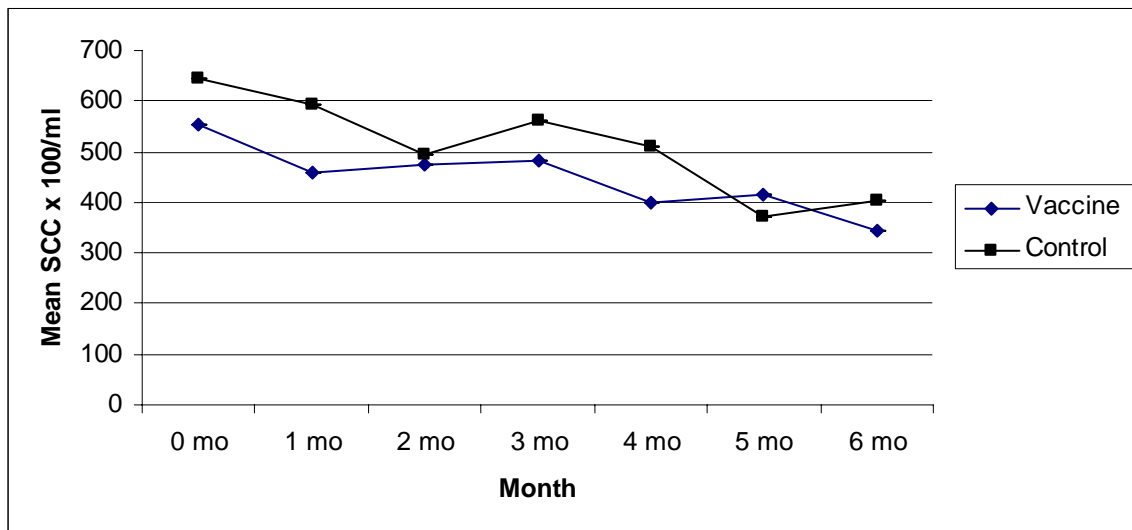
Figure 2. Individual SCC in the vaccinated and control groups.

Milk samples with CMT (+3) score were taken with the aim of bacteriological examination from 320 of 463 udder quarters which had not received intramammary antibiotic before sampling. Table-1 shows the bacteriological findings of the milk samples. The rates of *S. uberis* IMI were 10.2% in vaccinated and 9.0% in control groups at milk sampling. *Streptecoccus spp.* IMI was 10.8% in vaccinated group and 12.3% in the control groups. CNS IMI was detected from the samples of the vaccinated and the control groups were 15.5% and 10.9%, respectively. The bacterium (*S. agalactiae, S. dysgalactiae, S. pyogsenes, A. pyogenes* and *P. aeruginos*) that is covered by the vaccine had not been cultured in milk samples. IMI of *S. aureus* was not different between vaccinated (25.8%) and control (34.8%) groups ($P< 0.069$). IMI of *S. aureus* was significantly ($P< 0.05$) lower in vaccinated group (22.4%) than the control (groups) (32.4%) in pure culture, however, *S. aureus* growth in mixed culture was not included in statistical analysis. When herds were evaluated separately, *S. aureus* IMI was not different between groups. During the study, regardless of the treatments, the *S. aureus* infections were determined 33% and 59% in second and third farms at the milk samples. *S.aureus* IMI were detected, 13% in vaccinated and 21% in control group in second farm, 27% in

vaccinated and 32% in control group in third farm ($P< 0.08$). *S. aureus* IMI was numerically but not statistically decreased in vaccinated than control group in these farms.

Table 1. The bacterial isolates were obtained from individual quarter milk samples from cows whose California Mastitis Test score was 3

Herds	1. Herd		2. Herd		3. Herd		4. Herd		Total			
	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine	%	Control	%
S. aureus	2	0	15	24	25	29	6	1	48	25.8	54	34.8
E. coli	1	4	2	2	1	0	0	0	4	2.1	6	3.9
S. uberis	5	2	5	5	3	4	6	3	19	10.2	14	9.0
Staph. spp. (CNS)	14	3	8	8	3	4	4	2	29	15.5	17	10.9
*Strep. spp.***	8	5	10	12	1	1	1	1	20	10.8	19	12.3
Other***	10	5	7	6	1	2	5	3	23	12.3	16	10.3
No Growth	21	14	7	6	6	2	9	7	43	23.1	29	18.7
	61	33	54	63	50	42	31	17	186		155	
	94		117		92		48		341*			

* n (341) number was higher than n number (320) taken for bacteriological examination due to mixed cultres

***S. sangui, S. parasangui, S. epidermis*

*** *Serratia marcescens, Mikrococcus spp., Citrobacter fondi, Aerococcus spp.*

DISCUSSION

During the trial, CM was detected 26.1% of the vaccinated and 18.7 % of the control groups in all farms. High numbers of CM cases were detected between the first and second doses of vaccine in vaccinated group in first farm ($P<0.05$). The number of the cows with CM in the first farm was much higher than the other three farms in terms of incidence rate of CM (Figure 1). This condition which was not detected in other farms was considered to be coincidental. Only 18 of the 49 CM cases were observed during the trial. We have found that CM cases in the vaccinated group had less severity and duration of clinical sings. However, the difference in severity of CM between groups was not statistically evaluated, because of the insufficient case numbers. In several studies, it was shown that vaccination against mastitis caused to increase antibody titers in blood and milk whey, so enhanced opsonization of bacteria via antibody. The severity and duration of CM in vaccinated cows was better than the controls (Clark and Roekel 1994; Hogan et al 1992; Hogan et al 1999; Pankey et al 1985; Smith et al 1999).

In respect with previous studies (Calzolari et al 1997; Leitner et al 2003), the pozitive effects of vaccination on SCC were detected in this study ($P<0.056$). After the vaccination of heifers (Leitner et al. 2003), SCC was lower in vaccinated than control group for following two lactations period, 42.3% (first lactation) and 54% (second lactation). When udder health's of whole cows were examined, vaccinated cows were healthy than control cows. Researchers acclaimed that this condition might be herd prevalence of *S. aureus* and CNS IMI were very low during the study. However, Nordhaug et al. (1994) was shown that SCC was equally low in both groups during the lactation. Vaccination against *S. aureus* mastitis neither reduced nor decreased in mean SCC. The small reduction in the incidence of *S. aureus* mastitis did not influence the mean SCC in the vaccinated cows. In addition a numbers of authors have demonstrated that vaccination did not have any effect on SCC (Giraudo et al 1997; Hoedemaker et al 2001; Nickerson et al 2000; Tenhagen et al 2001; Watson et al 1996).

After the vaccination, SCC in both groups decreased toward the end of the study (P<0,0001). This reduction in SCC might be diminishing seasonal effect on udder quarters. In summer, it is easier to achieve milking hygiene than in winter because bedding material and the udder itself are drier. In addition, SCC in second and third farms was higher than (P<0,0001) other farms. This difference would be associated with farm management which appyling antibiotic treatments for dry cows and postmilking teat-dipping.

In this study, the vaccine containing staphylococcal and streptococcal antigens, (*S. pyogenes, S. agalactiae, S. dysgalactiae S. uberis)* did not protect the animals in vaccinated group against IMI of *Streptcoccocus spp.* and CNS. Similar findings had been reported in some field studies with both CNS and *Streptcoccocus spp.* IMI (Calzolari et al 1997; Giraudo et al 1997; Küçük and Alaçam 2003). CNS IMI was

higher in vaccinated group (15.5%) than control groups (10.9%) in this study. Giraudo et al. (1997) was used vaccine containing *S. aureus* and *Strepteoccus spp.* and found that CNS isolates in milk from quarters in vaccinated cows showed an increase over those from control cows. This increase might have resulted from the reduction *S. aureus* IMI in vaccinated cows, which could favor colonization by other microorganisms (CNS). In experimental studies, immunisation against *S. uberis* was providing effective protection against subsequent experimental challenge in the absence of an overt inflammatory reaction (Hill et al. 1994). This protective effect was confirmed by Finch et al (1997), at the same time. They demonstrated that this regimen was less effective against strains other than that administered as the immunizing antigen. These findings suggest that this variability of results from different studies may be due to bacterial strain variations.

In our study, we have found that the incidence of *S. aureus* IMI had not been different between vaccinated and control groups. Vaccination of cows did not cause to decrease udder quarter prevalence of *S. aureus* infections in comparison with controls. Similar to our findings, some researchers have demonstrated that vaccination was not effective on the control of *S. aureus* IMI (Hoedemaker et al 2001; Nourdhaug et al 1994; Tenhagen et al 2001). However several researchers acclaimed that vaccination against *S. aureus* was effective (Calzolari et al 1997; Giraudo et al 1997; Küçük and Alaçam 2003; Nickerson et al 1985; Watson et al 1996). Hoedemaker et al (2001) and Tenhagen et al (2001) have reported that vaccination containing autogenous *S. aureus* bacterins did not have desired effect on the prevalence of *S. aureus* IMI in heifers. Nourdhaug et al (1994) have demonstrated that the vaccination of *S. aureus* bacterins and toxoid did not have a protective effect when all cows were used as the unit of concern. These findings suggest that this variability of results from different field trials may be due to the *S. aureus* strain variations.

CONCLUSION

It was concluded that, polyvalent mastitis vaccine had not an enough clinical effect on prevention against all the mastitis pathogens. However; in farms that mastitis with an intensive problem, mastitis vaccination caused to decrease the incidence of CM and IMI of *S. aureus*. Further detailed studies are needed to clearly determine the factors affecting the success of vaccination.

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