

Field Trials of a Vaccine Against Bovine Mastitis.

2. Evaluation in Two Commercial Dairy Herds

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ABSTRACT

A vaccine against bovine mastitis was developed. The vaccine was based on inactivated, highly encapsulated *Staphylococcus aureus* cells; a crude extract of *Staph. aureus* exopolysaccharides; and inactivated unencapsulated *Staph. aureus* and *Streptococcus* spp. cells. In this study, the vaccine was evaluated in 164 cows from two commercial dairies (A and B) during a 4-mo period. Two doses of the vaccine were administered subcutaneously to 82 cows in the brachiocephalic muscle of the neck within a 4-wk interval. The results of this trial revealed significantly fewer intramammary infections caused by *Staph. aureus* at various levels of severity (clinical, subclinical, and latent) in cows that were vaccinated. The odds ratios of all types of intramammary infections caused by *Staph. aureus* for dairies A and B, which were determined by a logistic model, were 1.84 and 1.89, respectively, for quarters of vaccinated cows and quarters of control cows. The colony counts for *Staph. aureus* in milk from infected quarters of vaccinated cows were significantly lower than those in milk from infected quarters of control cows. Also, the somatic cell counts per milliliter in milk from vaccinated cows were significantly decreased when the initial somatic cell count was <500,000 cells/ml at the start of the trial. The vaccine had no observable effect on fat production in milk or on streptococcal infections.

(**Key words:** bovine mastitis, vaccine, *Staphylococcus aureus*, *Streptococcus* spp.)

INTRODUCTION

Staphylococcus aureus is the most important etiological agent of bovine mastitis. Many attempts to develop an effective vaccine against bovine mastitis caused by *Staph. aureus* (referred to as *Staph. aureus* mastitis throughout) have been reported (1, 3, 7, 8, 9, 10, 11, 12, 13). Two of these vaccines were evaluated recently under field conditions (8, 12).

A new vaccine was developed for use against bovine mastitis based on inactivated, highly encapsulated *Staph. aureus* cells; a crude extract of *Staph. aureus* capsular polysaccharides; and inactivated, unencapsulated *Staph. aureus* and *Streptococcus* spp. cells, and this vaccine was tested in heifers under controlled conditions (4). Results of that trial showed the effectiveness of the vaccine in controlling the incidence of IMI caused by *Staph. aureus* (*Staph. aureus* IMI) at various levels of severity. The vaccine was also shown to be effective in preventing new *Staph. aureus* IMI.

To validate the usefulness of the vaccine, its effectiveness against mastitis needed to be evaluated under commercial conditions using the whole herd. The purpose of the present study was the evaluation of the vaccine in two commercial dairy farms.

MATERIALS AND METHODS

Herds

The trial was carried out in two commercial dairy herds located in Córdoba, Argentina. At the start of the trial, dairy A had 90 milking cows and a mean annual milk production of 17 L/d per cow. The mean SCC in bulk milk was 580,000 cells/ml. Sixteen percent of the quarters were infected with *Staph. aureus*, and 6.3% were infected with *Streptococcus* spp. Ten percent of the teats had lesions resembling cowpox lesions. Dairy B had 85 milking cows and a mean annual milk production of 16 L/d per cow. The mean SCC in bulk milk was 600,000 cells/ml. Fifteen per-

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cent of the quarters were infected with *Staph. aureus*, and 2.7% were infected with *Streptococcus* spp. Fifteen percent of the teats showed lesions caused by overmilking and high vacuum levels. In both dairies, the hygienic milking conditions were deficient: teats were not properly washed before milking and were not disinfected after milking. Intramammary antibiotic therapy was applied to the quarters at drying off. None of the working conditions of the dairies were modified during the trial.

Vaccination

The vaccine was prepared as described previously (4). The batch employed in this study was the same used in the trial with heifers (4).

Fifty percent of the lactating cows and the cows ≥ 7 mo pregnant were vaccinated at random. Forty-two cows from dairy A (4 pregnant) and 40 cows from dairy B (3 pregnant) were vaccinated. Two doses of the vaccine were administered within a 4-wk interval by subcutaneous injection in the brachiocephalic muscle of the neck. Vaccinated cows were monitored weekly for 2 mo for the presence of reactions at the inoculation site starting the day after administration of the vaccine. No adverse reactions were caused by administration of the vaccine. Some cows developed a transitory swelling at the inoculation site that disappeared within 1 to 3 wk after injection.

Sampling and Laboratory Analysis

Milk samples were collected monthly for 4 mo after the administration of the second dose of the vaccine. The first stream of milk was discarded, and a

3- to 5-ml sample of the milk was collected in sterile tubes. The dairy farmer or veterinarian registered the clinical mastitis cases and collected the mastitic milk samples, which were then refrigerated and sent to our laboratory within the next 24 h. All other samples were collected and processed by the laboratory staff. The milk samples were kept at 4°C until processing. Samples were processed within 4 to 8 h of sampling.

The microbiological assays, evaluation of SCC, determinations of milk and fat production, and diagnosis of mastitis were carried out as indicated previously (4).

Culling

Some of the cows in the study were withdrawn from the trial for various reasons, such as drying off. Only those cows from which the four monthly milk samples from all quarters had been collected and analyzed were considered for microbiological analyses.

Statistical Analysis

The quarters that were infected with primary pathogens (*Staph. aureus* or *Streptococcus* spp.) at the start of the trial were excluded from the analyses. The Mantel-Haenszel or Fisher exact test was used to compare the rates of mastitis. The ANOVA was applied to compare mean fat production. The Epi-Info[®] software was employed for these analyses (2). A logistic model was performed (6) to determine the risk of infection between vaccinated and control cows. The model included the dairy herd and the time of lactation at which vaccination was carried out. These

TABLE 1. Frequencies of IMI caused by *Staphylococcus aureus* according to level of severity.¹

Diagnosis and prevalence	Herd A		Herd B		Total	
	Control	Vaccinated	Control	Vaccinated	Control	Vaccinated
Clinical mastitis	2.3	0.6 ^a	1.2	0.5	1.7	0.6 ^d
Subclinical mastitis	10.7	6.8 ^b	5.9	3.2 ^d	8.0	4.5 ^e
Clinical plus subclinical mastitis	13.0	7.4 ^c	7.1	3.7 ^e	9.7	5.1 ^e
Latent infection	2.6	2.5	1.0	1.1	1.7	1.6
Total <i>Staph. aureus</i> IMI	15.7	9.9 ^c	8.0	4.9 ^d	11.2	6.7 ^e
Quarters, no.	383	325	512	556	895	881

^a $P = 0.056$.

^b $P < 0.05$.

^c $P < 0.014$.

^d $P < 0.03$.

^e $P < 0.002$.

¹Determined using the Fisher exact test.

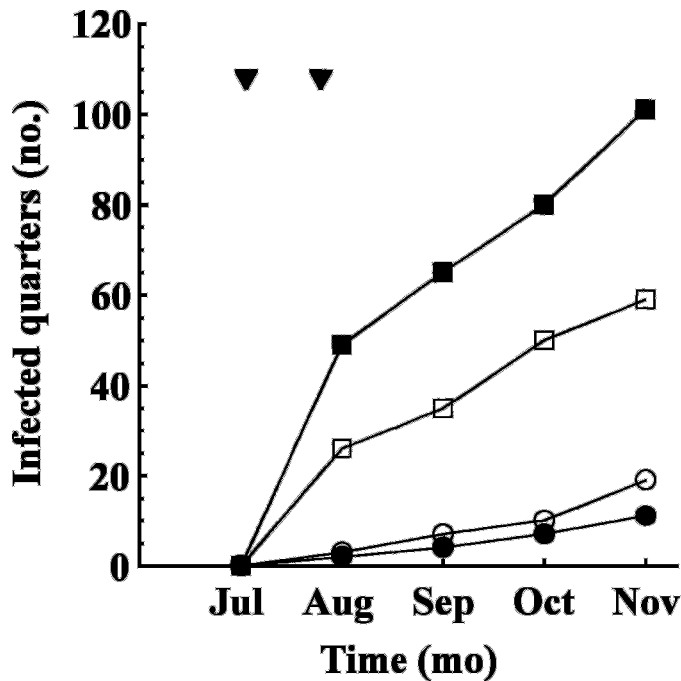


Figure 1. Cumulative incidence of *Staphylococcus aureus* isolated from the quarters of vaccinated (□) and control (■) cows and cumulative incidence of *Streptococcus* spp. isolated from the quarters of vaccinated (○) and control (●) cows. Triangles indicate the administration of the two doses of the vaccine.

factors were treated as potential confounders. The dependent variable was the infection status of the quarters sampled (0 = infected; 1 = uninfected). The independent variables were vaccination (0 = control; 1 = vaccinated), herd (0 = herd B; 1 = herd A), and time of lactation at which vaccination was carried out (0 = <101 d; 1 = ≥101 d and ≤200 d; 2 = >200 d). This analysis was performed with the Stata[®] software (5). In all statistical analyses, the unit of concern was the mammary quarter, except for fat production, for which the unit of concern was the cow. Significance was declared at $P < 0.05$.

RESULTS

The frequencies of *Staph. aureus* IMI in the quarters of the control and vaccinated cows were recorded for 4 mo after vaccination. For vaccinated cows, the frequencies of *Staph. aureus* IMI with different levels of severity were reduced in both dairy herds. As shown in Table 1, incidence of clinical mastitis decreased from 2.3% in the quarters of control cows to 0.6% ($P < 0.056$) in vaccinated cows in dairy A and from 1.2% in the quarters of control cows to 0.5% in those of vaccinated cows in dairy B. Also, subclinical

cases of *Staph. aureus* mastitis were reduced from 10.7% in the quarters of control cows to 6.8% in quarters of vaccinated cows in dairy A ($P < 0.05$) and from 5.9% in the quarters of control cows to 3.2% in the quarters of vaccinated cows in dairy B ($P < 0.05$). Total *Staph. aureus* IMI in both dairies were reduced from 11.2% in the quarters of control cows to 6.2% in those of vaccinated cows ($P < 0.002$). These results indicate an overall reduction of 40.1% for all *Staph. aureus* IMI, and a reduction of 65 and 44% for clinical and subclinical mastitis, respectively, in vaccinated cows of the two dairies compared with mastitis incidence in control cows. The response to the administration of the vaccine in each dairy was also analyzed by a logistic model discriminating for portions of the lactation during which vaccination occurred. Using this model, the calculated odds ratio was 1.84 ($P = 0.031$) for herd A and 1.89 ($P = 0.011$) for herd B. Mean lactation time was similar for vaccinated and control cows in each group. These values were 58 ± 33 d and 42 ± 39 d for the first lactation period, 142 ± 22 d and 138 ± 26 d for the second period, and 289 ± 74 d

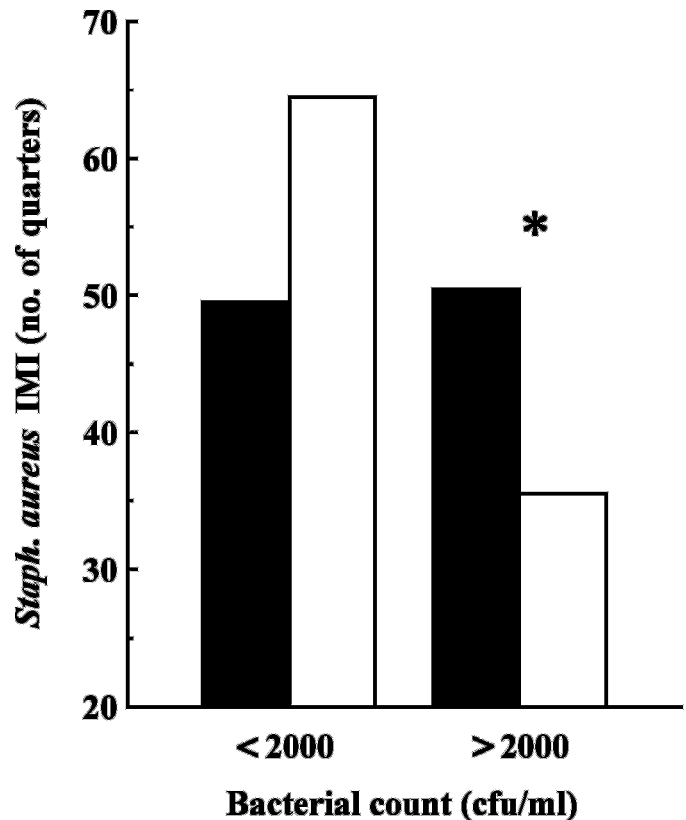


Figure 2. Total bacterial counts of quarters infected with *Staphylococcus aureus* of vaccinated (□) and control (■) cows. * $P = 0.0036$.

TABLE 2. Frequencies¹ of IMI caused by *Streptococcus* spp. according to level of severity.

Diagnosis	Control	Vaccinated		
			(%)	
Clinical mastitis	0.1	0		
Subclinical mastitis and latent infection	1.9	2.6		
Total <i>Streptococcus</i> spp. IMI	2.1	2.6		

¹Calculated on the basis of the analyzed milk samples: n = 895 for control cows and n = 881 for vaccinated cows.

and 280 ± 61 d for the third period for vaccinated and control cows, respectively.

Staphylococcus aureus IMI increased markedly during the initial month of the trial (Figure 1). This increase was higher for control cows than for vaccinated cows. During the subsequent 3 mo of the trial, the rate of increase of new *Staph. aureus* IMI was lower (Figure 1). The pattern of *Staph. aureus* IMI of each herd was similar to that shown in Figure 1.

Only 35% of *Staph. aureus* IMI in the quarters of all vaccinated cows had total bacterial counts >2000 cfu/ml; 50.5% of such infections in the quarters of control cows had similar counts ($P = 0.0036$; Figure 2).

The percentages of streptococcal IMI did not significantly differ between vaccinated and control cows in both dairy herds (Table 2 and Figure 1).

The frequencies of infections by other microorganisms were also studied (Table 3). The percentage of quarters infected with coagulase-negative staphylococci or *Corynebacterium bovis* was slightly, but not significantly, higher for the vaccinated cows than for the control cows.

Milk fat production in vaccinated and control cows from the two dairy herds was determined with the same number of cows in each group (n = 76); groups were matched by milk fat production at the start of the trial. Vaccinated cows showed only a slight increase (2.6%; $P = 0.11$) in total milk fat production over that of controls. The analysis of milk fat production of vaccinated cows in the first third of lactation (n = 32) showed an increase of 5.1% over that of controls.

The analysis of SCC in individual mammary quarters of vaccinated and control cows of the two herds was determined by separating these groups into two subgroups. One subgroup had an SCC <500,000/ml at the start of the trial, and the other subgroup had an SCC >500,000/ml at the start of the trial. The reduction (between 30 and 50%) was significant for cows with <500,000 cells/ml during the first 3 mo after

vaccination ($P \geq 0.05$; Figure 3). Differences were not significant between the vaccinated and control subgroups with SCC >500,000/ml (data not shown).

DISCUSSION

The results of this trial revealed significantly fewer *Staph. aureus* IMI; levels of severity of IMI were different between vaccinated cows of the two dairies. Thus, the odds ratios for all types of *Staph. aureus* (clinical, subclinical, and latent), for dairies A and B were 1.84 ($P < 0.031$) and 1.89 ($P < 0.011$), respectively, for the quarters of vaccinated and control cows.

In the previous trial of this vaccine (4), the odds ratio for heifers infected with *Staph. aureus* and vaccinated prepartum or postpartum were 3.22 and 3.37, respectively, compared with that for control heifers infected with *Staph. aureus* but not vaccinated. The apparent higher effectiveness of the vaccine with heifers than with whole herds might be accounted for by a higher initial basal immunity of the whole herd to *Staph. aureus*.

The results of this study showed a higher reduction in cases of clinical and subclinical *Staph. aureus* mastitis with vaccination than was shown by other researchers (8, 12). The studies of Watson and Schwartzkoff (12) evaluated five commercial dairies in Australia involving 582 cows and showed that vaccination reduced the incidence of clinical cases of mastitis by about 50% and reduced the incidence of subclinical cases by 25%. However, in our study, the reductions in incidence of clinical and subclinical mastitis were 65% ($P < 0.03$; Table 1) and 44% ($P < 0.002$; Table 1), respectively, for vaccinated groups in both dairies. The vaccine employed by Nordhaug et al. (8), which was assayed with 108 heifers in 16 herds, had a positive, but not significant, effect in reducing the incidence of *Staph. aureus* IMI (relative risk of not contracting mastitis of 0.4; $P = 0.14$) (8).

The vaccinations carried out in the present study caused a reduction in the rates of all types of *Staph. aureus* IMI and significant decreases in the microbial counts in milk from infected quarters (Figure 2) and

TABLE 3. Frequencies¹ of IMI caused by other microorganisms.

	Control	Vaccinated		
			(%)	
Coagulase-negative staphylococci	10.7	13.1		
<i>Corynebacterium bovis</i>	33.2	39.7		
Other microorganisms ²	7.1	8.0		

¹Calculated on the basis of the analyzed milk samples: n = 895 for control cows and n = 881 for vaccinated cows).

²Yeasts or Gram-positive or Gram-negative bacilli.

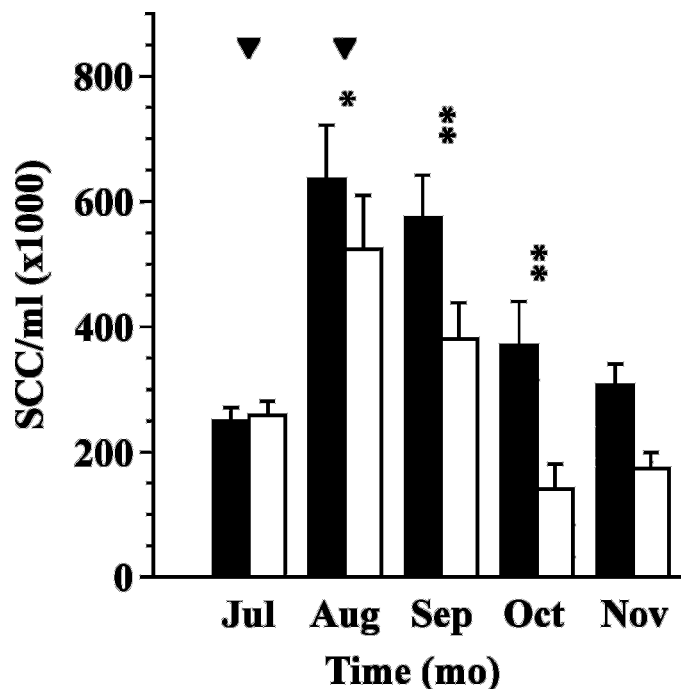


Figure 3. Somatic cell count per milliliter in milk of mammary quarters from a subgroup of vaccinated (\square) and control (\blacksquare) cows with an SCC $<500,000/\text{ml}$ at the start of the trial. * $P = 0.05$. ** $P = 0.005$. Triangles indicate the administration of the two doses of the vaccine.

in the SCC from vaccinated cows that had $<500,000$ SCC/ml at the start of the trial (Figure 3). These results indicate that the vaccine improves the quality of milk.

Furthermore, fat production was slightly higher for cows in the vaccinated group than that for cows in the control group, which is in agreement with the observations of the previous trial (4), which also revealed a slightly higher fat production for vaccinated heifers. Even a slight increase in fat production would represent an important economical benefit for the dairy producer.

In agreement with the results observed in the trial with heifers (4), no significant differences were found in this study in the percentages of quarters infected with *Streptococcus* spp. between the vaccinated and the control groups.

The results of the two trials with the vaccine under analysis, one under controlled conditions using heif-

ers (4) and the other under natural conditions of dairy management, as described in this study, suggest that this vaccine is very effective in controlling *Staph. aureus* IMI. Moreover, the efficacy of the vaccine could probably be improved if a continued program of vaccination is carried out (8, 12).

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