Field Trials of a Vaccine Against Bovine Mastitis. 1. Evaluation in Heifers

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ABSTRACT

A vaccine was developed against bovine mastitis based on inactivated, highly encapsulated *Staphylococcus aureus* cells; a crude extract of *Staph. aureus* exopolysaccharides; and inactivated, unencapsulated *Staph. aureus* and *Streptococcus* spp. cells. This vaccine was tested on 30 heifers during a 7-mo period. The 30 heifers were randomly assigned to three groups of 10 heifers each. The prepartum group received two injections of the vaccine at 8 and 4 wk before calving, and the postpartum group received two injections at 1 and 5 wk after calving. The control group received two injections of a placebo at 8 and 4 wk before calving. The vaccine or the placebo was administered subcutaneously in the brachiocephalicus muscle of the neck. The frequencies of intramammary infections caused by *Staph. aureus* were reduced from 18.8% for heifers in the control group to 6.7 and 6.0% for heifers in the prepartum and postpartum groups, respectively. This protective effect was maintained for at least 6 mo. The relative risk of mastitis caused by *Staph. aureus* was 0.31 and 0.28 for heifers in the prepartum and postpartum groups, respectively, compared with that for heifers in the control group. The results of the trial indicated the effectiveness of the vaccine in decreasing the incidence of intramammary infections caused by *Staph. aureus*. A slight but nonsignificant increase occurred in fat production in the milk of vaccinated cows. The vaccine had no observable effect on somatic cell count or streptococcal infections.

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

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Abbreviation key: **CNS** = coagulase-negative staphylococci.

INTRODUCTION

Bovine mastitis is the most important infectious disease of dairy cows that affects both the quality and quantity of milk. Worldwide, annual losses caused by this disease are nearly $35 billion (38); in Argentina, milk production losses reach $221 million annually (2).

Staphylococcus aureus is the most important etiological agent of bovine mastitis (10, 13, 37, 40, 44). According to recent estimates, 19 to 40% of dairy cows have mastitis caused by *Staph. aureus* (*Staph. aureus* mastitis) (8). Depending on the duration and severity of the infection, the productive performance of infected cows may be diminished permanently.

Streptococcus agalactiae and *Streptococcus uberis* are also predominant species that cause mastitis (10, 40, 44). Coliforms were less frequently isolated in Argentina than in other countries (10). In Argentina, dairy cows are maintained on pasture instead of being kept in barns, which could explain the lower incidence of mastitis caused by coliforms.

In addition, several preventive strategies have been applied to minimize bovine mastitis, including hygienic cleaning procedures, disinfection, antibiotic therapies, culling, and vaccination (12, 16). Numerous attempts at vaccination have been made that employ live or killed *Staph. aureus*, isolated peptidoglycan, toxoids, adhesins, or preparations of killed cells and toxoids (7, 22, 36, 41). These vaccines increased the spontaneous cure rate of infections and lessened the severity of the infections, but did not prevent the occurrence of new infections during the trial.

Several studies (14, 31, 33) have emphasized the importance of the antiphagocytic properties of the capsule of *Staph. aureus* as a determinant of virulence. The majority of the strains that were directly isolated from milk without subculturing were encapsulated (18, 24). In most studies, a capsule of anti-

genic type A was prevalent (1, 31, 46). However, another report (34) described the prevalence of antigenic types 5 and 8. Observations in Argentina have shown that the majority of the strains directly isolated from milk are of antigenic type A (27, 28).

When inoculated at high concentrations, microorganisms expressing the type A capsule can induce circulating antibodies that also appear in milk (26, 32). Vaccines developed on the basis of encapsulated bacteria have been shown to reduce the severity of IMI as well as the frequency of mastitis (23, 25, 39, 43, 48). Very few reports (5, 15, 20) exist on streptococcal vaccines against mastitis.

The present research describes the evaluation under field conditions of heifers that received a vaccine developed against *Staph. aureus* mastitis and mastitis caused by streptococci. The vaccine was based on crude extract of *Staph. aureus* exopolysaccharides, killed encapsulated *Staph. aureus* strains, and killed *Streptococcus* spp. strains. This vaccine has also been evaluated under natural conditions on two commercial dairies in Argentina (3).

MATERIALS AND METHODS

Strains

The strains used were *Staph. aureus* RC-1a, an encapsulated constitutive strain of antigenic type A that was isolated from a local clinical case of mastitis; *Staph. aureus* RC-1v, a heavily encapsulated variant of *Staph. aureus* RC-1a that was isolated in the laboratory after several subcultures; *Staph. aureus* RC-2, an isolate from a local clinical mastitis case; *Staph. aureus* Smith, a reference encapsulated strain provided by J. Hash (Vanderbilt University, TN); *Staph. aureus* RC8, which was isolated from a subclinical case of mastitis (37); *Staph. aureus* RN4220 (19); and *Streptococcus uberis* RC-3 and *Streptococcus agalactiae* RC-4, which were isolated from local cases of bovine clinical mastitis.

Determination of the Capsule

The presence of the capsule was verified by diffuse morphology in semisolid agar with serum and by the absence of clumping factor (6, 47). The larger capsule size of strain RC-1v was confirmed by transmission electron microscopy of cells stained with lanthanum and nitrate (29) and by a variation of a volumetric method (47).

Formulation of the Vaccine

The vaccine was prepared with cells of *Staph. aureus* RC-1v, *Staph. aureus* RC-2, *Strep. uberis* RC-3, and *Strep. agalactiae* RC-4 grown in brain-heart infusion broth at 37°C with aeration to stationary phase (determined by optical density). The cells were inactivated with formalin (0.4%, vol/vol), centrifuged (at 7000 rpm for 20 min at 4°C), and resuspended in saline (0.9% NaCl; pH 7.0). Each 5-ml dose contained 1×10^{10} cfu/ml of each strain of *Staph. aureus*, 4×10^{9} cfu/ml of each strain of *Streptococcus* spp., and a crude extract of the capsule from about 1×10^{10} cells/ml of *Staph. aureus* RC-1v. The crude extract was prepared as follows. Live cells were centrifuged as indicated previously, resuspended in a small volume of saline, and autoclaved for 20 min. This preparation was centrifuged under the same conditions, and the supernatant was added to the vaccine preparation at a concentration of approximately 5 mg of dry weight per dose. This extract contained about 70% reducing sugars, determined by the anthrone method (9). Sodium azide, thimerosal, and formalin were added as preservatives at final concentrations of 0.001% (wt/vol), 0.001% (wt/vol), and 0.4% (vol/vol), respectively. Aluminum hydroxide (3.5%, wt/vol) was added as an adjuvant. For the placebo, aluminum hydroxide and preservatives were added to saline at the same concentration used in the vaccine.

Quality Control of the Vaccine

Samples of the vaccine were assayed for sterility, side effects, and protection against *Staph. aureus* infection in mice. Side effects were determined by intraperitoneal administration of 1 ml of vaccine to 30 mice, by subcutaneous inoculation of the vaccine in the backs of five rabbits, and by simultaneous subcutaneous injection of three doses of the vaccine (two in the right side and one in the left side) in the brachiocephalicus muscle of the neck of two calves.

Protection Assays with Mice

The BALB/c mice, 21 to 25 d old, were supplied by the Universidad Nacional de Río Cuarto from stocks provided by the Centro Panamericano de Zoonosis, Argentina. The mice were immunized intraperitoneally with two 0.1-ml doses of the vaccine administered at 15-d intervals. The mice were then challenged 30 d after administration of the second dose with different strains of *Staph. aureus* (RC-1v, RC8, Smith, or RC-2) or *Streptococcus* spp. (RC-3 and RC-4). One milliliter of four different concentrations (10^{6} to 10^{9} cfu/ml) of each of these strains that had been grown in brain-heart infusion broth for 24 h at 37°C, centrifuged as indicated before, and resuspended in peptone solution [0.5% (wt/vol) of peptone and 0.5% NaCl] were inoculated intraperitoneally in vaccinated mice and in controls. The

number of dead mice was recorded on the 3rd d after the challenge. Groups of 5 mice were used for each bacterial concentration. These experiments were done in duplicate. The median lethal dose was calculated by the Reed and Muench method (45).

Herd

The trial was carried out on a dairy farm in Río Cuarto, Córdoba, Argentina during a 9-mo period from February to October. The dairy farm had 200 lactating Holstein cows and had a mean annual milk production of 18 L/d per cow. The cows were maintained on pasture at all times. Calving occurred throughout the entire year, but calving frequencies were higher in autumn and spring.

Machine milking was inadequate (high milk line and high vacuum levels and fluctuations during milking), the cows suffered overmilking frequently, and teat dipping was not practiced. Cows were routinely treated with antibiotics at drying off.

The mean bulk tank SCC was 600,000 cells/ml during the year prior to the start of the trial and varied between 480,000 and 730,000 cells/ml throughout the experimental period. Results from milk samples collected from quarters of 35% of the cows in the herd 2 mo prior to the start of the trial showed that 19% of the quarters were infected with *Staph. aureus*, 13% with coagulase-negative staphylococci (**CNS**), 1.7% with CAMP-positive streptococci, and 3.5% with CAMP-negative streptococci. The prevalance of IMI in the herd did not change throughout the trial.

Thirty heifers that were 24 to 26 mo old, 7 mo pregnant, and of similar BW were chosen for the trial out of 88 heifers. All of these heifers had been inseminated with semen from the same bull.

The heifers were maintained on pasture without contact with lactating cows and were incorporated into the herd about 10 d before calving. Heifers then received the same feed and treatment as the other cows. None of the management conditions that were applied in the dairy before the trial were modified during the trial.

Cases of clinical mastitis were diagnosed by the dairy farmer or the veterinarian, who sent a sample of milk to our laboratory for confirmation. After sampling, diagnosed cases were treated by intramammary administration of antibiotics. No quarters with clinical mastitis were detected in the heifers before calving.

Experimental Design

The 30 heifers were randomly assigned to three groups of 10 heifers each. The prepartum group received two injections of the vaccine at 8 and 4 wk before the estimated time of calving, and the postpartum group received two injections at 1 and 5 wk after calving. The control group received two injections of a placebo at 8 and 4 wk before calving. The vaccine or the placebo was administered subcutaneously in the brachiocephalicus muscle of the neck.

Sampling and Laboratory Analysis

Milk samples from all quarters were collected every 15 d during the first 3 mo after calving and every 30 d during subsequent months of the trial. Prior to sample collection, the tip of the teat was disinfected with alcohol; the first stream of milk was discarded, and a 3- to 5-ml sample of the milk was received in sterile tubes. The dairy farmer or the veterinarian collected the samples of colostrum and the milk from the quarters that were infected with clinical mastitis. All other samples were collected by the laboratory staff. The milk samples were kept at 4°C until processing in the laboratory. Samples were processed within 18 h.

For the microbiological assay, a 5-μl sample of milk was plated on sheep blood agar (7.5%, vol/vol) and incubated at 37°C for 40 h. The number and type of the colonies and the type of hemolysis were recorded. Gram-positive cocci were investigated for production of catalase. Presumptive *Staphylococcus* spp. were assayed for coagulase, and the CAMP assay was applied for presumptive *Streptococcus* spp. (10, 11, 13, 37). Classification of *Staph. aureus* was confirmed as described previously (37). The SCC was determined according to the technique recommended by the International Dairy Federation (17) as described previously (11, 37).

Monthly data for milk and fat production were provided by the Cooperativa de Inseminación Artificial of Venado Tuerto, Argentina, which routinely performed these determinations for the dairy farm. Fat production was calculated from only 9 heifers per group because 1 heifer in the control group died of tympanism before the end of the trial.

Diagnosis of Mastitis

The criteria used to classify subclinical mastitis were presence of a primary pathogen (*Staph. aureus* or *Streptococcus* spp.) in milk samples and a high SCC (25). For milk samples from Argentina, an SCC $>2.4 \times 10^5$ cells/ml was considered to be indicative of subclinical mastitis. This SCC value corresponds to the calculated geometric mean SCC per milliliter from quarters from which no microorganisms were detected (11). The presence of a primary pathogen without a corresponding increase in SCC was assessed as a latent infection (25).

TABLE 1. Median lethal dose of strains of *Staphylococcus aureus* injected intraperitoneally in mice that were immunized with the trial vaccine.

Strain	Control	Immunized	Ratio
RC-1v	7.1×10^6	7.9×10^7	11.1
RC-2	1.3×10^7	1.1×10^8	8.2
Smith	8.0×10^6	8.1×10^7	10.1
RC8	2.1×10^7	2.8×10^8	13.2
RN4220	4.5×10^7	4.3×10^8	9.6

Statistical 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 (4). The probability of not contracting *Staph. aureus* mastitis was analyzed with Quattro Pro® (35) in a Kaplan-Meyer plot (42). In all statistical analyses, the unit of concern was the mammary quarter, except for fat production, for which cow was the unit of concern. Significance was declared at $P < 0.05$.

RESULTS

Clinical Reactions After Vaccination

None of the 30 vaccinated mice died. Only 3 of the mice had bristled hair and decreased motility during the first 24 h after injection. None of the five vaccinated rabbits developed an inflammatory response or fever. The two calves that received three doses of the vaccine did not show any reaction after vaccination. About 20% of the heifers in the prepartum or postpartum groups showed a transitory swelling around the inoculation site that disappeared within 7 to 21 d after injection. No other adverse reaction in heifers was recorded.

Protection Assays in Mice

Protection conferred by vaccination was investigated in mice. After vaccination, mice became more resistant to different strains of *Staph. aureus* (Table 1). The median lethal dose for mice that were vaccinated was 10 times higher than that for the untreated mice (Table 1). The vaccination assay could not be carried out with streptococci because these bacteria were not pathogenic in mice in this type of assay.

IMI Caused by *Staph. aureus* in Heifers

Table 2 shows the cumulative incidence of IMI caused by *Staph. aureus* (*Staph. aureus* IMI) categorized by the severity of the IMI in the quarters of the 30 heifers during the 7-mo trial. Of the seven quarters with clinical *Staph. aureus* mastitis, five belonged to heifers in the control group, and only one belonged to a heifer in the vaccinated groups (Table 2). One quarter of a heifer in the control group suffered clinical mastitis twice; two of the other clinical mastitis cases were severe and led to total loss of quarter function. A mild case of clinical mastitis occurred at d 158 of lactation in the quarter of a heifer in the prepartum group; only a few clots were detected. One heifer in the postpartum group suffered two episodes of clinical mastitis in the same quarter.

TABLE 2. Cumulative incidence of IMI caused by *Staphylococcus aureus* according to level of severity in quarters of heifers.

	Treatment					
Diagnosis	Control (n = 40)		Vaccinated prepartum (n = 40)		Vaccinated postpartum (n = 40)	
	(%)	(no.)[1]	(%)	(no.)	(%)	(no.)
Clinical mastitis	12.5	5	2.5	1[a]	2.5	1[a]
Subclinical mastitis	40.0	16	22.5	9[b]	15.0	6[c]
Latent infection	22.5	9	7.5	3[d]	10.0	4
Total *Staph. aureus* IMI	75.0	30	32.5	13[e]	27.5	11[e]

[a]$P = 0.10$.

[b]$P = 0.07$.

[c]$P = 0.011$.

[d]$P < 0.057$.

[e]$P < 0.0001$.

[1]Numbers of quarters affected at one or more occasions.

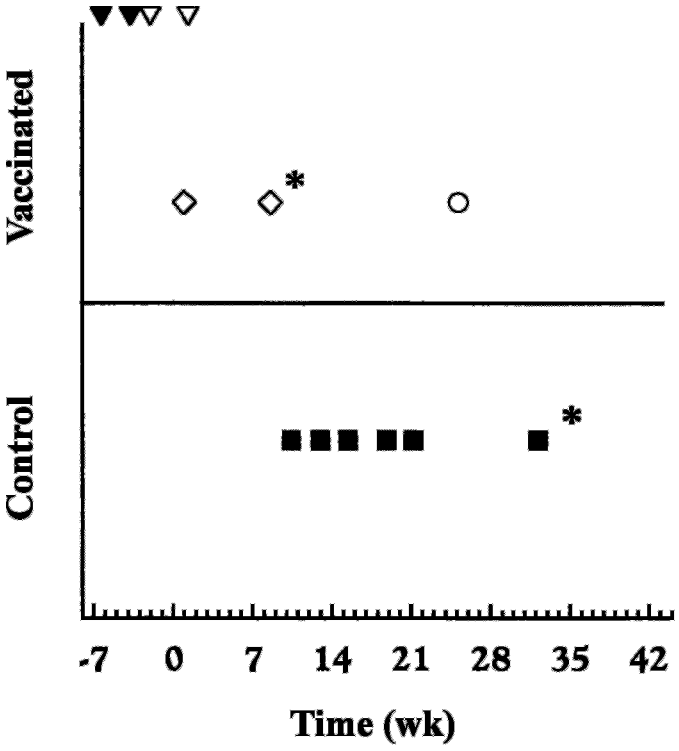

Figure 1. Episodes of clinical mastitis caused by *Staphylococcus aureus* recorded for control heifers (■) and for heifers vaccinated prepartum (○) or postpartum (◊). Asterisks indicate a second episode in the same quarter. Triangles indicate the administration of the two doses of the vaccine in prepartum (▼) and postpartum (▽) groups.

One episode occurred the day after receiving the first dose of the vaccine, and the second episode, which led to loss of the quarter, occurred 56 d later. Only one of the clinical episodes of mastitis occurred before the 6th wk after calving (Figure 1).

During the trial, 31 quarters were determined to have subclinical mastitis on one or more occasions (Table 2). The majority of those cases (n = 16) occurred in heifers in the control group. The number of quarters affected in heifers in the prepartum and postpartum groups was much lower; 9 and 6 cases, respectively. In the control group, 9 quarters had latent *Staph. aureus* IMI, but only 3 and 4 of the quarters of heifers in the prepartum and postpartum groups, respectively, had such IMI (Table 2).

The frequencies (as a percentage of total quarter samples) of *Staph. aureus* IMI with different levels of severity (clinical, subclinical, and latent) among the milk samples analyzed are indicated in Table 3. The frequencies of clinical and subclinical *Staph. aureus* mastitis were lower in the quarters of heifers in the prepartum and postpartum groups [4.1% ($P = 0.001$) and 2.5% ($P < 0.0001$), respectively] than those in the quarters of heifers in the control group (10.2%). Of all *Staph. aureus* IMI, 6.7 and 6.0% corresponded with quarters of heifers in the prepartum and postpartum groups, respectively; the percentage of quarters with *Staph. aureus* IMI for heifers in the control group was higher (18.8%; $P < 0.0001$). Data from Table 3 can be used to calculate that all *Staph. aureus* IMI decreased 64 and 68% for heifers in the prepartum and postpartum groups, respectively, compared with heifers in the control group. *Staphylococcus aureus* was not detected in any of the samples of colostrum that were collected from heifers during the first 2 d after calving.

The probabilities of not contracting *Staph. aureus* mastitis, calculated by the Kaplan-Meyer plot based on the number of clinical and subclinical cases of mastitis that occurred during the trial, are shown in Figure 2. A remarkable difference between the control group and the prepartum and postpartum groups was evident. In the period following the first 40 d after calving, the probabilities of not contracting *Staph. aureus* mastitis were noticeably higher for the two vaccinated groups than for the control group (Figures 1 and 2).

The relative risk of all *Staph. aureus* IMI was 0.31 and 0.28 ($P < 0.0001$) for heifers in the prepartum and postpartum groups, respectively, compared with heifers in the control group.

Streptococcal Infections

Only one case of clinical mastitis caused by *Streptococcus* spp. was diagnosed in the control group, and no cases were diagnosed in the two vaccinated groups

TABLE 3. Frequencies[1] of IMI caused by *Staphylococcus aureus* according to level of severity.

Diagnosis	Treatment		
	Control	Vaccinated prepartum	Vaccinated postpartum
	(%)		
Clinical mastitis	1.6	0.3[a]	0.6
Subclinical mastitis	8.6	3.8[b]	1.9[b]
Clinical plus subclinical mastitis	10.2	4.1[c]	2.5[b]
Latent infection	8.6	2.6[b]	3.5[b]
Total *Staph. aureus* IMI	18.8	6.7[b]	6.0[b]

[a]$P = 0.076$.

[b]$P < 0.0001$.

[c]$P = 0.001$.

[1]Calculated on the basis of the number of milk samples analyzed: n = 374 for control heifers, n = 344 for heifers vaccinated prepartum, and n = 317 for heifers vaccinated postpartum.

(Table 4). There were no significant differences in the number of subclinical and latent infections among groups.

Other Microorganisms

The numbers of milk samples from which CNS were isolated were higher ($P < 0.001$) for heifers in the prepartum (123 of 344; 35.8%) and postpartum (112 of 317; 35.3%) groups than for heifers in the control group (95 of 374; 25.4%). No IMI caused by CNS, coliforms, yeasts, or fungi were observed.

Fat Production

During the 7-mo trial, the total milk fat production of heifers in the prepartum and postpartum groups was 13.2% ($P = 0.09$) and 9.4% ($P = 0.11$, NS) higher, respectively, than that of heifers in the control group (Table 5).

SCC

No significant differences were found among the mean SCC from mammary quarters of the three groups studied (Figure 3).

DISCUSSION

The vaccine evaluated in this work was developed on the basis of a crude extract of *Staph. aureus* exopolysaccharides, inactivated, highly encapsulated *Staph. aureus* cells, unencapsulated *Staph. aureus*; and *Streptococcus* spp. cells. The vaccine conferred effective protection to mice against *Staph. aureus* strains RC-1v and RC-2, which had been used to prepare the vaccine, as well as against encapsulated (Smith strain) and other unencapsulated *Staph. aureus* strains (Table 1).

The results of this trial revealed that the frequencies of clinical and subclinical *Staph. aureus* mastitis in the quarters of heifers in the prepartum and postpartum groups (4.1 and 2.5%, respectively) were significantly lower than those of heifers in the control group (10.2%). Similarly, the frequencies of latent *Staph. aureus* IMI were also reduced for the heifers that had been vaccinated either prepartum or postpartum (2.6 and 3.5%, $P < 0.0001$, respectively) compared with the frequency for heifers in the control group (8.6%) (Table 3). All *Staph. aureus* IMI decreased 64 and 68% for heifers in the prepartum and postpartum groups, respectively, compared with heifers in the control group.

The relative risk of *Staph. aureus* mastitis in quarters of heifers in the prepartum and postpartum groups was 0.31 ($P = 0.0001$) and 0.28 ($P = 0.0001$), respectively. Data shown in Figures 1 and 2 indicate that the vaccine maintained its protective effect against *Staph. aureus* mastitis for a period of at least 6 mo.

A vaccine that was based on a killed, encapsulated *Staph. aureus* and α- and β-toxoids was recently developed and tested in heifers by Nordhaug et al. (25). This vaccine, tested on 58 heifers in a multicenter study involving 16 dairies, yielded a nonsignificant relative risk of *Staph. aureus* mastitis of 0.40 in vaccinated heifers compared with controls. According to these data, the vaccine used in this work seems to be more efficient than that evaluated by Nordhaug et al. (25) under the experimental conditions involved.

The high efficacy shown by this vaccine to reduce *Staph. aureus* IMI in heifers might be attributed to its high concentration of bacterial antigens and capsular polysaccharides. Opdebeeck and Norcross (32) reported that the administration of high concentra-

TABLE 4. Frequencies[1] of IMI caused by *Streptococcus* spp. according to level of severity.

Diagnosis	Treatment		
	Control	Vaccinated prepartum	Vaccinated postpartum
	(%)		
Clinical mastitis	0.3	. . .	. . .
Subclinical mastitis	1.3	1.5	1.6
Latent infection	2.7	2.0	2.8
Total streptococcal infections	4.3	3.5	4.4

[1]Calculated on the basis of the number of milk samples analyzed: n = 374 for control heifers, n = 344 for heifers vaccinated prepartum, and n = 317 for heifers vaccinated postpartum.

TABLE 5. Total milk fat production in heifers.

	Treatment		
	Control	Vaccinated prepartum	Vaccinated postpartum
Heifers, no.	9	9	9
Total fat production, kg	1350.0	1528.1	1478.1
$\bar{X}$[1]	21.4	24.3	23.5
SD	8.9	6.4	9.0
Increase in production, %[2]	. . .	13.2	9.4

[1]Milk fat production per heifer per month.

[2]Vaccinated/control.

tions of staphylococcal and streptococcal antigens to cows induced the appearance of specific antibodies in milk and that the concentrations of antibodies against *Staph. aureus* β-toxin and pseudocapsule significantly increased in serum and milk during lactation (39). Additionally, the beneficial effects of the vaccine studied here could have been accentuated by the high frequency of *Staph. aureus* IMI and the low sanitation standards that existed initially in the herds.

Contrary to the results of Nordhaug et al. (25), who found that most of the clinical cases of mastitis occurred during the first 3 to 4 wk after calving, our results indicated that most of the cases of clinical mastitis for heifers in the control group occurred following the 1st mo after calving. In the studies of Nordhaug et al. (25), heifers probably had a high initial level of IMI at calving as revealed by the high occurrence of mastitis during the 1st d postpartum. Conversely, under conditions of this study, heifers appeared to be free of IMI at calving because they did not show microorganisms in colostrum, and the occurrence of mastitis during the postpartum period was extremely low.

Several studies (21, 30) have presented advantages of administering a mastitis vaccine at drying off because of a better immune response and a higher susceptibility of the mammary gland during the 1st wk after calving. The results of both the prepartum and postpartum vaccinations, as carried out in this work, did not confirm those speculations, because both vaccinated groups showed similar decreases in the incidence of clinical, subclinical, and latent *Staph. aureus* IMI.

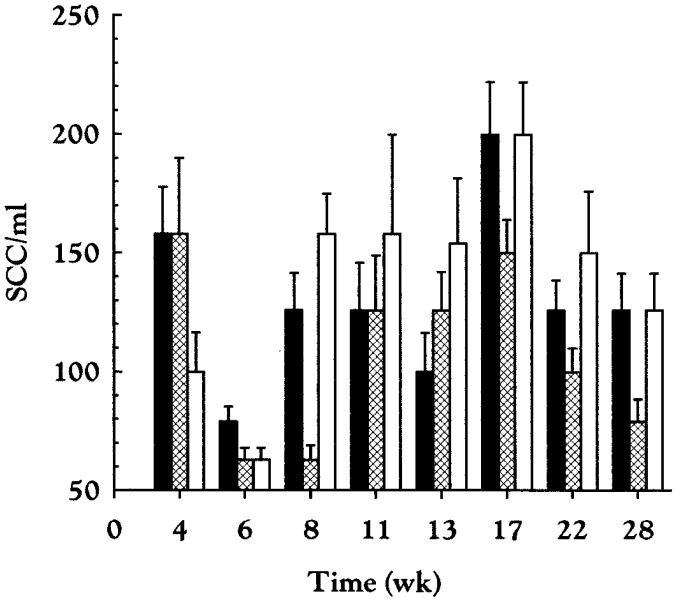

Figure 3. The SCC per milliliter of milk from quarters of control heifers (solid bar) and heifers vaccinated prepartum (patterned bar) and postpartum (open bar).

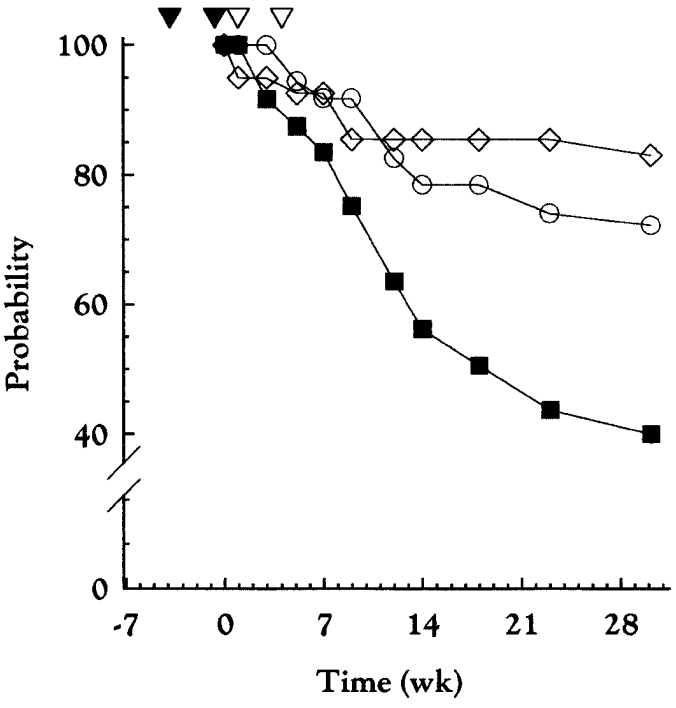

Figure 2. Probabilities of not contracting mastitis caused by *Staphylococcus aureus* at various times for control heifers (■) or heifers vaccinated prepartum (○) or postpartum (◊). Triangles indicate the administration of the two doses of the vaccine in prepartum (▼) and postpartum (▽) groups.

The CNS isolates in milk from quarters of heifers in the two vaccinated groups showed an increase over those in milk from quarters of heifers in the control group. This increase might have resulted from the reduction in *Staph. aureus* IMI in vaccinated cows, which could favor colonization by other microorganisms. These results do not agree with those of Yoshida et al. (48), who reported a significant reduction in the percentage of quarters infected with CNS in the group of vaccinated cows. A probable explanation for these differences is that the vaccine employed by Yoshida et al. (48) also contained capsular polysaccharides extracted from a strain of *Staphylococcus epidermidis*.

The vaccine evaluated in this study did not diminish the frequency of streptococcal mastitis because vaccinated and control heifers had similar percentages of infected quarters (Table 4). More studies are necessary to understand the failure of this vaccine to diminish or prevent streptococcal IMI. The development of an efficient vaccine against *Strep. uberis* has

been reported recently (5, 15). The success of this vaccine in inducing protection against streptococcal IMI could be explained by the intramammary route of administration and the employment of a live vaccine strain (5, 15).

The lack of effect of the vaccine on SCC could be due to the unusually low initial SCC. However, the lack of effect of vaccination on SCC is in agreement with the observations of other researchers (25, 39) who did not find significant effects on SCC with other vaccines against *Staph. aureus* mastitis. A slight, although not statistically significant, increase in fat was observed in milk from heifers in vaccinated groups (Table 5). The results reported in this study indicate the effectiveness of the vaccine used to decrease the incidence of *Staph. aureus* IMI in heifers.

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