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Veterinary Immunology and Immunopathology 93 (2003) 153–158
Veterinary immunology and immunopathology
www.elsevier.com/locate/vetimm

Development of a *Staphylococcus aureus* vaccine against mastitis in dairy cows. II. Field trial

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Received 16 May 2002; received in revised form 11 February 2003; accepted 4 March 2003

Abstract

A recently described new *Staphylococcus aureus* vaccine ''MASTIVAC I'' (Patent no. PTC/IL98/00627) against *S. aureus* udder infection elicited protection against experimentally induced infection in cows. In the present paper we describe a large-scale vaccination field trial. A total of 452 Israeli Holstein heifers were included in the study over two consecutive years. Approximately half of the heifers (228) were vaccinated while the others (224) served as a control group. Antibody response was detected in all vaccinated animals 4–5 weeks post-primary immunization and it was sustained throughout the experimental period (300–330 days). *S. aureus* infection could be detected in only 3 out of 228 animals (1.3%) in the vaccinated group and in 6 out of 224 (2.7%) in the control group. These numbers were too low to be statistically evaluated. However, when somatic cell counts (SCC) and milk yields were considered, a significant difference was found between the two groups, namely, the vaccinated cows in first and second lactation had 42 and 54%, respectively, lower SCCs and milk yields 0.5 kg per day higher than the non-vaccinated control cows. These results suggest that the new vaccine elicits a non-specific health improvement of the udder in addition to specific protection against *S. aureus*.
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Keywords: Cattle; Mastitis; Vaccine; *S. aureus*

1. Introduction

Staphylococcus aureus is one of the major pathogenic agents of the udder, in which it causes mainly subclinical infection. This type of mastitis impairs alveolar function, reduces milk yield and has a deleterious effect on milk composition, including increased milk somatic cell counts (SCC) (Gudding et al., 1984; Nickerson, 1989). Much effort has been devoted to the development of a vaccine against *S. aureus* mastitis in bovines (Yoshida et al., 1984; Watson and Schwartzkoff, 1990; Nelson et al., 1991; Watson, 1992; Watson et al., 1996; Mamo et al., 1994; Nordhoug et al., 1994; Calzolari et al., 1997; Giraudo et al., 1997) but a substantial solution has not been achieved so far. Recently we described

Abbreviations: SCC, somatic cell count; *S. aureus*, *Staphylococcus aureus*; IDF, International Dairy Federation; CC, Coulter® Counter; LSCC, $\log_2$ SCC; CNS, coagulase-negative staphylococci

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0165-2427/03/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0165-2427(03)00062-X

the development of a the new *S. aureus* vaccine, designated "MASTIVAC I" (Patent no. PTC/IL98/00627 and Leitner et al., this issue, mouse model). The new vaccine is composed of three field strains, which exhibit a broad spectrum of antigenic and immunogenic properties. Upon administration of the vaccine, no side effects or pathogenic findings were observed. In controlled experiments the vaccine was found effective against challenge with a virulent field strain of *S. aureus* (Leitner et al., this issue, challenge trials) the results showed significant protection from infection (70%). Moreover, all quarters challenged in the vaccinated cows, regardless of whether or not they were successfully infected with *S. aureus*, demonstrated only very mild inflammatory reactions in their udder tissues as expressed in their low SCCs (<100 000) (Leitner et al., this issue, challenge trials).

In the present paper we describe a large-scale field study, involving 452 Israeli Holstein heifers from seven dairy farms, over two consecutive years.

2. Materials and methods

2.1. Animals

A total of 473 Israeli Holstein heifers from seven dairy farms located in different regions of Israel were included in the study. *S. aureus* was prevalent in all herds, being present in 8–25% of the animals. Data on the phenotypic characteristics of the *S. aureus* strains revealed several different subtypes among the seven dairy farms (Table 1). All heifers included in this study underwent serological tests for *S. aureus* antibodies and only those which did not possess specific antibodies (452 animals) were included. Post-partum, cows were milked three times daily. Food was offered in mangers located in free stall barns.

Table 1
Hemolysis patterns and phage types of the local *S. aureus* isolates from cows in each of the seven experimental farms

Farm	Hemolysis	Phage type
19700	α + β	$D11^+/HK_2$, 84 [90]
27300	β	6, 42E, 47, 53, 54, 75, 83A, 85, 90, $D11^+/HK_2$
32700	β	3A/3C, 55, 71
38300	β	80/55 [29, 52, 52A, 3/C, 92]
44700	–	3A/3C, 71 [55]
57200	ND	ND
69400	α + β	84, 92 [53, 75, $D11^+/HK_2$]

2.2. Vaccine and vaccination

A comprehensive description of the vaccine has been described under Patent nos. 122829 and PTC/IL98/00627 and in the previous manuscript "*Staphylococcus aureus* vaccine against mastitis in dairy cows, composition and evaluation in mouse model" (Leitner et al., companion paper, this issue). The vaccine is derived from three field strains of *S. aureus*; it comprises insoluble bacterial fragments (derived by mechanical agitation) of two of the strains and a supernatant fraction containing secreted antigens of the third strain, and contains a broad spectrum of antigenic determinants. Each cow was immunized subcutaneously under the tail root with 1 ml of the vaccine emulsified with incomplete Freund's adjuvant and in the area of the supra-mammary lymph node with 1 ml of the vaccine without the adjuvant. A second dose of the vaccine without adjuvant was administered by means of the same procedure, 40–60 days after the primary immunization.

2.3. Bacteriological examinations

Bacteriological examinations were conducted as described in a companion paper (Leitner et al., this issue. 1. Challenge trials).

2.4. Milk content and SCC

SCCs were determined with a Coulter® Counter (CC) Model Z1 (Coulter Electronics Ltd., Beds., UK) according to the revised protocol of the A2B subgroup of the Mastitis Experts of the IDF (1991) or with the Fossomatic 360 instrument at the Israeli Cattle Breeders Association, Milk Control Laboratory in Caesarea.

2.5. Immunological assay

ELISA: The assay was performed as previously described (Leitner et al., 2000a), with VLVL8407 *S. aureus* as antigen. To each plate, positive and negative serum standards were added. The optical

density of each tested sample was normalized by linear regression in order to calculate the titer.

2.6. Study layout

In each of the seven farms, the heifers were divided into groups according to the time of expected calving. Ten to eight weeks prior to the first calving in each group, the heifers were bled and assigned randomly into two subgroups: immunized and unimmunized (control). Heifers in the immunized subgroup were vaccinated at that time; 4–5 weeks later, all heifers were bled and the immunized animals were revaccinated.

After first calving, each animal in the experiment was bled and milk samples were collected two or three times: at 2–4 weeks, 2–3 months and 5–6 months postpartum. The data on farm, year and treatment of the 452 heifers included in this experiment are summarized in Table 2. Data on milk composition, SCC, and yield for 7–9 months from the time of were obtained from the Israeli Cattle Breeders' Association's Herd Book. On two of the farms (27 300 and 38 300), all heifers that remained for the second calving were bled at the time of drying-off and 26 cows from the immunized group were revaccinated. This formed three new groups of second-lactating cows: vaccinated as a heifer and revaccinated as a cow (26 animals), vaccinated only as a heifer (13 animals) and unvaccinated control (45 animals) (Table 4). SCC and milk yield over 7–9 months from the time of second parturition were obtained from the Israeli Cattle Breeders' Association's Herd Book and analyzed as for the first calving.

Table 2
Distribution of heifers according to farm, year, and treatment

Farm	Year	Vaccinated	Control	Total
19700	1	12	11	23
27300	1	28	28	56
	2	51	32	83
32700	1	24	30	54
38300	1	34	36	70
	2	19	16	35
44700	1	12	13	25
57200	1	14	25	39
69400	1	34	33	67
Total		228	224	452

2.7. Statistical analysis

Data were analyzed by means of the SAS general linear model (GLM) procedure (SAS, 1990). Dependent variables were: milk yield (kg), fat, protein, and lactos contents, SCC, and SCC transformed to $\log_2$ (LSCC). The independent variables were: treatment, herd, cow, number of days in milk, and (number of days in milk) squared. The effects on the dependent variables were examined with the model:

$$Y_{ijkl} = \mu + T_i + H_j + R_k + C_{ijk}(\mathrm{THR}) + \mathrm{DIM} + \mathrm{DIM}^2 + e_{ijkl}$$

where Y is the dependent variables; μ the overall mean; T_i the treatment, $i = 1, 2$; H_j the herd, $j = 1, \ldots, 7$; R_k the year, $k = 1, 2$; C_{ijk} the cow at treatment$_i$ in herd$_j$ in year$_k$; DIM the days in milk; and $\mathrm{DIM}^2 = \mathrm{DIM} \times \mathrm{DIM}$.

3. Results

During the entire period of the experiment, none of the cows showed any abnormal symptoms of sensitivity to the vaccine except for local swelling for up to 10 days after vaccination. Of the new calves 95% were born alive and healthy, with no teratogenic features. No difference could be detected in calf survival rate between the immunized and the control cows. Antibody response to antigen of VLVL8407 *S. aureus* could be detected in all vaccinated animals when first tested, 4–5 weeks after the primary immunization (Fig. 1), and the highest antibody level was detected at that time. After calving, antibody titer decreased moderately, with some fluctuations up to 150 days post-vaccination (Fig. 1). Further tests were carried out in two of the seven experimental farms at the time of drying-off (300–330 days): all heifers tested (53% of all heifers vaccinated) at that time maintained antibodies at a level similar to that determined at 150 days. Although all vaccinated cows responded to the vaccine, the mean antibody level varied among the herds, and in two of the herds the overall response was lower than in the others. The numbers of cows from whose milk *S. aureus* was isolated were 3 out of

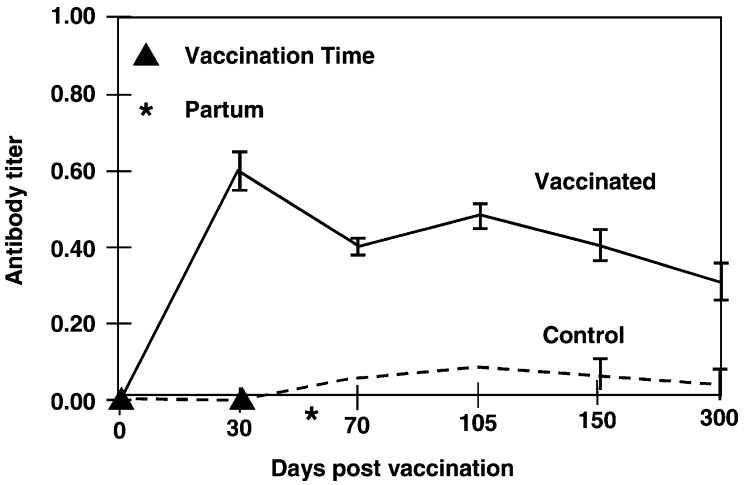

Fig. 1. Mean and S.E. of *S. aureus*-specific antibody titers of 228 heifers from seven farms, vaccinated against this agent and 224 control.

228 (1.3%) in the vaccinated groups and 6 out of 224 (2.7%) in the control groups. Coagulase-negative staphylococcus (CNS) was the predominant bacterial isolate from the milk samples, with no difference observed between the frequencies of isolation from vaccinated and control cows.

The SCCs and milk yields (Herd Book) for 7–9 months from the time of parturition were analyzed, and large differences in SCC were found among the herds, but in all seven herds the vaccinated heifers had lower SCCs than the control animals. Vaccinated heifers had a mean (over herd and year) value of 101.1×10^3 cells/ml (S.E. 12.1), compared with 175.2×10^3 cells/ml (S.E. 14.8) in the controls. This difference was found to be significant ($P < 0.0001$) (Table 3). Thus, vaccinated heifers had 42.3% lower SCCs than the control cows. There were also differences among herds in their milk yields; however; in all seven herds the vaccinated heifers had either the same or higher milk yield than the controls: the mean milk yield of the vaccinated heifers was significantly higher ($P < 0.01$), by 0.5 kg per day than that of the control animals (Table 3).

During the second lactation, six of the cows (from the three groups) became clinically infected with Gram negative bacteria and therefore, were not considered for the analysis. Revaccinated cows exhibited a mean (over herd) value of 100.8×10^3 cells/ml (S.E. 28.8), compared with 137.6×10^3 cells/ml (S.E. 45.0) for vaccinated cows and 220.7×10^3 cells/ml (S.E. 45.6) for the controls (Table 4). Thus, the SCCs of revaccinated cows were 54.33% lower than that of the control cows and 26.7% lower than that of the vaccinated cows; the SCC of the vaccinated cows was

Table 3
Daily milk yield (kg) and SCC in milk of vaccinated and control heifers

Group	Milk (kg per day)		SCC ($\times 10^3$)	
	Mean	S.E.	Mean	S.E.
Control	34.8	0.19	175.2	14.8
Vaccinated	35.3	0.23	101.1	12.1
Vaccinated – control	+0.5 (1.4%)		−74.1 (42.3%)	
$P > F$	0.01		0.0001	

Table 4
Distribution of second-lactating cows according to treatment: revaccinated, vaccinated, and control animals evaluated according to their SCC during the lactation

Group	SCC ($\times 10^3$)	
	Mean	S.E.
Control (45)	220.7	45.6
Vaccinated (13)	137.6	45.0
Revaccinated (26)	100.8	28.8
Vaccinated – control	−83.1 (37.7%)	
Revaccinated – control	−119.9 (54.3%)	
Revaccinated – vaccinated	−36.8 (26.7%)	
$P > F$	0.05	

37.7% lower than that of the control animals. However, the only significant differences were those between revaccinated or vaccinated cows and the controls; the difference between the vaccinated and the revaccinated cows was not significant (Table 4). No significant differences in daily milk yield were found among the groups.

4. Discussion

Vaccination with the experimental vaccine against *S. aureus* mastitis did not affect pregnancy and no difference was observed between vaccinated and control heifers in the numbers of healthy calves delivered. All heifers responded to the vaccine with the production of specific antibodies, but the peak distributions of antibody levels were based on observations at the herd level rather than at the individual animal level (Fig. 1), therefore, the possibility that local herd factors influence the immune response to antigens cannot be ruled out. In spite of these local differences between herds, the difference in udder health between the vaccinated and control cows, as indicated by the SCCs, were significant for each herd considered separately as well as for the total of 452 animals tested.

Although at the time of vaccination the prevalence of *S. aureus* infection in the cows of tested herds ranged between 8 and 25%, the numbers of first-lactating heifers and second-lactating cows infected with *S. aureus* in this study were low, therefore, it is not possible to analyze the rate of specific protection stimulated by the vaccine. It was shown previously (Leitner et al., 2000b) that the SCC in an uninfected mammary gland is lower than 50×10^3 cells/ml and that the presence in an udder quarter of most contaminating bacteria, including CNS (Chaffer et al., 1999), causes an increase in the SCC, to 10^5 to 10^6 cells/ml. Moreover, contaminants in one quarter have no influence regarding SCC on the other quarters of the same cow; therefore, the total SCC in the milk indicates the health status of the udder at whole. Hence, the more quarters infected the higher SCC in the bulk milk. The present study has shown that the SCCs in vaccinated heifers and second-lactating cows were significantly lower than those determined in control animals, though not extremely high. Nevertheless, the high number of animal tested in the current experiment indicates that the overall udder health of vaccinated cows was clearly better than that of the control group. These results are in agreement with those of a previous challenge trial (Pankey et al., 1985) found that the vaccinated cows had significantly lower SCCs than the control cows. The significantly lower SCCs in the second-lactating cows that were not revaccinated before the second calving, together with the detection of the specific antibody to *S. aureus* in those cows, indicate that vaccination before first calving resulted in a persistently healthy udder condition. It seems, however, that revaccination strengthens the likelihood that the mammary gland will remain uninfected. Unfortunately, it was impossible to determine which quarters of a cow were contaminated with CNS, because all the quarters were sampled together. Nevertheless, the significantly lower SCCs in vaccinated than in unvaccinated cows suggest that either the number of quarters infected with CNS was lower or that the cow's response to the presence of the CNS infection in a quarter was less than that in unvaccinated cows.

There was a non-specific effect of the vaccine on udder health. Nevertheless, the vaccine appears to have a heterologous effect since the *S. aureus* types on the seven farms were quite different.

Therefore, it seems, that the vaccine maintains the SCCs similar to those of the uninfected mammary gland.

The 0.5 kg per day increases in milk yield in the vaccinated heifers in the first lactation may have resulted from the overall improvement of udder health in vaccinated cows. Thus, the novel vaccine seems to exert positive effects on both the quantity and the quality of the milk production. The significance of such results to the dairy industry is clearly considerable.

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