STUDY OF THE HUMORAL IMMUNOLOGICAL RESPONSE AFTER VACCINATION WITH A *Staphylococcus aureus* BIOFILM-EMBEDDED BACTERIN IN DAIRY COWS: POSSIBLE ROLE OF THE EXOPOLYSACCHARIDE SPECIFIC ANTIBODY PRODUCTION IN THE PROTECTION FROM *Staphylococcus aureus* INDUCED MASTITIS.

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OBJECTIVE

The objective of the present study was to analyze an extracellular component from *Staphylococcus aureus (S. aureus)*, which we refer to as Slime Associated Antigenic Complex (SAAC), and to investigate the role of SAAC-specific antibody production in protection from *S. aureus* bovine mastitis.

INTRODUCTION

Data on the specific role of biofilm matrix polysaccharides in the development of a protective immune response against *S. aureus* mastitis are limited. Recently, Pérez et al. (2009) found that bacterins from strong biofilm-producing bacteria triggered the highest production of antibodies to PNAG and conferred the highest protection against infection and mastitis in an immunization-challenge study in sheep, compared with weak biofilm-producing bacteria, crude extract or purified PNAG. It was concluded that bacterins from strong biofilm bacteria, rather than purified polysaccharide, could be a cost-efficient vaccination approach against *S. aureus* ruminant mastitis. Here, we used two bacterin preparations, with different SAAC content, to perform an immunization-challenge study against *S. aureus* mastitis in cows.

MATERIALS AND METHODS

SAAC characterization: the slime producing phenotype in Congo Red agar (CRA) plates, the biofilm formation ability on polystyrene microtiter plates (late adherence test) and the SAAC production (ELISA inhibition method) were determined in thirteen isolates of *S. aureus.* Purified SAAC was chemically analyzed in order to characterize the free sugars, free amino acids, total sugars after acidic hydrolysis and total amino acids after acidic hydrolysis. **Animals, vaccinations and challenge:** 12 healthy pregnant heifers were randomly assigned to the following groups: Group 1 animals (n = 4) were vaccinated with an *S. aureus* bacterin with very limited SAAC content (2 x 10^{10} bacteria per dose), Group 2 (n = 4) received a *S. aureus* bacterin with high SAAC content (2 x 10^{10} bacteria per dose), Group 3 animals (n = 4) were inoculated with PBS and served as non-immunized controls. Additionally, vaccines from Group 1 and Group 2 also contained inactivated *Escherichia coli* O111:B4 (J5 strain) bacterial cells (10^{10} bacteria per dose) and an oil-based adjuvant. Seronegative animals were vaccinated intramuscularly at 45 days before the expected day of parturition and revaccinated 35 days later. Each heifer was challenged by intramammary infusion in two contralateral quarters with 8 x 10^3 CFU of a virulent CP type 8 *S. aureus* strain (C195+) approximately on day 23 of lactation. **Sample collection and analysis:** milk bacterial cell counts (CFU/ml) were determined on Columbia blood agar plates. Serum samples were assayed for IgG SAAC-specific antibodies using a SAAC based indirect ELISA. *S. aureus*-specific antibodies in milk were determined using a commercial ELISA kit (VMRD). **Statistical analysis:** antibody production and bacterial cell count were analyzed using a Factorial Mixed Model for repeated measures.

RESULTS AND DISCUSSION

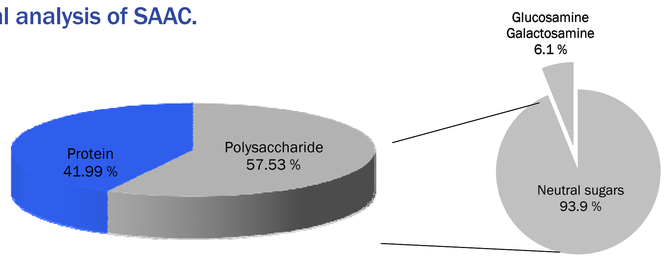
SAAC characterization and analysis: all the slime-producing strains showed different levels of SAAC production, whereas no to weak production was detected in non slime-producing strains The correlation between SAAC expression and biofilm forming ability was statistically evaluated, and a significant correlation ($P < 0.05$) was found between the two parameters ($R = 0.882$). The SAAC composition is shown in figure 1.

Table 1. Slime-producer phenotype determination (+/-), biofilm formation ability (OD_{492} in the biofilm assay) and SAAC production (mg SAAC / mg total protein) in *S. aureus* isolates.

S. aureus isolate	Slime-producer phenotype	OD_{492} in the biofilm assay (SE[1])	SAAC production (SE[1])
SA1H	+	1.444 (0.04)	54.0 (0.012)
SA2H	+	1.597 (0.02)	63.3 (0.015)
SA3H	+	0.385 (0.03)	20.8 (0.011)
SA4H	+	1.499 (0.04)	60.5 (0.012)
SA5H	+	1.521 (0.03)	27.6 (0.015)
SA6H	-	0.088 (0.01)	2.2 (0.011)
SA7H	+	1.030 (0.02)	26.5 (0.012)
SA8H	-	0.388 (0.06)	Nd[2]
SA9H	-	0.200 (0.02)	Nd[2]
SA10H	-	0.145 (0.01)	0.1 (0.010)
SA11H	-	0.130 (0.01)	Nd[2]
SA12H	-	0.235 (0.01)	Nd[2]
SA13H	+	0.632 (0.02)	6.9 (0.013)

[1]SE : standard error of the mean
[2]Nd. : non-detected

Figure 1. Chemical analysis of SAAC.



Efficacy of immunization with *S. aureus* bacterins containing SAAC: immunization with a high SAAC content in the *S. aureus* bacterin (Group 2) significantly enhanced antibody titers against SAAC (in serum and milk) and reduced the *S. aureus* concentration in milk during the post-challenge period compared to Group 1 and Group 3. Moreover, a significant negative correlation was observed between SAAC antibody production on the day of the challenge and the *S. aureus* count in milk by 1 day after challenge. However, there was no evidence of a difference between vaccinated and control groups with regard to clinical signs of mastitis following the challenge. Nevertheless, the SAAC antibody concentration on the day of the challenge negatively correlated with the mastitis score in quarters infected with *S. aureus* at 2 days post-challenge.

Figure 2. SAAC-specific total IgG response in the serum of cattle.

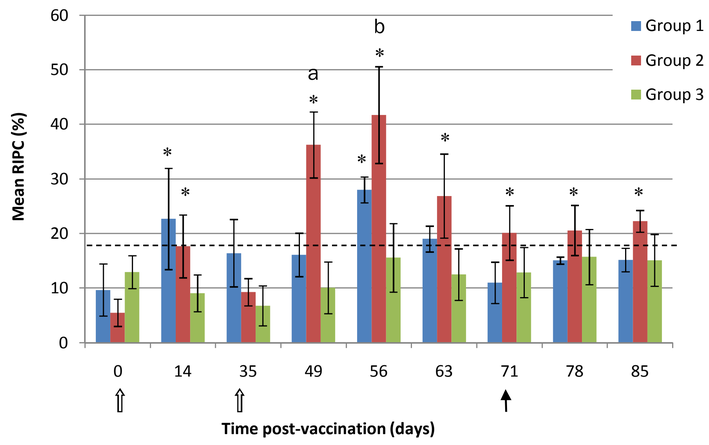


Figure 3. *S. aureus*-specific IgG (A), SAAC-specific total IgG (B), IgG1 (C) and IgG2 (D) levels in milk.

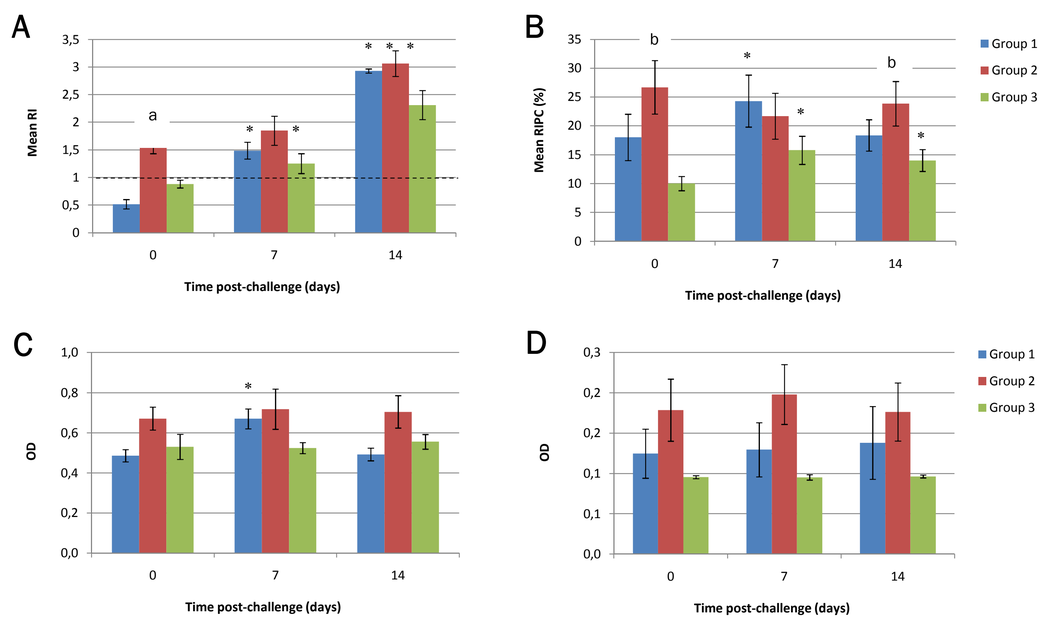
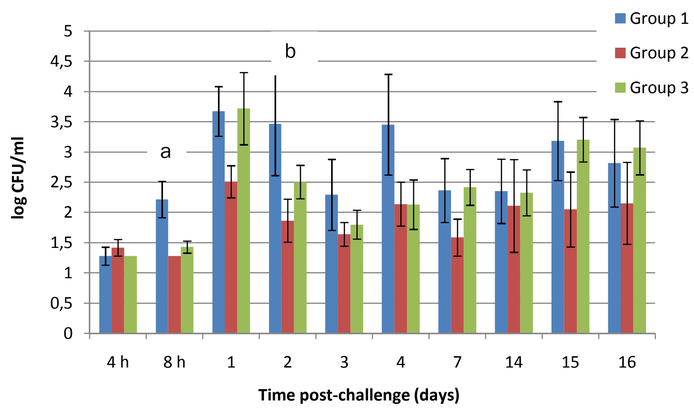


Figure 4. Bacterial cell counts in milk from challenged quarters following intramammary infection with *S. aureus* C195.



CONCLUSIONS

These results indicate that immunization with an *S. aureus* bacterin with high SAAC content was able to reduce *S. aureus* multiplication in the mammary gland after challenge and suggests that the SAAC specific antibody response could be involved in the protection against *S. aureus* intramammary infection. In this way, bacterins from strong biofilm-producing bacteria are proposed as a cost-efficient vaccine design against bovine mastitis, just as Pérez et al. (2009) previously demonstrated in an efficacy study with sheep. This information may be relevant for developing more efficacious commercial staphylococcal vaccines based on bacterins surrounded by their own biofilm matrix containing SAAC.

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