Efficacy evaluation of a new vaccine against bovine mastitis: field trials results

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1. Vaccine

The vaccine used was STARTVAC® (HIPRA), containing inactivated *Escherichia coli* J5 and inactivated *Staphylococcus aureus* SP140 strain. The *S. aureus* inactivated cells contain an extracellular component that we refer to as Slime Associated Antigenic Complex (SAAC), related to the slime producing phenotype and the biofilm formation ability.

2. Deciding the composition of the Startvac vaccine

Biofilm refers to the microbial communities attached to a solid surface and enclosed in a extracellular matrix. Some advantages of biofilm formation are that promote the catalytic functions, affords protection from the environmental challenges (UV exposure, metal toxicity, acid exposure, dehydratation and salinity, phagocytosis and several antibiotics and antimicrobial agents).

S. aureus adhesins are involved in the establishment of infection through early attachment to host tissues (Clarke and Foster, 2006). A second step in virulence results in intercellular adhesion among bacterial cells and subsequent development of a biofilm, leading to chronic infections and bacterial resistance to phagocytosis (Monleon et al., 1997; Costerton et al., 1999; Kampen et al., 2005). A major constituent of the staphylococcal biofilm matrix is the poly-*N*-acetyl β-1,6 glucosamine surface polysaccharide, synthesized by proteins encoded by the intercellular adhesion *ica* operon (Cramton et al., 1999).

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 a immunization-challenge study in sheeps, compared with weak biofilm-producing bacteria, crude extract or purified PNAG, concluding that bacterins from strong biofilm bacteria, rather than purified polysaccharide could be a cost-efficient vaccination approach against *S. aureus* ruminant mastitis.

The *E. coli* J5 strain included in the vaccine is a natural mutant strain that lacks the enzyme responsible for binding the outer polysaccharide to the core region of the LPS, so the core antigen is exposed in the external membrane of this strain. The interesting thing of this strain is that the core region of LPS is highly conserved among a broad spectrum of Gram-negative bacteria.

The objective of the present study was to analyse the efficacy this new vaccine against bovine mastitis in a field trial.

3. Field trials

The trial was carried out in six dairy farms with a known history of staphylococcal, and /or coliforms mastitis within the last year. The farms included in the study had different type of milking, working procedures, park design, nutrition, etc. The type of management employed in these farms (housing conditions, feeding, type of milking, parameterisation...) as well as the genetics of the animals used and the habitual mastitis problems found are common in the dairy farms around Europe. None of the

management conditions that were applied in the dairy before the trial were modified during the trial.

Experimental design

The field trial was conducted following Good Clinical Practices (GCP) (VICH) and was multicentric, randomized, double blind, controlled (with a parallel negative control group) and stratified (primiparous and multiparous). A total of 386 gestating cows (198 vaccinated with STARTVAC® and 188 inoculated with a Placebo) were included. Cows and heifers at an age of 22 months onwards were immunised in accordance with the proposed immunisation schedule by intramuscular route either with the vaccine STARTVAC® or with a placebo: 1st injection 45 days before the expected parturition date, 2nd injection 35 days thereafter (corresponding to 10 days before the expected parturition date) and 3rd injection 62 days after the 2nd injection (corresponding to 52 days after the expected parturition date). The following variables were examined and evaluated:

- Incidence of clinical mastitis (appearance of new cases of mastitis) by means of evaluating the general clinical symptoms and local clinical symptoms.

- Incidence of subclinical mastitis by means of the aseptic taking of milk per cow (from the 4 quarters) for microbiological analysis and somatic cell count and individual recording of the daily milk production in the totality of the animals included in the trial.

- Severity of the symptoms, analysing the Somatic Cell Counts (SCC), general clinical signs, local clinical signs (milk and quarter appearance), dead cows due to mastitis or severe mastitis and mastitis treatments with pharmacological products. All the variables were recorded weekly in all of the animals included in the study and throughout the entire observation period (130 days post-partum), but they were statistically analysed to assess the reduction of the severity of the symptoms only in those animals in which clinical or subclinical mastitis were diagnosed according to the study plan criteria.

- Spontaneous cure rate. That means the cured cases of mastitis per number of infected animals. Cured cases of mastitis were considered all those animals that recovered from mastitis spontaneously, without the administration of any pharmacological mastitis treatment.

4. Results

Table 1 shows the total number of cases of intramammary infections (IMI), with clinical or subclinical manifestations. 27 cows of the placebo group and 7 of the STARTVAC® group showed clinical mastitis caused by *Staphylococcus aureus*, coliforms or CNS. 79 cows of the placebo group and 32 of the STARTVAC® group showed subclinical mastitis caused by *Staphylococcus aureus*, coliforms or CNS.

Table 1. Number of cases of intramammary infection (IMI) (clinical or subclinical).

		PLACEBO	STARTVAC	Significance (P-value)
S. aureus				
	Total cows	18	2	0.001 (***)
	Primiparous	7	1	0.023 (*)
	Multiparous	11	1	0.006 (**)
Coliforms				

	Total cows	31	7	0.001 (***)
	Primiparous	12	3	0.010 (*)
	Multiparous	19	4	0.002 (**)
CNS				
	Total cows	56	28	0.001 (***)
	Primiparous	30	16	0.007 (**)
	Multiparous	26	12	0.033 (*)

* p<0.05;** p<0.01; *** p<0.001

The SCC was significantly lower in the vaccinated group compared to the placebo group (Table 2). In relation with clinical signs of mastitis (milk and quarter appearance), there was a 24.03 % of cows with abnormal quarter appearance in the placebo group, whereas in the STARTVAC® group the percentage of cows with abnormal quarter appearance was 14.44 %. In the placebo group there was 19.79 % of cows with abnormal milk appearance, whereas in the STARTVAC® group the percentage with abnormal milk appearance was 11.42 % (Table 3). Furthermore, the number of cows that needed to be treated with pharmacological products was 40 in the placebo group and 24 in the STARTVAC® group..

Table 2. Somatic Cell Counts results.

TREATMENT	**N***	**SCC x 10^3**	**SCS***	**H0:LSMean1=LSMean2**
		LSMEAN [1]	**LSMEAN [1]**	**Pr > |t|**
PLACEBO	2729	548.6	2.68	< 0.0001
STARTVAC	2578	328.2	2.53	

[1]Least square mean * N: no. of milk samples

Table 3. Clinical signs results of mammary gland and milk appearance.

TREATMENT	**QUARTER APPEARANCE**		**P value (treatment effect)**	**MILK APPEARANCE**		**P value (treatment effect)**
	0[1]	**≥1**		**0[1]**	**≥1**	
PLACEBO	75.97 %	24.03 %	0.026*	80.21 %	19.79 %	0.031*
STARTVAC	85.56 %	14.44 %		88.58 %	11.42 %	

[1]0: Normal quarter and milk ≥ 1: Quarter inflammation and abnormal milk (serous yellowish secretion with the presence of lumps and or blood.). * Statistically significant results

Another variable assessed was the spontaneous cure rate, that means the cases cured of mastitis per number of infected animals during the observation period. It has to be clarified that cured cases of mastitis were considered all those animals that recovered from mastitis spontaneously, without the administration of any pharmacological mastitis treatment. Taking into account the total number of cows (multiparous + primiparous) and all the pathogens together, STARTVAC® vaccinated cows showed a cure rate of 51.43 % whereas the placebo cows showed a cure rate of 32.18 %, so there were 19.25 % more cows cured in the STARTVAC® group than in the placebo group (Table 4).

Table 4. Number of cured cows [percentage over the total mastitis cases] after an intramammary infection (clinical or subclinical) for all the different pathogens together.

	Multiparous	**Primiparous**	**Total cows**
PLACEBO	9 [20.45]	19 [44.19]	28 [32.18]
STARTVAC	8 [53.33]	10 [50]	18 [51.43]
P value (Chi-square test [1])	< 0.05 *	> 0.05	< 0.05 *

[1]Association test between treatment and cure rate. * Significant differences.

5. Conclusions

All these results indicate that the immunisation program, as well as the dosage of 2 ml/animal and the administration route of the vaccine, is efficacious in the reduction of the incidence of intramammary infection due to *S. aureus*, coliforms or coagulase-negative staphylococci, with clinical or subclinical manifestations in cows (multiparous) and heifers (primiparous) in the period of maximum incidence, i.e. post parturition. Immunisation also significantly reduces the severity of the symptoms, causes a significant increase in the spontaneous cure rate of the infected cows, significantly reduces the number of cows that need to be treated for mastitis and has positive effects on both the quantity and quality of milk production.